

# AnnotSV Manual

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## **Version 3.1.3**

AnnotSV is a program for annotating and ranking structural variations from genomes of several organisms. This README version is dedicated to the human genome.

<https://lbgf.fr/AnnotSV/>

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## LEXIQUE

1000g: 1000 Genomes Project (phase 3)

2G = "Digenic inheritance"

ACMG: American College of Medical Genetics and Genomics

AD = "Autosomal dominant"

AR = "Autosomal recessive"

ADm = "Autosomal dominant with maternal imprinting"

ADp = "Autosomal dominant with paternal imprinting"

BED: Browser Extensible Data

bp: base pair

CDS: CoDing Sequence

CNV: Copy Number Variation

DDD: Deciphering Developmental Disorders

DECIPHER: DatabasE of genomic varlation and Phenotype in Humans using Ensembl Resources

DEL: Deletion

DGV: Database of Genomic Variants

DNA: DesoxyriboNucleic Acid

DUP: Duplication

ENCODE: Encyclopedia of DNA Elements

ExAC: Exome Aggregation Consortium

GenCC: Gene Curation Coalition

GH: GeneHancer

GRCh37: Genome Reference Consortium Human Build 37

GRCh38: Genome Reference Consortium Human Build 38

HI: Haploinsufficiency

hom: homozygous

htz: heterozygous

ID: Identifier

indel: Insertion/deletion

INS: Insertion

INV: Inversion

IPVE = "Incomplete Penetrance and/or Variable Expressivity"

LoF: Loss of Function

MCNV: multiallelic CNV

MEI: Mobile Element Insertion

misZ = Z score indicating gene intolerance to missense variation

moi: mode of inheritance

MT = "Mitochondrial"

NAHR: Non-Allelic Homologous Recombination

OMIM: Online Mendelian Inheritance in Man

pLI: score indicating gene intolerance to a loss of function variation

sD = "Semidominant"

SNV: Single Nucleotide Variation

SOM = "Somatic mosaicism"

SV: Structural Variations

synZ = Z score indicating gene intolerance to synonymous variation

TAD: Topologically Associating Domains

Tcl: Tool Command Language

TS: Triplosensitivity

Tx: transcript

VCF: Variant Call Format

XL = "X-linked"

XLD = "X-linked dominant"

XLR = "X-linked recessive"

YL = "Y-linked"

YLD = "Y-linked dominant"

YLR = "Y-linked recessive"

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## 1. INTRODUCTION

AnnotSV (Geoffroy et al., 2021, 2018) is a program designed for annotating and ranking Structural Variations (SV). This tool compiles functionally, regulatory and clinically relevant information and aims at providing annotations useful to i) **interpret SV potential pathogenicity** and ii) **filter out SV potential false positives**.

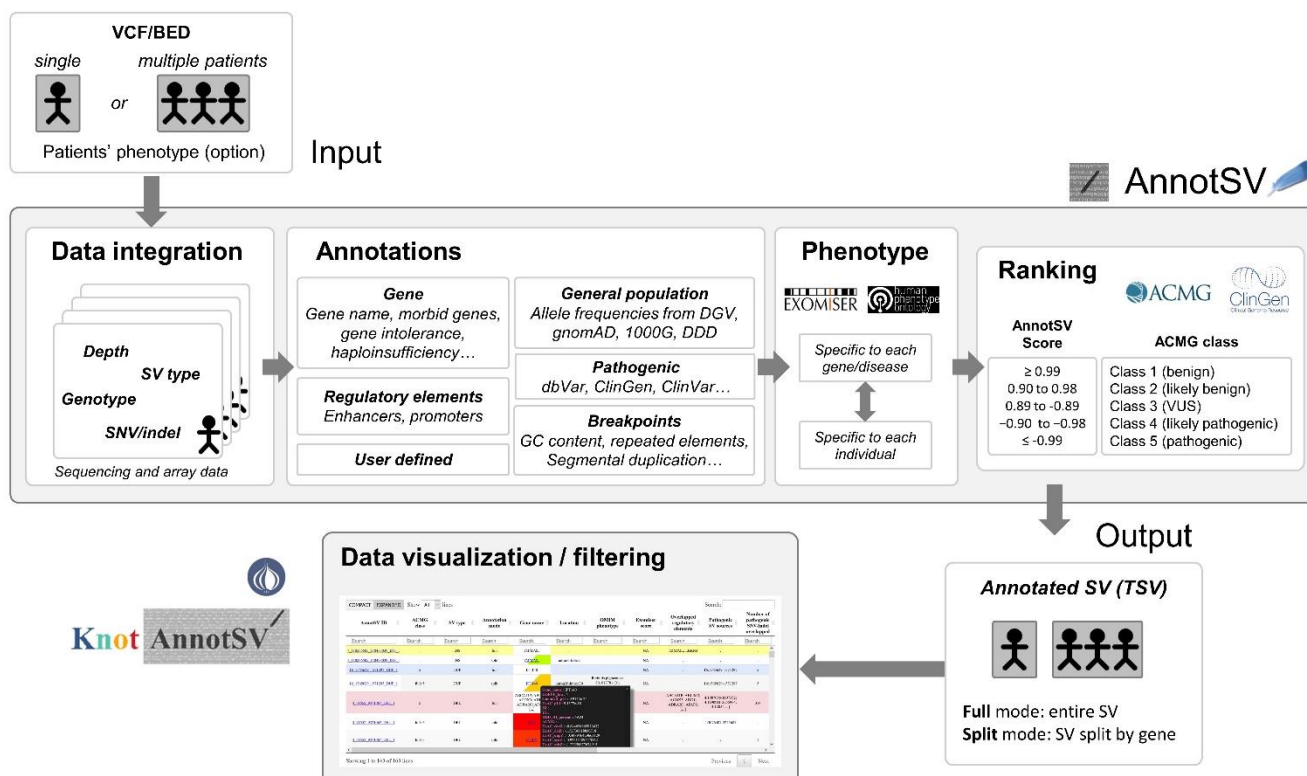
Different types of SV exist including deletions, duplications, insertions, inversions, translocations or more complex rearrangements. They can be either balanced or unbalanced. When unbalanced and resulting in a gain or loss of material, they are called Copy Number Variations (CNV). CNV can be described by coordinates on one chromosome, with the start and end positions of the SV (deletions, insertions, duplications). Complex rearrangements with several breakends can arbitrary be summarized as a set of novel adjacencies, as described in the Variant Call Format specification [VCF v4.3](#) (Jun 2020).

### a) Overview

AnnotSV takes as an input file a classical BED or VCF file describing the SV coordinates, as well as the patients' phenotype (optional). The outputfile contains the overlaps of the SV with relevant genomic features where the genes refer to RefSeq or ENSEMBL genes (user defined). AnnotSV provides numerous additional relevant annotations:

- Gene-based annotations (GenCC, OMIM, Gene intolerance, Haploinsufficiency...)
- Annotations with features overlapping the SV (DGV, 1000genomes...)
- Annotations with features overlapped with the SV (pathogenic SV from dbVar, promoters, enhancers, TAD...)
- Annotations of the SV breakpoints (GC content, repeats...)

In addition to these annotations, AnnotSV also provide a systematic SV classification/ranking using the same type of categories delineated by the American College of Medical Genetics and Genomics (ACMG) and ClinGen (Richards et al., 2015; Riggs et al., 2020).



It is important to notice that, in order to reduce or at least not to expand too much the list of annotation columns, we have decided to specifically report the information of the corresponding SV type.  
Ex: A deletion of interest will be annotated with pathogenic SV using only the deletion data in details.

## b) Supported organisms

AnnotSV is mainly dedicated for the annotation and ranking of structural variations from human genomes. However, since version 2.2 AnnotSV supports also the mouse genome. If you are interested, please see the specific mouse README file.

## 2. INSTALLATION/REQUIREMENTS

### a) Tcl (required)

The AnnotSV program is written in the Tcl language. Modern Unix systems have this scripting language already installed (otherwise it can be downloaded from <https://www.activestate.com/activetcl/downloads>).

AnnotSV requires **the latest release of the Tcl distribution starting with version 8.5** as well as the following 4 packages "http", "json", "tar" and "csv".

The "http" and the "json" packages are used for the phenotype-driven analysis.

The "tar" and "csv" packages are used only when data sources are updated.

## b) bedtools (required)

The “[bedtools](#)” toolset (developed by Quinlan AR) needs to be locally installed.

Add the path of the bedtools bin directory to your PATH and save the settings in your .cshrc or .bashrc file:

- In csh, you can define it with the following command line:  
`setenv PATH {${PATH}}:/somewhere'/bedtools-2.25.0/bin`
- In bash, you can define it with the following command line:  
`export PATH=${PATH}:/somewhere'/bedtools-2.25.0/bin`

**Warning:** the minimum bedtools version compatible with AnnotSV is version 2.25. To check if bedtools exists and if the version is the good one, run:

`bedtools --version`

## c) bcftools (required)

The “[bcftools](#)” toolset (Li, 2011) needs to be locally installed if using VCF input file(s).

Add the path of the bcftools bin directory to your PATH and save the settings in your .cshrc or .bashrc file:

- In csh, you can define it with the following command line:  
`setenv PATH {${PATH}}:/somewhere'/bcftools-1.9/bin`
- In bash, you can define it with the following command line:  
`export PATH=${PATH}:/somewhere'/bcftools-1.9/bin`

**Warning:** the minimum bcftools version compatible with AnnotSV is version 1.10. To check if bcftools exists and if the version is the good one, run:

`bcftools --version`

## d) Java (optional)

In order to use the phenotype-driven analysis based on one Exomiser module, a minimal Java 8 installation is required.

Moreover, the Exomiser module writes in the /tmp/spring.log file that must, therefore, have write permissions.

## e) AnnotSV source code (required)

Since the 2.3 version, “**AnnotSV source code**” is only downloadable on GitHub at the following address (under the GNU GPL license):

<https://github.com/lgmgeo/AnnotSV>

### **Install:**

The sources can be cloned to any directory:

`cd /somewhere/`



git clone <https://github.com/lgmgeo/AnnotSV.git>

Then, the user can choose either to easily set the install by default in /usr/local:  
make install

or to define \$PREFIX as a specific installation directory:  
make PREFIX='somewhere\_else'/AnnotSV\_'version'/ install

or to define \$PREFIX as the actual directory:  
make PREFIX=. install

The AnnotSV installation directory (/path\_of\_AnnotSV\_installation) will be either set to:

/usr/local

or: /somewhere\_else'/AnnotSV\_'version'/

or: /somewhere'/AnnotSV\_'version'/

Thus, the AnnotSV executable will be located in:

/path\_of\_AnnotSV\_installation/bin/AnnotSV

Then, the annotations requested by the user (human, mouse or both) need to be installed with the following command lines:

make PREFIX=... install-human-annotation

make PREFIX=... install-mouse-annotation

make PREFIX=... install-mouse-annotation install-human-annotation

make PREFIX=... install-all-annotations

Finally, the installation requires simply to set the following environment variable:

\$ANNOTSV: "AnnotSV installation directory"

And to save the settings in your .cshrc or .bashrc file.

- In csh, you can define it with the following command line:  
setenv ANNOTSV /path\_of\_AnnotSV\_installation/
- In bash, you can define it with the following command line:  
export ANNOTSV=/path\_of\_AnnotSV\_installation/

Make sure the program correctly finds the Tcl interpreter. By default, the best way to make a Tcl script executable is to put the following as the first line of the main script (already done in the AnnotSV executable):

#!/usr/bin/env tclsh

It can be changed to any other path like:

#!/usr/local/ActiveTcl/bin/tclsh

## f) Filesystem Hierarchy Standard (FHS)

AnnotSV follows the Filesystem Hierarchy Standard (FHS) that defines the directory structure and directory contents in Linux distributions.

### **AnnotSV installation directory:**

By default, the AnnotSV installation directory looks like this:

<code>\${DESTDIR}\${PREFIX}</code>	<code>#the program installation directory (default = /usr/local)</code>
<code> </code>	
<code> ----- bin/</code>	<code>#where the executable script is stored</code>
<code> </code>	
<code> ----- etc/AnnotSV/</code>	<code>#where a configfile example is stored, that can be copied to any</code>
<code> </code>	<code>#analysis directory for modification purpose</code>
<code> ----- Makefile</code>	
<code> </code>	
<code> ----- share/</code>	<code>#Architecture-independent (shared) data</code>
<code>       ----- AnnotSV</code>	<code>#where annotation files are stored (Genes, OMIM...)</code>
<code>             ----- Annotations_Exomiser</code>	
<code>             ----- Annotations_Human</code>	
<code>             ----- Annotations_Mouse</code>	
<code>             ----- jar</code>	
<code>       </code>	
<code>       ----- bash</code>	<code>#where bash files are stored</code>
<code>       ----- doc/AnnotSV/</code>	
<code>             ----- Example</code>	<code>#command/input/output examples</code>
<code>             ----- changeLog.txt</code>	<code>#description of AnnotSV changes</code>
<code>             ----- commandLineOptions.txt</code>	<code>#command line usage</code>
<code>             ----- License.txt</code>	<code>#GNU GPL license</code>
<code>             ----- README.AnnotSV_*.pdf</code>	<code>#this file</code>
<code>       </code>	
<code>       ----- tcl*/AnnotSV/</code>	<code>#where the procedures .tcl files are stored</code>

### 3. ANNOTATION SOURCES

AnnotSV requires different data sources for the annotation of SV. **In order to provide a ready to start installation of AnnotSV, each annotation source listed below (that do not require a commercial license) is automatically downloaded during the installation. Two exceptions need to be noticed with specific licence required in case the GeneHancer and/or the COSMIC resources are of any interest to you.** The aim and update of each of these sources are explained below. Annotation can be performed using either the GRCh37 or GRCh38 build of the human genome (user defined, see USAGE/OPTIONS), but depending on the availability of some data sources there might be some limitations. Some of the annotations are linked to the gene name and thus provided independently of the genome build.

**IMPORTANT NOTE:** To update the data sources, please download the latest available files (not the ones given as an example in the README).

#### a) Gene annotations

Each gene overlapped by the SV to annotate is reported (even with 1bp overlap).

##### Aim:

The “Gene annotation” aims at providing information for the overlapping known genes with the SV. This will result in gene list from the well annotated [RefSeq](#) or [ENSEMBL](#) databases. These annotations include the definition of the genes and corresponding RefSeq transcripts from NCBI (default value). Transcripts from

ENSEMBL can be user defined with the "-transcript" option, (see in USAGE/OPTIONS). This will also integrate the length of the CoDing Sequence (CDS) and of the transcript, the location of the SV in the gene (e.g. « txStart-exon3 ») and the coordinates of the intersection between the SV and the transcript.

#### **Annotation columns:**

Add 15 annotation columns: "Gene\_name", "Gene\_count", "Tx", "Tx\_start", "Tx\_end", "Overlapped\_tx\_length", "Overlapped\_CDS\_length", "Frameshift", "Exon\_count", "Location", "Location2", "Dist\_nearest\_SS", "Nearest\_SS\_type", "Intersect\_start", "Intersect\_end".

#### **Method:**

For each gene, only a single transcript from all transcripts available for this gene is reported in the following order of preference:

- The transcript selected by the user with the "-txFile" option is reported
- The transcript with the longest overlapped CDS is reported
- If there is no difference in CDS length, the longest overlapped transcript is reported.

#### **Updating the data source from RefSeq (if needed):**

- Remove the "genes.RefSeq.sorted.bed" file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Genes/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Genes/GRCh38" directories.
- Download and place the "refGene.txt.gz" file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Genes/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Genes/GRCh38" directories.

The latest update of this file is available for free download at:

*Genome build GRCh37:*

<http://hgdownload.cse.ucsc.edu/goldenPath/hg19/database/refGene.txt.gz>

*Genome build GRCh38:*

<http://hgdownload.cse.ucsc.edu/goldenPath/hg38/database/refGene.txt.gz>

After the update, this refGene.txt.gz file will be processed by AnnotSV during the first run (it will take longer than usual AnnotSV runtime).

#### **Updating the data source from ENSEMBL (if needed):**

- Remove the "genes.ENSEMBL.sorted.bed" file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Genes/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Genes/GRCh38" directories.
- Download:

*Genome build GRCh37:*

```
bash
cd $ANNOTSV/share/AnnotSV/Annotations_Human/Genes/GRCh37/
wget http://ftp.ensembl.org/pub/release-75/gtf/homo\_sapiens/Homo\_sapiens.GRCh37.75.gtf.gz
wget http://hgdownload.cse.ucsc.edu/admin/exe/linux.x86\_64/gtfToGenePred
chmod +x gtfToGenePred
gunzip Homo_sapiens.GRCh37.75.gtf.gz
./gtfToGenePred -genePredExt -geneNameAsName2 Homo_sapiens.GRCh37.75.gtf refGene.txt
for i in 1 10 11 12 13 14 15 16 17 18 19 2 20 21 22 3 4 5 6 7 8 9 M MT X Y; do \
    awk -v chr=$i '$2 ==chr {print $2"\t"$4"\t"$5"\t"$3"\t"$12"\t"$1"\t"$6"\t"$7"\t"$9"\t"$10}' \
    \
    refGene.txt | sed 's/^MT/M/' | sort -k1,1 -k2,2n -k3,3n >> genes.ENSEMBL.sorted.bed; done
rm gtfToGenePred Homo_sapiens.GRCh37.75.gtf refGene.txt
```

Genome build GRCh38:

```
bash
cd $ANNOTSV/share/AnnotSV/Annotations_Human/Genes/GRCh38/
wget http://ftp.ensembl.org/pub/release-107/gtf/homo\_sapiens/Homo\_sapiens.GRCh38.107.chr.gtf.gz
wget http://hgdownload.cse.ucsc.edu/admin/exe/linux.x86\_64/gtfToGenePred
chmod +x gtfToGenePred
gunzip Homo_sapiens.GRCh38.107.chr.gtf.gz
./gtfToGenePred -genePredExt -geneNameAsName2 Homo_sapiens.GRCh38.107.chr.gtf
refGene.txt
for i in 1 10 11 12 13 14 15 16 17 18 19 2 20 21 22 3 4 5 6 7 8 9 M MT X Y; do \
    awk -v chr=$i '$2 ==chr {print $2"\t"$4"\t"$5"\t"$3"\t"$12"\t"$1"\t"$6"\t"$7"\t"$9"\t"$10}' \
    refGene.txt | sed 's/^MT/M/' | sort -k1,1 -k2,2n -k3,3n >> genes.ENSEMBL.sorted.tmp.bed;
done
grep -v "none" genes.ENSEMBL.sorted.tmp.bed > genes.ENSEMBL.sorted.bed
rm gtfToGenePred Homo_sapiens.GRCh38.107.chr.gtf refGene.txt genes.ENSEMBL.sorted.tmp.bed
```

**NOTE:**

It is to notice that the **promoter's annotations update** will be done at the same time (without supplementary update command).

## b) Regulatory Elements annotations

**Aim:**

The contribution of SV affecting promoters/enhancers/miRNA to disease etiology is well established. Affecting possibly gene expression, understanding the consequences of these regulatory variants on the human transcriptome remains a major challenge.

**Method:**

AnnotSV reports the list of the genes whose promoters/enhancers/miRNA are overlapped (even with 1bp overlap) by the SV. When available, the regulated gene name is detailed with associated haploinsufficiency (HI), triplosensitivity (TS) and exomiser (EX) score as well as morbid and candidate gene annotation. The data source of the regulatory elements is also reported (RefSeq, ENSEMBL, EnhancerAtlas (EA), GeneHancer (GH) and/or miRTargetLink (mTL)).

**Options:**

Since overlapping so many regulated genes is a problem, AnnotSV restricts by default the report of the regulated genes to highlight the most relevant ones (i.e. to explain the patient's phenotype):

- By default, only the genes fulfilling at least one of the following criteria are reported (see the "**RSelect1**" option in USAGE/OPTIONS to keep all the regulated genes):
  - OMIM morbid genes
  - HI genes (ClinGen HI = 3)
  - TS genes (ClinGen TS = 3)

- Phenotype matched genes (Exomiser gene score > 0.7)
- User candidate genes (see the “-candidateGenesFile” option in USAGE/OPTIONS)
- By default, only the genes not present in "Gene\_name" (see the “-REselect2” option in USAGE/OPTIONS) are reported.

### **Sources:**

- **Gene data (RefSeq or ENSEMBL):** Promoters are defined by default as 500 bp upstream from the transcription start sites of the RefSeq or ENSEMBL databases (see the "-transcript" option in USAGE/OPTIONS). Nevertheless, the user can define a different bp size with the "promoterSize" option (see USAGE/OPTIONS)
- **EnhancerAtlas 2.0** (Gao and Qian, 2020): Genes regulated by enhancers are reported from [EnhancerAtlas 2.0](#). Based on the enhancer consensus and gene expression data, EnhancerAtlas predicted the target genes of enhancers for many tissue/cell types in human
- **GeneHancer** (Fishilevich et al., 2017): Genes regulated by promoters/enhancers are reported from four different databases: the Encyclopedia of DNA Elements (ENCODE), the Ensembl regulatory build, the functional annotation of the mammalian genome (FANTOM) project and the VISTA Enhancer Browser
- **miRTargetLink** (Kern et al., 2021): Genes regulated by validated miRNA are reported from the miRTargetLink database kindly provided by the authors.

### **WARNING:**

GeneHancer data is under a specific licence that prevent the systematic availability in AnnotSV sources. Users need to request the up-to-date GeneHancer data dedicated to AnnotSV (“GeneHancer\_<version>\_for\_annotsv.zip”) by contacting directly the GeneCards team:

- Academic users: [genecards@weizmann.ac.il](mailto:genecards@weizmann.ac.il)
- Commercial users: [support@lifemapsc.com](mailto:support@lifemapsc.com)

### **Annotation columns:**

Add 1 annotation column (only in the “full” lines): “RE\_gene”.

### **NOTE:**

- Depending on the "tx" option setting, either RefSeq (default) or ENSEMBL gene name are reported
- To come back to the regulatory elements coordinates, the user can set the “REreport” option to 1 (default = 0) which will allow to report this information in an “\*.SV\_RE\_intersect.report” output file.

### **Updating the data source (if needed):**

- Remove all the files in the “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/RegulatoryElements/GRCh37” and/or “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/RegulatoryElements/GRCh38” directories.
- Gene data:  
Promoters will be automatically updated by using the Gene annotations.
- Download EnhancerAtlas files:  
You can freely download the GRCh37 EnhancerAtlas TXT files from <http://www.enhanceratlas.org/downloadv2.php>. Click the “Download enhancer-gene interactions” section. Download the 114 human files (\*\_EP.txt) in the “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/RegulatoryElements/GRCh37”.  
To do this:

```
tcsh
```

```
foreach tissuOrCell {A375 A549 AML_blast Astrocyte BJ Bronchia_epithelial Caco-2 Calu-3 CD14+ CD19+ CD20+  
CD34+ CD36+ CD4+ CD8+ Cerebellum CUTLL1 DOHH2 ECC-1 ESC_neuron Esophagus Fetal_heart Fetal_kidney  
Fetal_muscle_leg Fetal_placenta Fetal_small_intestine Fetal_spinal_cord Fetal_stomach Fetal_thymus FT246  
FT33 GM10847 GM12878 GM12891 GM12892 GM18505 GM18526 GM18951 GM19099 GM19193 GM19238  
GM19239 GM19240 H1 H9 HCC1954 HCT116 HEK293T HEK293 Hela-S3 Hela HepG2 HFF HL-60 hMADS-3 HMEC  
hNCC HSMM HT1080 HT29 HUVEC IMR90 Jurkat K562 Kasumi-1 KB Keratinocyte Left_ventricle LHCN-M2 Liver  
LNCaP-abl LNCaP Lung MCF-7 MCF10A ME-1 Melanocyte melanoma Mesendoderm MS1 Myotube Namalwa NB4  
NHDF NHEK NHLF NKC OCI-Ly7 Osteoblast Ovary PANC-1 Pancreas Pancreatic_islet PBMC PC3 PrEC  
SGBS_adipocyte SK-N-SH SK-N-SH_RA Skeletal_muscle Small_intestine Sperm Spleen T47D T98G th1 Thymus U2OS  
VCaP ZR75-30} {  
  catch {eval exec wget http://www.enhanceratlas.org/data/AlIEPs/hs/${tissuOrCell}_EP.txt}  
}
```

These GRCh37 files will be computed then removed the first time AnnotSV is executed after the update. After processing, you need to lift over the resulting GRCh37 file to GRCh38 with the [UCSC web server](#) and to move it in the “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/RegulatoryElements/GRCh38” directory.

- **GeneHancer data:**

Put the “GeneHancer\_<version>\_for\_annotsv.zip” file in the following directory: “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/RegulatoryElements”

Unzip this GRCh37/GRCh38 file:

```
cd $ANNOTSV/share/AnnotSV/Annotations_Human/  
cd FtIncludedInSV/RegulatoryElements/  
unzip GeneHancer_<version>_for_annotsv.zip  
  Archive: GeneHancer_<version>_for_annotsv.zip  
  inflating: ReadMe.txt  
  inflating: GeneHancer_elements.txt  
  inflating: GeneHancer_gene_associations_scores.txt  
  inflating: GeneHancer_hg19.txt  
  inflating: GeneHancer_tissues.txt
```

These files will be computed then removed the first time AnnotSV is executed after the update.

- **miRTargetLink data:**

Put the “Validated\_miRNA-gene\_pairs\_hsa\_miRBase\_<version>\_GRCh38\_location\_augmented.tsv” file (provided by the authors) in the following directory:

“\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/RegulatoryElements”.

This file will be computed then removed the first time AnnotSV is executed after the update.

After processing, you need to lift over the resulting GRCh38 file to GRCh37 with the [UCSC web server](#) and to move it in the “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/RegulatoryElements/GRCh37” directory.

## c) Gene-based annotations

These annotations are linked to the **gene name** and thus provided independently of the genome build. They give additional information on each gene overlapped by a SV.

## Gene-Disease relationship annotations

### *GenCC gene annotations*

#### **Aim:**

The [GenCC](#) (Gene Curation Coalition) (DiStefano et al., 2022) develops standards and consistent terminologies for describing gene-disease validity. GenCC encompasses:

- organizations that currently provide online resources (e.g. ClinGen, DECIPHER (Firth et al., 2011, 2009), Genomics England PanelApp, OMIM, Orphanet, PanelApp Australia, TGM1's G2P)
- diagnostic laboratories that have committed to sharing their internal curated gene-level knowledge (e.g. Ambry, Illumina, Invitae, Myriad Women's Health, Mass General Brigham Laboratory for Molecular Medicine).

#### **Annotation columns:**

Add 5 annotation columns (only in the "split" lines): "GenCC\_classification", "GenCC\_moi", "GenCC\_disease", "GenCC\_pmid".

#### **Updating the data source (if needed):**

- Remove all the \*GenCC\* files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/GenCC" directory.
- Download and place the "submissions-export-tsv" GenCC file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/GenCC" directory. The latest update of this file is available for free download at:

<https://search.thegencc.org/download/action/submissions-export-tsv>

This file will be computed the first time AnnotSV is executed after the update.

### *OMIM annotations*

#### **Aim:**

[OMIM \(Online Mendelian Inheritance in Man\)](#) (Hamosh et al., 2000) focuses on the relationship between phenotype and genotype. Moreover, a morbid genes list is provided.

#### **Annotation columns:**

Add 3 annotation columns: "OMIM\_ID", "OMIM\_morbid" and "OMIM\_morbid\_candidate".

Add 2 other annotation columns (only in the "split" lines): "OMIM\_phenotype" and "OMIM\_inheritance".

#### **Method:**

The "morbidGenes" and "morbidGenesCandidates" are described in the "Disorder" column of the Gene Map file as follows:

- morbidGenes: the number in parentheses after the name of each disorder is set to (3) or (4):

(3) indicates that the molecular basis of the disorder is known; a mutation has been found in the gene.

(4) indicates that a contiguous gene deletion or duplication syndrome, multiple genes are deleted or duplicated causing the phenotype.

- **morbidGenesCandidates:** the number in parentheses after the name of each disorder is set to (3) or (4) AND the symbol in front of the name of each disorder is set to "{ }" or "?:

"{ }", indicates mutations that contribute to susceptibility to multifactorial disorders (e.g., diabetes) or to susceptibility to infection (e.g., malaria).

"?", before the phenotype name indicates that the relationship between the phenotype and gene is provisional.

#### **Updating the data source (if needed):**

- Remove all the files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/OMIM" directory.
- Download and place the "**genemap2.txt**" and "**morbidmap.txt**" OMIM files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/OMIM" directory.  
The latest updates of these files are available for download following a registration and review process (<https://omim.org/downloads/>). "**genemap2.txt**" is a tab-delimited file containing OMIM's synopsis of the Human gene map including additional information such as genomic coordinates and inheritance. "**morbidmap.txt**" is a tab-delimited file of OMIM's Synopsis of the Human Gene Map (same as genemap.txt above) sorted alphabetically by disorder

#### ACMG annotations

##### **Aim:**

The American College of Medical Genetics and Genomics has published recommendations for reporting incidental or secondary findings in genes with a medical benefit (Miller et al., 2022). The most recent version of the recommendations is the [ACMG SF v3.1](#) including 78 genes.

##### **Annotation columns:**

Add 1 annotation column (only in the "split" lines): "ACMG".

#### Gene intolerance annotations

##### gnomAD annotations

##### **Aim:**

Gene intolerance annotations from the [gnomAD](#) dataset give the significant deviation from the observed and the expected number of variants for each gene.

pLI is a score indicating the probability that a gene is intolerant to a loss of function variation (Nonsense, splice acceptor/donor variants due to SNV/indel). A gene with pLI $\geq$ 0.9 is considered as an extremely LoF intolerant gene.

LOEUF stands for the "loss-of-function observed/expected upper bound fraction."

- Low LOEUF scores (e.g. 0) indicate strong selection against predicted loss-of-function (pLoF) variation in a given gene
- High LOEUF scores (e.g. 9) suggest a relatively higher tolerance to inactivation.

LOEUF advantage over pLI is that it can be used as a continuous value rather than a dichotomous scale (e.g. pLI > 0.9) - if such a single cutoff is still desired, pLI is a perfectly fine metric to use. At large sample sizes, the observed/expected ratio will be a more appropriate measure for selection, but at the moment, LOEUF provides a good compromise of point estimate and significance measure.



### **Annotation columns:**

Add 3 annotation columns: "LOEUF\_bin", "GnomAD\_pLI" and "ExAC\_pLI".

### **Updating the data source (if needed):**

- Remove the file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/gnomAD" directory.
- Download, uncompress and place the "gnomad.v2.1.1.lof\_metrics.by\_gene.txt.bgz" gnomAD file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/gnomAD" directory. The latest update of this file is available for free download in the "pLoF Metrics by Gene TSV" section at: <https://gnomad.broadinstitute.org/downloads#v2-constraint>  
This file will be computed the first time AnnotSV is executed after the update.

For genes with several transcripts, the maximal "LOEUF\_bin" score is reported.

### *ExAC annotations*

#### **Aim:**

Gene intolerance annotations from the [ExAC](#) (Lek et al., 2016) give the significance deviation from the observed and the expected number of variants for each gene:

Column name	Constraint from ExAC	Score	Indication
synZ_ExAC	Synonymous	Z score	Positive Z scores indicate gene intolerance to synonymous variation.
misZ_ExAC	Missense	Z score	Positive Z scores indicate gene intolerance to missense variation.
delZ_ExAC	Deletion	Z score	Higher positive values indicate greater intolerance (a lower than expected rate of CNVs for that gene).
dupZ_ExAC	Duplication	Z score	
cnvZ_ExAC	CNV	Z score	

### **Annotation columns:**

Add 5 annotation columns: "ExAC\_synZ", "ExAC\_misZ", "ExAC\_delZ", "ExAC\_dupZ" and "ExAC\_cnvZ".

### **Updating the data source (if needed):**

- Remove all the files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/ExAC" directory.
- Download and place the "fordist\_cleaned\_nonpsych\_z\_pli\_rec\_null\_data.txt" and the "exac-final-cnv.gene.scores071316" ExAC files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/ExAC" directory. The latest update of this file is available for free download at: [ftp://ftp.broadinstitute.org/pub/ExAC\\_release/release0.3.1/functional\\_gene\\_constraint/fordist\\_cleaned\\_nonpsych\\_z\\_pli\\_rec\\_null\\_data.txt](ftp://ftp.broadinstitute.org/pub/ExAC_release/release0.3.1/functional_gene_constraint/fordist_cleaned_nonpsych_z_pli_rec_null_data.txt)  
[ftp://ftp.broadinstitute.org/pub/ExAC\\_release/release0.3.1/cnv/exac-final-cnv.gene.scores071316](ftp://ftp.broadinstitute.org/pub/ExAC_release/release0.3.1/cnv/exac-final-cnv.gene.scores071316)

This file will be reprocessed the first time AnnotSV is executed after the update.

### Haploinsufficiency (HI) and triplosensitivity (TS) scores annotations

### *DDD HI annotations*

#### **Aim:**

Haploinsufficiency, wherein a single functional copy of a gene is insufficient to maintain normal function, is a major cause of dominant disease. As detailed in [DECIPHER](#), over 17,000 protein coding genes have been scored according to their predicted probability of exhibiting haploinsufficiency:

- High ranks (e.g. 0-10%) indicate a gene is more likely to exhibit haploinsufficiency

- Low ranks (e.g. 90-100%) indicate a gene is more likely to NOT exhibit haploinsufficiency.

#### **Annotation columns:**

Add 1 annotation column: "DDD\_HI\_percent".

#### **Updating the data source (if needed):**

- Remove the "\*\_HI.tsv.gz" file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/DDD" directory.
- Download and place the "HI\_Predictions\_Version3.bed.gz" DECIPHER file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/DDD" directory. The latest update of this file is available for free download at:  
<https://decipher.sanger.ac.uk/about#downloads/data>

This file will be computed the first time AnnotSV is executed after the update.

#### *ClinGen HI and TS annotations*

##### **Aim:**

The [ClinGen Consortium Rating System](#) is curating genes and regions of the genome to assess whether there is evidence to support that these genes/regions are dosage sensitive. Haploinsufficiency and triplosensitivity scorings are ranged as follow:

Score	Possible Clinical Interpretation
3	Sufficient evidence for dosage pathogenicity
2	Some evidence for dosage pathogenicity
1	Little evidence for dosage pathogenicity
0	No evidence for dosage pathogenicity
40	Evidence suggests the gene is not dosage sensitive
30	Gene associated with autosomal recessive phenotype

#### **Annotation columns:**

Add 2 annotation columns: "HI" and "TS".

Concerning annotations on the "full" length of SV covering several genes, only the most pathogenic score is reported if any.

#### **Updating the data source (if needed):**

- Remove all the files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/ClinGen/" directory.
- Download and place the "ClinGen\_gene\_curation\_list\_GRCh37.tsv" ClinGen file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/ClinGen/" directory. The latest update of this file is available for free download at:  
[ftp://ftp.clinicalgenome.org/ClinGen\\_gene\\_curation\\_list\\_GRCh37.tsv](ftp://ftp.clinicalgenome.org/ClinGen_gene_curation_list_GRCh37.tsv)

This file will be computed the first time AnnotSV is executed after the update. The annotations selected by AnnotSV are genome build independent and only based on the gene name (extracted from the "ClinGen\_gene\_curation\_list\_GRCh37.tsv" file).

## Phenotype-driven analysis powered by Exomiser

### **Aim:**

To score genes overlapped with a SV on biological relevance to the individual phenotype, AnnotSV rely on the Exomiser code (Smedley et al., 2015) and HPO (Köhler et al., 2019).

For a given phenotype, a HPO-based score corresponding to a damaging probability is provided for each gene overlapped with an SV so that:

- Genes previously associated with disease can be highlighted easily
- Genes not previously associated with disease can be highlighted
- Genes associated with diseases that have little or no similarity to the observed phenotypes can be removed along

### **HPO:**

AnnotSV uses the Human Phenotype Ontology (version reported in the AnnotSV output).

Find out more at <http://www.human-phenotype-ontology.org>.



Please cite the 3 following articles if you use these data in your work:

- AnnotSV: An integrated tool for Structural Variations annotation. Geoffroy V., *et al*, Bioinformatics (2018) doi: [doi:10.1093/bioinformatics/bty304](https://doi.org/10.1093/bioinformatics/bty304)
- Next-generation diagnostics and disease-gene discovery with the Exomiser. Smedley D., *et al*, Nature Protocols (2015) [doi:10.1038/nprot.2015.124](https://doi.org/10.1038/nprot.2015.124)
- Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources. Köhler S., *et al*, Nucleic Acids Research (2019) [doi:10.1093/nar/gky1105](https://doi.org/10.1093/nar/gky1105)

### **Annotation columns:**

Add 4 annotation columns: "Exomiser\_gene\_pheno\_score", "Human\_pheno\_evidence", "Mouse\_pheno\_evidence" and "Fish\_pheno\_evidence".

### **Usage:**

The user enters a human phenotype as a list of HPO terms (see "hpo" option in USAGE/OPTIONS). The HPO terms need to be as specific as possible.

According to our own (limited) experience, a known disease gene with an Exomiser\_gene\_pheno\_score  $\geq 0.7$  can be considered to be associated with the disease. For a gene that has not been previously associated with a disease, the threshold can be lowered to 0.5.

If not provided, the Exomiser\_gene\_pheno\_score is set to "-1.0". For SV overlapping several genes, the highest Exomiser\_gene\_pheno\_score is reported in the full annotation.

### **Updating the data source (if needed):**

AnnotSV needs matching between the "HGNC symbols" and "NCBI gene ID".

- Remove all the files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/NCBIGeneID/" directory.
  - Download and place your NCBI gene ID file ("results.txt") in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Gene-based/NCBIGeneID/" directory
- This file is available for free download at:

[https://biomart.genenames.org/martform/#!/default/HGNC?datasets=hgnc\\_gene\\_mart](https://biomart.genenames.org/martform/#!/default/HGNC?datasets=hgnc_gene_mart)

In the "Attributes" / "HGNC data" section:

- Select only the "Approved symbol", the "Alias symbol" and the "Previous symbol".

In the "Attributes"/"Gene resources" section:

- Select only the "NCBI gene ID".

Click the "Go >>" button.

Then, click the "Download data" button to download the "results.txt" file.

Exomiser data can be updated (e.g. with the 2202 version):

```
cd $ANNOTSV/share/AnnotSV/Annotations_Exomiser/
mkdir -p 2202/2202_hg19
cd 2202/2202_hg19
cp
$ANNOTSV/share/AnnotSV/Annotations_Exomiser/1902/1902_hg19/1902_hg19_genom
e.h2.db 2202_hg19_genome.h2.db
cp
$ANNOTSV/share/AnnotSV/Annotations_Exomiser/1902/1902_hg19/1902_hg19_trans
cripts_ensembl.ser 2202_hg19_transcripts_ensembl.ser
cp
$ANNOTSV/share/AnnotSV/Annotations_Exomiser/1902/1902_hg19/1902_hg19_varia
nts.mv.db 2202_hg19_variants.mv.db
cd ..
wget https://data.monarchinitiative.org/exomiser/data/2202_phenotype.zip
unzip 2202_phenotype.zip
rm 2202_phenotype.zip 2202_phenotype.sha256
```

Then, check the \$ANNOTSV/etc/AnnotSV/application.properties file is pointing to the correct versions:

exomiser.phenotype.data-version=2202

exomiser.hg19.data-version=2202

Then, update the \$ANNOTSV/Makefile with the new Exomiser version (e.g. 2202).

## d) Known pathogenic genes or genomic regions annotation

AnnotSV searches for known pathogenic genes or genomic regions **completely overlapped** with the SV to annotate.

### **Aim:**

According to the ACMG technical standards (Riggs et al., 2020), a SV completely overlapping an established pathogenic CNV region would be classified as pathogenic (if sharing the same SV type).

### **Annotation columns:**

Add 12 annotation columns:

"P\_gain\_phen", "P\_gain\_hpo", "P\_gain\_source", "P\_gain\_coord",

"P\_loss\_phen", "P\_loss\_hpo", "P\_loss\_source", "P\_loss\_coord",

"P\_ins\_phen", "P\_ins\_hpo", "P\_ins\_source", "P\_ins\_coord",

### **Pathogenic dataset creation:**

For each SV type (Loss, Gain, Ins, Inv), different sources of pathogenic genes or genomic regions have been merged in AnnotSV:

- ClinVar
- ClinGen
- dbVar
- OMIM

### **Updating the data source (if needed):**

- Remove all the files in the following directories:  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/PathogenicSV/GRCh37"  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/PathogenicSV/GRCh38"
- Download and place the files of the different sources in the following directories:  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/PathogenicSV/GRCh37"  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/PathogenicSV/GRCh38"

These files will be computed then removed the first time AnnotSV is executed after the update.

**NOTE:** It is to notice that, for this type of pathogenic SV annotations, a reciprocal overlap cannot be used (See the "Aim" section for explanation). The "-reciprocal" option can only be used with custom annotations with features overlapping the SV.

### [ClinVar pathogenic SV annotations](#)

#### **Aim:**

[ClinVar](#) gives access to the relationships asserted between human variants and observed health status.

#### **Method:**

Pathogenic SV are selected based on the following criteria:

- "pathogenic" or "pathogenic/likely pathogenic" clinical significance (CLNSIG)
- "criteria\_provided", "\_multiple\_submitters" or "reviewed\_by\_expert\_panel" SV review status (CLNREVSTAT)
- "Deletion" or "Duplication" SV type (CLNVC)
- $\geq 50$  bp in size.

#### **Source files:**

The latest update of the ClinVar files are available for free download at:

*Genome build GRCh37:*

[https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf\\_GRCh37/clinvar\\_20220903.vcf.gz](https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh37/clinvar_20220903.vcf.gz)

*Genome build GRCh38:*

[https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf\\_GRCh38/clinvar\\_20220903.vcf.gz](https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh38/clinvar_20220903.vcf.gz)

### [Dosage sensitive genes/regions annotation \(ClinGen\)](#)

#### **Aim:**

The ClinGen Consortium Rating System is curating genes and regions of the genome to assess whether there is evidence to support that these genes/regions are dosage sensitive.

A haploinsufficiency (HI) score of 3 suggests the gene/region to be dosage sensitive for a loss, associated with clinical phenotype.

A triplosensitivity (TS) score of 3 suggests the gene/region to be dosage sensitive for a gain, associated with clinical phenotype.

**Method:**

Genes and regions with a score of 3 are selected.

**Source files:**

The latest update of the ClinGen files are available for free download at:

*Genome build GRCh37:*

[ftp://ftp.clinicalgenome.org/ClinGen\\_gene\\_curation\\_list\\_GRCh37.tsv](ftp://ftp.clinicalgenome.org/ClinGen_gene_curation_list_GRCh37.tsv)

[ftp://ftp.clinicalgenome.org/ClinGen\\_region\\_curation\\_list\\_GRCh37.tsv](ftp://ftp.clinicalgenome.org/ClinGen_region_curation_list_GRCh37.tsv)

*Genome build GRCh38:*

[ftp://ftp.clinicalgenome.org/ClinGen\\_gene\\_curation\\_list\\_GRCh38.tsv](ftp://ftp.clinicalgenome.org/ClinGen_gene_curation_list_GRCh38.tsv)

[ftp://ftp.clinicalgenome.org/ClinGen\\_region\\_curation\\_list\\_GRCh38.tsv](ftp://ftp.clinicalgenome.org/ClinGen_region_curation_list_GRCh38.tsv)

[dbVarNR pathogenic SV annotations](#)

**Aim:**

dbVar is the NCBI's database of genomic structural variation collecting insertion/deletion/duplications/mobile elements insertions/translocations data from large initiative including also medically relevant variations. A non-redundant version of the database, dbVar non-redundant SV (NR SV) datasets include more than 2.2 million deletions, 1.1 million insertions, and 300,000 duplications. These data are aggregated from over 150 studies including 1000 Genomes Phase 3, Simons Genome Diversity Project, ClinGen, ExAC, and others.

By selecting pathogenic SV records from the dbVar NR SV database, AnnotSV obtained a clinically relevant human SV dataset. Nevertheless, associated phenotypes are not provided.

**Source files:**

The latest update of the pathogenic dbVarNR files are available for free download at:

*Genome build GRCh37:*

[https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv\\_datasets/nonredundant/deletions/GRCh37.nr\\_deletions.pathogenic.tsv.gz](https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv_datasets/nonredundant/deletions/GRCh37.nr_deletions.pathogenic.tsv.gz)

[https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv\\_datasets/nonredundant/duplications/GRCh37.nr\\_duplications.pathogenic.tsv.gz](https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv_datasets/nonredundant/duplications/GRCh37.nr_duplications.pathogenic.tsv.gz)

[https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv\\_datasets/nonredundant/insertions/GRCh37.nr\\_insertions.pathogenic.tsv.gz](https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv_datasets/nonredundant/insertions/GRCh37.nr_insertions.pathogenic.tsv.gz)

*Genome build GRCh38:*

[https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv\\_datasets/nonredundant/deletions/GRCh38.nr\\_deletions.pathogenic.tsv.gz](https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv_datasets/nonredundant/deletions/GRCh38.nr_deletions.pathogenic.tsv.gz)

[https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv\\_datasets/nonredundant/duplications/GRCh38.nr\\_duplications.pathogenic.tsv.gz](https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv_datasets/nonredundant/duplications/GRCh38.nr_duplications.pathogenic.tsv.gz)

[https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv\\_datasets/nonredundant/insertions/GRCh38.nr\\_insertions.pathogenic.tsv.gz](https://ftp.ncbi.nlm.nih.gov/pub/dbVar/sandbox/sv_datasets/nonredundant/insertions/GRCh38.nr_insertions.pathogenic.tsv.gz)

These files will be computed then removed the first time AnnotSV is executed after the update.

## OMIM morbid genes

### **Aim:**

The complete deletion of a morbid gene would be classified as pathogenic.

### **Method:**

The “morbidGenes” are selected and only added to the pathogenic loss SV dataset in AnnotSV.

### **Source files:**

The latest update of the OMIM morbid gene should have been done during the OMIM Gene-based annotation (see section "3.c Gene-based annotation").

*Genome build GRCh37 and GRCh38:*

To update the known pathogenic loss SV with the morbid gene, run the following commands:

```
cd $ANNOTSV/share/AnnotSV/Annotations_Human/FtIncludedInSV/PathogenicSV/GRCh37
cp $ANNOTSV/share/AnnotSV/Annotations_Human/Gene-based/OMIM/*_morbid.tsv.gz .
cd $ANNOTSV/share/AnnotSV/Annotations_Human/FtIncludedInSV/PathogenicSV/GRCh38
cp $ANNOTSV/share/AnnotSV/Annotations_Human/Gene-based/OMIM/*_morbid.tsv.gz .
```

The OMIM morbid gene coordinates are computed through their gene names and the AnnotSV gene annotations.

## e) Known pathogenic SNV/indel annotations

AnnotSV searches for pathogenic SNV/indel from ClinVar completely overlapped with the SV to annotate.

### **Aim:**

The presence of pathogenic variants indicates the region is critical to protein function.

### **Method:**

Pathogenic SNV/indel with all the following requirements are selected:

- “pathogenic” or “pathogenic/likely pathogenic” clinical significance (CLNSIG)
- “criteria\_provided”, “\_multiple\_submitters” or “reviewed\_by\_expert\_panel” SV review status (CLNREVSTAT)
- < 50 bp in size.

### **Annotation columns:**

Add 2 annotation columns: “P\_snvindl\_nb” and “P\_snvindl\_phen”.

### **Updating the data source (if needed):**

- Remove all the files in the following directories:  
“\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/PathogenicSNVindel/GRCh37”  
“\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/PathogenicSNVindel/GRCh38”
- Download and place the files of the different sources in the following directories:

“\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/PathogenicSNVindel/GRCh37”  
“\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/PathogenicSNVindel/GRCh38”

The latest update of the ClinVar files are available for free download at:

*Genome build GRCh37:*

[https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf\\_GRCh37/clinvar\\_20220903.vcf.gz](https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh37/clinvar_20220903.vcf.gz)

*Genome build GRCh38:*

[https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf\\_GRCh38/clinvar\\_20220903.vcf.gz](https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh38/clinvar_20220903.vcf.gz)

These files will be computed then removed the first time AnnotSV is executed after the update.

## f) Known benign genes or genomic regions annotation

AnnotSV searches for benign genomic regions completely overlapping the SV to annotate.

### **Aim:**

According to the ACMG technical standards (Riggs et al., 2020), a SV completely contained within an established benign CNV region would be classified as benign (if sharing the same SV type).

### **Annotation columns:**

Add 12 annotation columns: “B\_gain\_source”, “B\_gain\_coord”, “B\_gain\_AFmax”, “B\_loss\_source”, “B\_loss\_coord”, “B\_loss\_AFmax”, “B\_ins\_source”, “B\_ins\_coord”, “B\_ins\_AFmax”, “B\_inv\_source”, “B\_inv\_coord” and “B\_inv\_AFmax”.

### **Benign dataset creation:**

For each SV type (Loss, Gain, Ins, Inv), different sources of benign genes or genomic regions have been merged in AnnotSV:

- gnomAD (The “B\_\*\_source” output values begin with “gnomAD”)
- ClinVar (The “B\_\*\_source” output values begin with “CLN”)
- ClinGen (The “B\_\*\_source” output values begin with “TS40” or “HI40”)
- DGV Gold Standard (The “B\_\*\_source” output values begin with “dgv”, “nsv” or “esv”)
- DDD (The “B\_\*\_source” output values begin with “DDD”)
- 1000 genomes (The “B\_\*\_source” output values begin with “1000g”)
- Ira M. Hall’s lab (The “B\_\*\_source” output values begin with “IMH”)
- Children’s Mercy Research Institute (The “B\_\*\_source” output values begin with “CMRI”)

### **Updating the data source (if needed):**

- Remove all the files in the following directories:  
“\$ANNOTSV/share/AnnotSV/Annotations\_Human/SVincludedInFt/BenignSV/GRCh37”  
“\$ANNOTSV/share/AnnotSV/Annotations\_Human/SVincludedInFt/BenignSV/GRCh38”
- Download and place the files of the different sources in the following directories:



“\$ANNOTSV/share/AnnotSV/Annotations\_Human/SVincludedInFt/BenignSV/GRCh37”  
“\$ANNOTSV/share/AnnotSV/Annotations\_Human/SVincludedInFt/BenignSV/GRCh38”

These files will be computed then removed the first time AnnotSV is executed after the update.

**NOTE:** It is to notice that, for this type of benign SV annotations, a reciprocal overlap cannot be used. The “-reciprocal” option can only be used with custom annotations with features overlapping the SV.

### [gnomAD benign SV annotations](#)

#### **Aim:**

A reference atlas of SV from deep WGS of 14,891 individuals across diverse global populations has been constructed as a component of the gnomAD database (Collins et al., 2020). The publicly available SV data represents a relatively diverse collection of unrelated individuals that should have rates of most severe diseases equivalent to, if not lower than, the general population.

#### **Method:**

Putatively benign variants from gnomAD with all the following requirements are selected:

- at least one population allele frequency > 0.01 (i.e. 1%) (default, see the “-benignAF” option in USAGE/OPTIONS) **OR** at least 5 homozygous individuals
- “DUP” or “DEL” SV type

#### **Data sources:**

*Genome build GRCh37:*

The gnomAD data are based on the genome build GRCh37/hg19. They can be freely downloaded at:

[https://storage.googleapis.com/gcp-public-data-gnomad/papers/2019-sv/gnomad\\_v2.1\\_sv.sites.bed.gz](https://storage.googleapis.com/gcp-public-data-gnomad/papers/2019-sv/gnomad_v2.1_sv.sites.bed.gz)

*Genome build GRCh38:*

The GRCh38 gnomAD SV dataset is not yet available from the gnomAD team.

However, the GRCh37 gnomAD SV dataset has been lifted over to GRCh38 with the [UCSC web server](#) and is provided as it by AnnotSV.

### [ClinVar benign SV annotations](#)

#### **Aim:**

[ClinVar](#) gives access to the relationships asserted between human variants and observed health status.

#### **Method:**

Benign SV with all the following requirements are selected:

- “benign” or “benign/likely benign” clinical significance (CLNSIG)
- “criteria\_provided”, “\_multiple\_submitters” or “reviewed\_by\_expert\_panel” SV review status (CLNREVSTAT)
- “Deletion” or “Duplication” SV type (CLNVC)
- ≥ 50 bp in size.

#### **Source files:**

The latest update of the ClinVar files are available for free download at:

*Genome build GRCh37:*

[https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf\\_GRCh37/clinvar\\_20220903.vcf.gz](https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh37/clinvar_20220903.vcf.gz)

*Genome build GRCh38:*

[https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf\\_GRCh38/clinvar\\_20220903.vcf.gz](https://ftp.ncbi.nlm.nih.gov/pub/clinvar/vcf_GRCh38/clinvar_20220903.vcf.gz)

### Not dosage sensitive genes/regions annotation (ClinGen)

#### **Aim:**

The ClinGen Consortium Rating System is curating genes and regions of the genome to assess whether there is evidence to support that these genes/regions are dosage sensitive.

An haploinsufficiency (HI) score of 40 suggests the gene/region is not dosage sensitive for a loss.

A triplosensitivity (TS) score of 40 suggests the gene/region is not dosage sensitive for a gain.

#### **Method:**

Genes and regions with a score of 40 are selected.

#### **Source files:**

The latest update of the ClinGen files are available for free download at:

*Genome build GRCh37:*

[https://ftp.clinicalgenome.org/ClinGen\\_gene\\_curation\\_list\\_GRCh37.tsv](https://ftp.clinicalgenome.org/ClinGen_gene_curation_list_GRCh37.tsv)

[https://ftp.clinicalgenome.org/ClinGen\\_region\\_curation\\_list\\_GRCh37.tsv](https://ftp.clinicalgenome.org/ClinGen_region_curation_list_GRCh37.tsv)

*Genome build GRCh38:*

[https://ftp.clinicalgenome.org/ClinGen\\_gene\\_curation\\_list\\_GRCh38.tsv](https://ftp.clinicalgenome.org/ClinGen_gene_curation_list_GRCh38.tsv)

[https://ftp.clinicalgenome.org/ClinGen\\_region\\_curation\\_list\\_GRCh38.tsv](https://ftp.clinicalgenome.org/ClinGen_region_curation_list_GRCh38.tsv)

### DGV benign SV annotations

#### **Aim:**

The Database of Genomic Variants ([DGV](#)) (MacDonald et al., 2014) provides SV defined as DNA elements with a size >50 bp. The content of DGV is only representing SV identified in healthy control samples from large cohorts published and integrated by the DGV team. The annotations will give information about whether your SV is a rare or a benign common variant.

#### **Method:**

Putatively benign variants from DGV with all the following requirements are selected:

- Allele frequency > 0.01 (i.e. 1%) (default, see the “-benignAF” option in USAGE/OPTIONS)
- ≥ 500 individuals tested
- “Loss” or “Gain” SV type

Loss allele frequency is computed as ‘observedlosses’ / (2 x ‘samplesize’).

Gain allele frequency is computed as ‘observedgains’ / (2 x ‘samplesize’).

#### **Source files:**

The latest update of the DGV files are available for free download at <http://dgv.tcag.ca/dgv/app/downloads>.

*Genome build GRCh37:*

**GRCh37\_hg19\_variants\_2020-02-25.txt** (see "DGV Variants" section)

*Genome build GRCh38:*

**GRCh38\_hg38\_variants\_2020-02-25.txt** (see "DGV Variants" section)

## DDD benign SV annotations

### **Aim:**

AnnotSV takes advantage of the common copy-number variants and their frequencies, as used and displayed in [DECIPHER](#).

### **Method:**

Putatively benign variants from DDD with all the following requirements are selected:

- Allele frequency > 0.01 (i.e. 1%) (default, see the “-benignAF” option in USAGE/OPTIONS)
- ≥ 500 individuals tested
- “Deletion” or “Duplication” SV type

### **Source files:**

The latest update of the “**population\_cnv.txt.gz**” DECIPHER files is available for free download at: <https://www.deciphergenomics.org/about/overview> (via the “Downloads” then “Data” tabs).

*Genome build GRCh37:*

[https://www.deciphergenomics.org/files/downloads/population\\_cnv\\_grch37.txt.gz](https://www.deciphergenomics.org/files/downloads/population_cnv_grch37.txt.gz)

*Genome build GRCh38:*

[https://www.deciphergenomics.org/files/downloads/population\\_cnv\\_grch38.txt.gz](https://www.deciphergenomics.org/files/downloads/population_cnv_grch38.txt.gz)

## 1000 genomes benign SV annotations

### **Aim:**

The goal of the [1000 Genomes Project](#) (Sudmant et al., 2015) was to find most genetic variants with frequencies of at least 1% in the populations studied. Analyses were conducted looking at both the short variations (up to 50 base pairs in length) and the SV. Most of the 1000 genomes data is already included in the gnomAD dataset.

### **Method:**

Putatively benign variants from 1000 genomes with all the following requirements are selected:

- at least one population allele frequency > 0.01 (i.e. 1%) (default, see the “-benignAF” option in USAGE/OPTIONS)
- “Gain” or “Loss” SV type

### **Source files:**

The latest updates of these files are available for free download at:

*Genome build GRCh37:*

[ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase3/integrated\\_sv\\_map/ALL.wgs.mergedSV.v8.20130502.svs.genotypes.vcf.gz](ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase3/integrated_sv_map/ALL.wgs.mergedSV.v8.20130502.svs.genotypes.vcf.gz)

*Genome build GRCh38:*

[http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase3/integrated\\_sv\\_map/supporting/GRCh38\\_positions/ALL.wgs.mergedSV.v8.20130502.svs.genotypes.GRCh38.vcf.gz](http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase3/integrated_sv_map/supporting/GRCh38_positions/ALL.wgs.mergedSV.v8.20130502.svs.genotypes.GRCh38.vcf.gz)

This file will be computed the first time AnnotSV is executed after the update.

### [Ira M. Hall's lab benign SV annotations](#)

#### **Aim:**

Ira M. Hall's lab characterized SV in 17,795 deeply sequenced human genomes from common disease trait mapping studies (Abel et al., 2020). They publicly released SV frequency annotations to guide SV analysis and interpretation in the era of WGS.

#### **Method:**

Putatively benign variants from Ira M. Hall's lab with all the following requirements are selected:

- Allele frequency (AF) > 0.01 (i.e. 1%) (default, see the "-benignAF" option in USAGE/OPTIONS)
- "DUP" or "DEL" SV type

#### **Data sources:**

Supplementary files 1 and 2 from (Abel et al., 2020) are available for free download at:

<https://www.biorxiv.org/content/10.1101/508515v1.supplementary-material>

Download, uncompress and keep the following files:

*Genome build GRCh37:*

Supplementary File 2: Media-2/B37.callset.public.bedpe.gz

*Genome build GRCh38:*

Supplementary File 1: Media-1/B38.callset.public.bedpe.gz

### [Children's Mercy Research Institute Benign SV annotations](#)

#### **Aim:**

The [Children's Mercy Research Institute](#) (CMRI) is undertaking a research initiative to collect genomic data and health information for 30,000 children and their families over the next seven years, creating a database of nearly 100,000 genomes (high-quality long read pacbio sequencing data).

#### **Method:**

Putatively benign variants from the CMRI with all the following requirements are selected:

- Allele frequency (AF) > 0.01 (i.e. 1%) (default, see the "-benignAF" option in USAGE/OPTIONS)
- ≥ 500 individuals tested
- "DUP", "DEL", "INS" or "INV" SV type

#### **Data sources:**

The latest update of the "pb\_joint\_merged.sv.vcf" files is available for free download at:

*Genome build GRCh37:*

The GRCh37 SV CMRI dataset is not available.

You need to lift over the GRCh38 file to GRCh37 with the [UCSC web server](#) and to move it in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/SVincludedInFt/BenignSV/GRCh37" directory.

*Genome build GRCh38:*

Download and uncompress:

[https://github.com/ChildrensMercyResearchInstitute/GA4K/blob/main/pacbio\\_sv\\_vcf/pb\\_joint\\_merged.sv.vcf.gz](https://github.com/ChildrensMercyResearchInstitute/GA4K/blob/main/pacbio_sv_vcf/pb_joint_merged.sv.vcf.gz)

## **g) Breakpoints annotations**

## GC content annotations

### **Aim:**

GC content (as well as repeated sequences, DNA sequence identity and concentration of the PRDM9 homologous recombination hot spot motif 5'-CCNCCNTNNCCNC-3') is positively correlated with the frequency of non allelic homologous recombination (NAHR). Indeed, NAHR hot spots have a significantly higher GC content (Dittwald et al., 2013). This information with others could help identifying a novel locus for recurrent NAHR-mediated SV.

### **Method:**

The GC content is calculated using bedtools around each SV breakpoint (+/- 100bp) then reported.

### **Annotation columns:**

Add 2 annotation columns: "GC\_content\_left", "GC\_content\_right".

### **Updating the data source (if needed):**

AnnotSV needs the human reference genome FASTA file to run the "bedtools nuc" command.

- Remove all the files in the  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/GCcontent/GRCh37"  
and/or  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/GCcontent/GRCh38"  
directories.
- Download and place the human reference genome FASTA file in the  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/GCcontent/GRCh37"  
and/or  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/GCcontent/GRCh38"  
directories.

The latest update of this file is available for free download at:

*Genome build GRCh37:*

<http://hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/chromFa.tar.gz>

*Genome build GRCh38:*

<http://hgdownload.cse.ucsc.edu/goldenPath/hg38/bigZips/hg38.chromFa.tar.gz>

This FASTA file will be reprocessed during the first time AnnotSV is executed after the update.

**Warning:** This update requires the "tar" Tcl package.

## Repeated sequences annotations

### **Aim:**

Repeated sequences (as well as GC content, DNA sequence identity and presence of the PRDM9 homologous recombination hotspot motif 5'-CCNCCNTNNCCNC-3') play a major role in the formation of structural variants.

### **Method:**

The overlapping repeats are identified using bedtools at the SV breakpoint (+/- 100bp) and reported (coordinates and type).

**Annotation columns:**

Add 4 annotation columns: "Repeat\_coord\_left", "Repeat\_type\_left", "Repeat\_coord\_right" and "Repeat\_type\_right".

**Updating the data source (if needed):**

AnnotSV needs a UCSC Repeat BED file.

- Remove all the files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/Repeat/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/Repeat/GRCh38" directories.
- You can freely download the BED file from the "<http://genome.ucsc.edu/cgi-bin/hgTables>". There are many output options, here are the changes that you'll need to make: "GRCh37" or "GRCh38" assembly, "Repeats" group and "Repeatmasker" track, "genome" region. Select output format as BED. Choose the following output filename: Repeat.bed. Then, click the get output button.
- Download and place the BED file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/Repeat/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/Repeat/GRCh38" directories.

This BED file will be reprocessed during the first time AnnotSV is executed after the update.

### Segmental duplication annotations

**Aim:**

Segmental duplications are large duplications of >1Kb of non-RepeatMasked sequence and  $\geq 90\%$  identity normally present in the human genome. They are associated with the non-allelic homologous recombination mechanisms (NAHR). Homologous recombination is thought to be a classical mechanism for promoting either genetic diversity or genomic disease. Moreover, these regions might also cause issues for read-depth SV detection methods. Reads located in a segmental duplication can perfectly map onto two or more genomic positions and lead to a coverage overestimation at these positions.

The SV breakpoints overlap with segmental duplications can therefore give a clue to explain the SV mechanism, but also a clue to filter out false positives in case of read-depth SV detection methods.

**Method:**

The Segmental Duplications coordinates are reported.

**Annotation columns:**

Add 2 annotation columns: "SegDup\_left" and "SegDup\_right".

**Updating the data source (if needed):**

AnnotSV needs a UCSC SegDup BED file.

- Remove all the files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/SegDup/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/SegDup/GRCh38" directories.

- You can freely download the BED file from the "<http://genome.ucsc.edu/cgi-bin/hgTables>". There are many output options, here are the changes that you'll need to make:  
"GRCh37" or "GRCh38" assembly, "All Tracks" group, "Segmental Dups" track, "genomicSuperDups" table and "genome" region. Select output format as BED. Choose the following output filename: SegDup.bed. Then, click the get output button.
- Download and place the BED file in the  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/SegDup/GRCh37" and/or  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/SegDup/GRCh38"  
directories.

This BED file will be reprocessed during the first time AnnotSV is executed after the update.

### [ENCODE blacklist annotations](#)

#### **Aim:**

The human ENCODE blacklist is a comprehensive set of regions that have anomalous, unstructured, or high signal in next-generation sequencing experiments independent of cell line or experiment. The removal of the ENCODE blacklist is an essential quality measure when analyzing functional genomics data.

If you use the blacklist, please cite:

Amemiya, H.M., Kundaje, A. & Boyle, A.P. The ENCODE Blacklist: Identification of Problematic Regions of the Genome. Sci Rep 9, 9354 (2019). <https://doi.org/10.1038/s41598-019-45839-z>

#### **Method:**

The ENCODE Blacklist regions and their characteristics are reported.

#### **Annotation columns:**

Add 4 annotation columns: "ENCODE\_blacklist\_left", "ENCODE\_blacklist\_characteristics\_left", "ENCODE\_blacklist\_right" and "ENCODE\_blacklist\_characteristics\_right".

#### **Updating the data source (if needed):**

AnnotSV needs a ENCODE blacklist BED file.

- Remove all the files in the  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/ENCODEblacklist/GRCh37"  
and/or  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/ENCODEblacklist/GRCh38"  
directories.
- A current version of the blacklists for hg19 and hg38 ("hg\*-blacklist.v2.bed.gz") are available in the "lists" folder of: <https://github.com/Boyle-Lab/Blacklist/>
- Download, uncompress and place the BED file, renamed "ENCODEblacklist.bed", in the  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/ENCODEblacklist/GRCh37"  
and/or  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/ENCODEblacklist/GRCh38"  
directories.

This BED file will be reprocessed during the first time AnnotSV is executed after the update.

### GAP annotations

#### **Aim:**

Depending on the genome build, several regions of the genome are not yet available. Therefore, they can be misinterpreted due to bad alignment in case of NGS data or badly called in array analysis and then generating false positives calls.

#### **Annotation columns:**

Add 2 annotation columns: "Gap\_left" and "Gap\_right".

#### **Updating the data source (if needed):**

AnnotSV needs a UCSC GAP BED file.

- Remove all the files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/Gap/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/Gap/GRCh38" directories.
- You can freely download the BED file from the "<http://genome.ucsc.edu/cgi-bin/hgTables>". There are many output options, here are the changes that you'll need to make:  
  
"GRCh37" or "GRCh38" assembly, "All Tracks" group, "Gap" track, "Gap" table and "genome" region. Select output format as BED. Choose the following output filename: Gap.bed. Then, click the get output button.
- Download and place the BED file in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/Gap/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/BreakpointsAnnotations/Gap/GRCh38" directories.

This BED file will be reprocessed during the first time AnnotSV is executed after the update.

### Cytoband

#### **Aim:**

CytoBand stands for cytogenic bands. This annotation source gives the approximate location of these bands as seen on Giemsa-stained chromosomes for each SV breakpoint.

#### **Annotation columns:**

Add 1 annotation column: "CytoBand".

#### **Updating the data source (if needed):**

- Remove all the files in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/AnyOverlap/CytoBand/GRCh37" and/or "\$ANNOTSV/share/AnnotSV/Annotations\_Human/AnyOverlap/CytoBand/GRCh38" directories.
- The txt cytoband files are available for free download at:  
Genome build GRCh37:  
<http://hgdownload.cse.ucsc.edu/goldenpath/hg19/database/cytoBand.txt.gz>  
Genome build GRCh38:  
<http://hgdownload.cse.ucsc.edu/goldenpath/hg38/database/cytoBand.txt.gz>



- Download and place the txt file in the  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/AnyOverlap/CytoBand/GRCh37" and/or  
"\$ANNOTSV/share/AnnotSV/Annotations\_Human/AnyOverlap/CytoBand/GRCh38" directories.
- Then run the following commands:

*Genome build GRCh37:*

```
gunzip cytoBand.txt.gz
echo "#chrom\tstart\tend\tCytoBand" > cytoBand_GRCh37.bed
cut -f 1-4 cytoBand.txt | grep -v "^chrM" >> cytoBand_GRCh37.bed
rm cytoBand.txt
```

*Genome build GRCh38:*

```
gunzip cytoBand.txt.gz
echo "#chrom\tstart\tend\tCytoBand" > cytoBand_GRCh38.bed
cut -f 1-4 cytoBand.txt | grep -v "^chrM" >> cytoBand_GRCh38.bed
rm cytoBand.txt
```

These BED files will be reprocessed during the first time AnnotSV is executed after the update.

## h) TAD boundaries annotations

### **Aim:**

The spatial organization of the human genome helps to accommodate the DNA in the nucleus of a cell and plays an important role in the control of the gene expression. In this non-random organization, topologically associating domains (TAD) emerge as a fundamental structural unit able to separate domains and define boundaries. Disruption of these structures especially by SV can result in gene misexpression (Lupiáñez et al., 2016).

### **Method:**

A TAD boundary is reported if i) the SV overlaps at least 100% of this TAD boundary (user defined, see the "overlap" option in USAGE/OPTIONS) or ii) if the SV is an insertion included in the TAD.

### **Annotation columns:**

Add 2 annotation columns: "TAD\_coordinate", "ENCODE\_experiment".

They contain i) the overlapping TAD coordinates with a SV and ii) the ENCODE experiments from which the TAD have been defined.

Very large SV (e.g. 30Mb) can sometime overlap too many TAD locations (e.g. more than 2600). It appears that depending on the visualisation program used (spreadsheet programs mostly) this annotation can be truncated. In order to avoid such embarrassing glitch and maybe also because overlapping so many TAD is already a problem, AnnotSV restrict the number of overlapping reported TAD to 20 (including their associated ENCODE experiments).

### **Updating the data source (if needed):**

AnnotSV needs ENCODE experiments in BED format for the TAD annotations.

- Remove all the files in the “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/TAD/GRCh37” and/or “\$ANNOTSV/share/AnnotSV/Annotations\_Human/TAD/GRCh38” directories.
- Download and place your ENCODE BED files in the “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/TAD/GRCh37” and/or “\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/TAD/GRCh38” directories.

These files (GRCh37 and GRCh38) are available for free download at:

[https://www.encodeproject.org/search/?type=Experiment&assay\\_title=Hi-C&files.file\\_type=bed+bed3%2B](https://www.encodeproject.org/search/?type=Experiment&assay_title=Hi-C&files.file_type=bed+bed3%2B)

Click the "bed bed3+" button on your link (else the "file.txt" is blank). Then, click the “Download” button to download a “files.txt” file that contains a list of URLs. Keep only the \*.bed URLs in your “files.txt”.

Then use the following command to download all the BED files in the list:

```
xargs -n 1 curl -O -L < files.txt
```

Finally, dispatch the downloaded files in either the GRCh37 or the GRCh38 directory.

These BED files will be reprocessed during the first time AnnotSV is executed.

## i) COSMIC annotations (not distributed)

### **Aim:**

[COSMIC](#) (Tate et al., 2019), the Catalogue Of Somatic Mutations In Cancer, is the world's largest and most comprehensive resource for exploring the impact of somatic mutations in human cancer.

### **WARNING:**

COSMIC data cannot be redistributed. Thus, COSMIC annotation cannot be supplied as part of the AnnotSV sources. Users are required to register in order to download COSMIC data files. More information can be found on their [licensing page](#).

### **Method:**

A COSMIC CNV is reported if the SV overlaps 100% of this feature (user defined, see the "overlap" option in USAGE/OPTIONS).

### **Annotation columns:**

Add 2 annotation columns “Cosmic\_ID” and “Cosmic\_mut\_typ”.

### **Installing the data source:**

AnnotSV needs the “CosmicCompleteCNA.tsv.gz” (2 genome versions available) file from <https://cancer.sanger.ac.uk/cosmic/download>

- Put the “CosmicCompleteCNA.tsv.gz” file in the corresponding directory:  
“\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/COSMIC/GRCh37/  
or  
“\$ANNOTSV/share/AnnotSV/Annotations\_Human/FtIncludedInSV/COSMIC/GRCh38/

These files will be reprocessed and then removed the first time AnnotSV is executed.

#### 4. VERSIONS OF THE ANNOTATION SOURCES

Annotations source	Version
<b>...Gene annotations</b>	
Gene annotations (RefSeq)	2020-08-17 (GRCh37) 2020-08-17 (GRCh38)
Gene annotations (ENSEMBL)	2014-02-07 (GRCh37.75) 2022-06-04 (GRCh38.107)
<b>...Regulatory Elements annotations</b>	
Promoter data (RefSeq)	2020-08-17 (GRCh37) 2020-08-17 (GRCh38)
Promoter data (ENSEMBL)	GRCh37.75 (2014-02-07) GRCh38.107 (2022-06-04)
EnhancerAtlas	2019-06-11 (v2.0)
GeneHancer	Downloaded by the user
miRTargetLink	2020-12-11 (v2.0, data provided by the authors)
<b>...Gene-based annotations</b>	
GenCC	2022-09-02
OMIM	2022-09-03
ACMG	ACMG SF v3.1
Gene intolerance (gnomAD)	V2.1.1
Gene intolerance (ExAC)	2016-08-23
Haploinsufficiency (DDD)	2020-07-13
Haploinsufficiency and triplosensitivity (ClinGen)	2022-09-05
Exomiser	2022-09-06 (v2202)
NCBI gene ID	2022-09-06
<b>...Annotations with known pathogenic genes or genomic regions</b>	
ClinVar	2022-09-03
ClinGen	2022-09-06
dbVar	2022-08-03
OMIM	2022-09-03
<b>...Annotations with known pathogenic SNV/indel</b>	
ClinVar	2022-09-03
<b>...Annotations with known benign genes or genomic regions</b>	
gnomAD (GRCh37)	2020-08-20 (v2.1)
ClinVar	2022-09-03
ClinGen	2022-09-08
DGV annotations	2020-02-25
DDD annotations (GRCh37)	September, 2015 (v9.2)
1000 genomes annotations (GRCh37)	2017-05-21
1000 genomes annotations (GRCh38)	2017-05-21
Ira M. Hall's lab annotations	2018-12-31
Children's Mercy Research Institute annotations	2021-10-27
<b>...Annotations with features overlapping the SV</b>	
<b>...Annotations with features overlapped with the SV</b>	
COSMIC annotations	Downloaded by the user
TAD boundaries annotations	2017-10-24
<b>...Breakpoints annotations</b>	

GRCh37 FASTA genome	2009-03-20
GRCh38 FASTA genome	2014-01-23
Repeated sequences annotations	2021-10-12 (GRCh37) 2022-09-08 (GRCh38)
Segmental Duplication annotations	2021-10-15
ENCODE blacklist annotations	2018 (v2)
GAP regions annotations	2021-10-15
Cytoband (GRCh37)	2009-06-14
Cytoband (GRCh38)	2019-03-11

## 5. SV RANKING/CLASSIFICATION

In order to assist the clinical interpretation of SV, AnnotSV provides on top of the annotations a ranking score to assess SV pathogenicity. This score is an adaptation of the work provided by the joint consensus recommendation of ACMG and ClinGen (Riggs et al., 2020). We especially payed attention to scoring as much as possible recessive SV observed in various datasets (NGS, array based...)

### **Scoring:**

- |                         |                                   |         |
|-------------------------|-----------------------------------|---------|
| • $\geq 0.99$           | Pathogenic                        | Class 5 |
| • 0.90 to 0.98 points   | Likely pathogenic                 | Class 4 |
| • 0.89 to -0.89 points  | Variant of uncertain significance | Class 3 |
| • -0.90 to -0.98 points | Likely benign                     | Class 2 |
| • $\leq -0.99$          | Benign                            | Class 1 |

### **Method:**

The comprehensive and detailed scoring guidelines are available in the AnnotSV\_Scoring\_Criteria.xlsx file (see Table1 for loss SV and Table2 for gain SV). In each section, only 1 criterion (from the most pathogenic to the least) is assigned.

To explicit which criteria have been used to support the ranking score, decision criteria are reported by default in the output file (in the "ranking decision criteria" column).

### **Annotation columns:**

Add 3 annotation columns: "AnnotSV\_ranking\_score", "AnnotSV\_ranking\_criteria" and "ACMG\_class".

## 6. SV TYPE

In order to be able to classify the SV and to provide relevant annotations, AnnotSV requires that the type of SV is provided (duplication, deletion...) in the input SV file (BED or VCF).

### **Using a VCF containing SV as input file:**

The INFO keys used for structural variants should follow at least the VCF version [4.3](#) (Jun 2020) specifications:

- The "SVTYPE" values should be one of DEL, INS, DUP, INV, CNV, BND, LINE1, SVA, ALU.
- The <CN0>, <CN2>, <CN3>... angle-bracketed ID from the "ALT" column should be used in case of SVTYPE=CNV in the INFO column.

### Using a BED containing SV as input file:

The column number with the SV type information should be indicated (see the -svtBEDcol option). The "SV\_type" values should be one of the following:

- Deletion: DEL, deletion, loss or <CN0>
- Duplication: DUP, duplication, gain, MCNV, <CN2>, <CN3>...
- Insertion: INS, insertion, ALU, LINE, SVA or MEI
- Inversion: INV or inversion
- Breakend record: BND, breakpoint, breakend

## 7. INPUT

AnnotSV takes several arguments as input including options that are detailed in the "USAGE / OPTIONS" section. The different arguments can be passed to the program in three ways (order of priority):

- Using the command line
- Using a "configfile" located in the same directory as your input file
- Using a "configfile" directly in the installation directory in \$ANNOTSV/etc/AnnotSV/configfile

Five types of INPUT files are detailed below:

### a) SV input file (required)

AnnotSV supports either the [VCF](#) (Variant Call Format) or the [BED](#) (Browser Extensible Data) formats as input files to describe the SV to annotate. It allows the program to be easily integrated into any bioinformatics pipeline dedicated to NGS analysis.

- **VCF format:**

It contains meta-information lines (prefixed with "##"), a header line (prefixed with "#"), and data lines each containing information about a position in the genome and genotype information on samples for each position (text fields separated by tabs). The specifications are described at <https://samtools.github.io/hts-specs/VCFv4.3.pdf>. AnnotSV supports either native or gzipped VCF file.

By default, AnnotSV extracts and reports from the VCF input file the following information:

- The REF, ALT, FORMAT and samples columns
- The SVTYPE value from the INFO column and only this one
- All other columns (QUAL, FILTER and INFO)

This report is user defined, see the "SVinputInfo" option in USAGE/OPTIONS.

**Warning: AnnotSV will not report (and annotate) SV described with a non-official nomenclature.**

**Warning: AnnotSV will report in the "Samples\_ID" output column the list of the samples ID for which the SV was called (based on the genotype (GT) information).**

- **BED format.**

Every single line of the BED file defines a SV including the obligatory first 3 fields to describe its coordinates:

1. *chrom* - The name of the chromosome (e.g. 3, Y, ...) - Preferred without "chr".
2. *chromStart* - The starting position of the SV on the chromosome. According to the format, the base count starts at base "0".

3. *chromEnd* - The ending position of the SV on the chromosome. The *chromEnd* base is not included in the display of the feature. For example, the first 100 bases of a chromosome are defined as *chromStart*=0, *chromEnd*=100, and span the bases numbered 0-99.

Two supplementary fields are **highly recommended**:

1. *SVTYPE* - The SV type (DEL, DUP...)  
=> The column number of the BED file with the SV type information should be indicated (see the *-svtBEDcol* option) in order to be able to classify the SV.
2. *Samples\_ID* - The list of the samples ID for which the SV was detected  
=> The column number with the *Samples\_ID* information should be indicated (see the *-samplesidBEDcol* option)

Additional fields from the BED file are optional and can be reported in the AnnotSV outputfile (user defined). It can be used to store quality, read depth or other metrics produced by the SV caller. By default, AnnotSV reports the additional fields from the BED input file. This report is user defined, see the "SVinputInfo" option in USAGE/OPTIONS.

When the additional fields from the BED file are reported, the user can provide a BED of which the first line begins with a "#", is tab separated and describe the columns header. The following example has been set to provide the SV coordinates associated to their SV type (DEL, DUP...) and score:

#Chrom	Start	End	SV_type	Score
1	2806107	107058351	DEL	5.0256
12	25687536	25699754	DUP	1.3652

## b) Custom annotations: SNV/indel input files - for DELETION filtering (optional)

AnnotSV can take VCF file(s) with SNV/indel calls from any sequencing experiment as input to the command line. These annotations report the counts and ratio of homozygous and heterozygous SNV/indel identified from the patients NGS data (user defined samples) and presents in the interval of the **deletion** to annotate.

### Usage:

The command line can be completed with the 2 following options: "*-snvIndelFiles*" and "*-snvIndelSamples*" (cf USAGE/OPTIONS).

### Annotation columns:

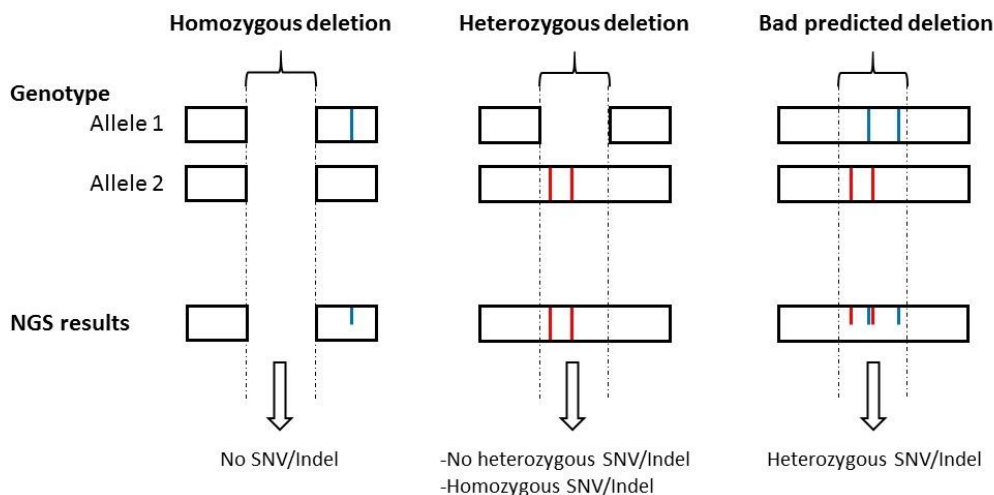
Add the "*Count\_hom(sample)*", "*count\_htz(sample)*", "*Count\_htz/allHom(sample)*", "*Count\_htz/total(cohort)*" and "*Count\_total(cohort)*" annotation columns.

- **Count\_hom(sample):** Count of homozygous SNV/indel called from the sample and present in the interval of the deletion
- **Count\_htz(sample):** Count of heterozygous SNV/indel called from the sample and present in the interval of the deletion
- **Count\_allHom(sample):** Count of homozygous SNV/indel called from the sample, including homozygous WT SNV/indel (extracted from VCF input file, GT=0/0), and present in the interval of the deletion
- **Count\_total(cohort):** Total count of SNV/indel called from all the samples of the cohort and present in the interval of the deletion

It is to notice that AnnotSV reports only 1 count if 2 SNV/indel are located at the same position (e.g. chr1:1234567 C>A and chr1:1234567 C>G).

### **Aim:**

These annotations can be used by the user to filter out false positive SV calls or to confirm events as following:



- **Homozygous deletion:** No SNV/indel is expected in the region. Homozygous deletion can be identified as a false positive by noting the presence of SNV/indel called at the predicted locus of the deletion in a sample. So we expect a zero “#htz/allHom(sample)” and “#htz/total(cohort)” ratio.

- **Heterozygous deletion:** All SNV/indel are expected to be homozygous. Heterozygous deletion can be identified as a false positive by noting the presence of heterozygous SNV/indel called at the predicted locus of the deletion in a sample. So we expect small “#htz/allHom(sample)” and “#htz/total(cohort)” ratio. However, threshold for these ratio is dependent on sequencing protocols and calling/filtering strategies and cannot be determined as a standard.

### **Warning:**

In the VCF file(s), the genotype of each SNV/indel should be indicated in the FORMAT column under the “GT” field.

**A deletion QC** can be performed by checking both ratio, ONLY if:

- analysing a cohort VCF where all samples have been jointly called.
- there is a minimum number of SNV/indel located in the SV. So, AnnotSV reports these ratio only if #total(cohort) > 50; otherwise the ratio will be set to "NA" (not applicable).

The deletion QC do not apply to standard VCF for single sample, since homozygous reference positions are not usually reported.

## c) Custom annotations: filtered SNV/indel input files - for compound heterozygosity analysis (optional)

### **Aim:**

AnnotSV can take a VCF file(s) with SNV/indel as input to the command line that is already filtered for genotype, frequency and effects on protein level. AnnotSV can report the heterozygous SNV/indel called (by any sequencing experiment) in the gene overlapped by the SV to annotate, as well in 'healthy' and 'affected' samples (user defined samples). AnnotSV offers an efficient way to highlight compound heterozygotes with one heterozygous SNV/indel and one heterozygous SV in the same gene. Indeed, in recessive genetic disorders, both copies of the gene are malfunctioning. This means that the maternally as well as the paternally inherited copy of an autosomal gene harbors a pathogenic variation. In addition, if the parents are non-consanguineous, compound heterozygosity is the best explanation for a recessive disease.

### **Annotation columns:**

Add 1 annotation columns for each sample: **compound\_htz(sample)**.

### **Usage:**

To add the "**compound\_htz(sample)**" annotation column, the command line can be completed with the 2 following options: "-candidateSnpIndelFiles" and "-candidateSnpIndelSamples" (cf USAGE/OPTIONS).

### **User challenge:**

The user challenge in filtering variants for compound heterozygotes is to know whether the two heterozygous variants (the SNV/indel and the SV) are in *cis* or in *trans*. Especially, when sequencing data of more than one family member is available, one can exclude certain variants based on the expected Mendelian inheritance (transmitted in a compound heterozygous mode from parents to the patient(s)). A specific feature (barcode) will be implemented soon for this.

**Warning:** In the SV and the SNV/indel VCF file(s), the genotype should be indicated in the FORMAT column as "GT".

## d) Custom annotations: External BED annotation files (optional)

### **Aim:**

Several users might want to add their own private region annotations to the one already provided by AnnotSV.

### **Inputs:**

AnnotSV can integrate external annotations for specific regions that will be imported from a BED file into the output file. Each external BED annotation file should be **copied or linked** in:

Genome build GRCh37:

➔ "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Users/GRCh37/**FtIncludedInSV**" directory



- or
- ➔ "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Users/GRCh37/**SVincludedInFt**" directory
- or
- ➔ "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Users/GRCh37/**AnyOverlap**" directory

*Genome build GRCh38:*

- ➔ "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Users/GRCh38/**FtIncludedInSV**" directory
- or
- ➔ "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Users/GRCh38/**SVincludedInFt**" directory
- or
- ➔ "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Users/GRCh38/**AnyOverlap**" directory

It is to notice that:

- **By placing the BED file in the "FtIncludedInSV" directory**, only the features overlapped at 100% with the SV will be reported.

e.g. Useful for a BED file of known pathogenic genomic regions.

- **By placing the BED file in the "SVincludedInFt" directory**, only the features overlapping 100% of the SV will be reported. In this case, a reciprocal overlap can be used (see "reciprocal" and "overlap" options in USAGE/OPTIONS).

e.g. Useful for a BED file of known benign genomic regions.

In both cases (FtIncludedInSV and SVincludedInFt), the user can modify the default behaviour of the overlap by using a different percentage (see the "-overlap" option in USAGE/OPTIONS).

- **By placing the BED file in the "AnyOverlap" directory**, any feature overlapped with the SV (even with 1bp overlap) will be reported. In this case, a reciprocal overlap can be used (see "reciprocal" option in USAGE/OPTIONS).

**Warning:** After a formatting step, the copy and/or linked users file(s) will be deleted the first time AnnotSV is executed after an update.

#### **Header:**

Each external BED annotation file (e.g. 'User'.bed) can start with a first line beginning with a "#" and describing the header of these new annotations.

#### **Examples:**

- This first example has been set to provide the SV overlap with frequency (Freq) of internal cohort regions:

The 'UserYYY'.bed file contains:

#Chrom	Start	End	Freq
1	2806107	107058351	0.0018
12	25687536	25699754	0.0023

The additional "Freq" annotation column is then made available in the output file.

- This second example has been set to provide the SV overlap with Regions of Homozygosity (RoH) of 2 individuals (sample1 and sample2):

The 'UserXXX'.bed file contains:

#Chrom	Start	End	RoH
1	2806107	107058351	sample1, sample2
12	25687536	25699754	sample2

The additional "RoH" annotation column is then made available in the output file.

## e) Custom annotations: External gene annotation files (optional)

In order to further enrich the annotation for each SV gene, AnnotSV can integrate external annotations imported from tab separated values file(s) into the output file. The first line should be a header including a column entitled "genes". The following example has been set to provide annotation for the interacting partners of a gene.

genes	Interacting_genes
BBS1	BBS7, TTC8, BBS5, BBS4, BBS9, ARL6, BBS2, RAB3IP, BBS12, BBS10

"Interacting\_genes" annotation column is then available in the output file.

Each external gene annotation file (\*.tsv) should be located in the "\$ANNOTSV/share/AnnotSV/Annotations\_Human/Users/" directory.

It is to notice that these files should not contain any of these 2 specific characters "{" and "}" (that would be replaced by "(" and ")"). AnnotSV supports either native or gzipped tsv file.

Moreover, you need to use a configfile (see INPUT section) and to define there the output column names you want to be added.

## 8. OUTPUT

### a) Output format

Giving a SV input file, AnnotSV produces a tab-separated values file that can be easily integrated in bioinformatics pipelines or directly read in a spreadsheet program.

### b) Output file path(s) and name(s)

Two options (-outputDir and -outputFile) can be used to specify the output directory and/or file name. The output file extension should be ".tsv" (tab separated values).

By default, an output directory is created where AnnotSV is run ('YYYYMMDD'\_AnnotSV). As an example, an input SV file named "mySVinputFile.vcf" will produce by default an output file named "'date'\_AnnotSV/mySVinputFile.annotated.tsv".

AnnotSV can also create another output file: a report of unannotated variants (".unannotated.tsv" file). Indeed, AnnotSV does not annotate variants from a VCF input file:

- If the variant is an indel (variant length < SVminSize)
- If the SV is not well formatted
- If the "END" of the SV is not defined

## c) "Annotation mode" column

A typical AnnotSV use would be to first look at the annotation and ranking of each SV as a whole (i.e. "full") and then focus on the content of that SV by genes. This is possible thanks to the way AnnotSV can present the data. Indeed, there are 2 types of lines provided by AnnotSV (cf the "Annotation\_mode" output column):

- An annotation on the "full" length of the SV. Every SV are reported, even those not covering a gene. This type of annotation gives an estimate of the SV itself.

- An annotation of the SV "split" by gene. This type of annotation gives an opportunity to focus on each gene overlapped by the SV. Thus, when a SV spans over several genes, the output will contain as many annotations lines as genes covered (cf example in FAQ). This latter annotation is extremely powerful to shorten the identification of mutation implicated in a specific gene.

Considering the "full" length annotation of one SV, AnnotSV does not report the Gene-based annotation (value is set to empty), except for scores and percentages where AnnotSV reports the most pathogenic score or the maximal percentage.

## d) Annotation columns available in the output file

In the following table, we describe the annotations that are available in the AnnotSV output file. It is to notice that, since AnnotSV can be configured to output the annotations using 2 different annotation modes (full or split), in some cases specific gene annotations are only present while using one of the two modes.

**Nomenclature:** All the column names begin with an upper case and contain no space character.

Column name	Annotation	Full	Split	BED input	VCF input
AnnotSV_ID	AnnotSV ID	X	X	X	X
SV_chrom	Name of the chromosome	X	X	X	X
SV_start	Starting position of the SV in the chromosome	X	X	X	X

<b>SV_end</b>	Ending position of the SV in the chromosome	X	X	X	X
<b>SV_length</b>	Length of the SV (bp) (deletions have negative values)	X	X	X	X
<b>SV_type</b>	Type of the SV (DEL, DUP, ...)	X	X	X	X
<b>Samples_ID</b>	List of the samples ID for which the SV was called (according to the SV input file)	X	X	X	X
<b>REF</b>	Nucleotide sequence in the reference genome (extracted only from a VCF input file)	X	X		X
<b>ALT</b>	Alternate nucleotide sequence (extracted only from a VCF input file)	X	X		X
<b>FORMAT</b>	The FORMAT column from a VCF file	X	X		X
<b>'SampleID'</b>	The sample ID column from a VCF file	X	X		X
<b>Annotation_mode</b>	Indicate the type of annotation lines generated: - annotation on the SV full length ("full") - annotation on each gene overlapped by the SV ("split")	X	X	X	X
<b>Gene_name</b>	Gene symbol	X	X	X	X
<b>Gene_count</b>	Number of overlapped genes with the SV	X		X	X
<b>Tx<sup>1</sup></b>	Transcript symbol		X	X	X
<b>Tx_start</b>	Starting position of the transcript		X	X	X
<b>Tx_end</b>	Ending position of the transcript		X	X	X
<b>Overlapped_tx_length</b>	Length of the transcript (bp) overlapping with the SV		X	X	X
<b>Overlapped_CDS_length</b>	Length of the CoDing Sequence (CDS) (bp) overlapped with the SV		X	X	X
<b>Overlapped_CDS_percent</b>	Percent of the CoDing Sequence (CDS) (bp) overlapped with the SV		X	X	X
<b>Frameshift</b>	Indicates if the CDS length is not divisible by three (yes or no)		X	X	X
<b>Exon_count</b>	Number of exons of the transcript		X	X	X
<b>Location</b>	SV location in the gene's Values: txStart, txEnd, exon'i', intron'i' e.g. « txStart-exon3 »		X	X	X
<b>Location2</b>	SV location in the gene's coding regions Values: UTR (no CDS in the gene), 5'UTR (before the CDS start), 3'UTR (after the CDS end), CDS (between the CDS start and the CDS end, can be in an exon or an intron). e.g. « 3'UTR-CDS »		X	X	X
<b>Dist_nearest_SS<sup>2</sup></b>	Absolute distance to nearest splice site after considering exonic and intronic SV breakpoints		X	X	X
<b>Nearest_SS_type</b>	Nearest splice site type: 5' (donor) or 3' (acceptor)		X	X	X
<b>Intersect_start</b>	Start position of the intersection between the SV and a transcript		X	X	X
<b>Intersect_end</b>	End position of the intersection between the SV and a transcript		X	X	X
<b>RE_gene</b>	Name of the genes regulated by a regulatory element overlapped with the SV to annotate. When available, the regulated gene name is detailed with associated haploinsufficiency (HI), triplosensitivity (TS), Exomiser (EX) scores, OMIM and candidate genes. (For the filtering output, see the -REselect1 and -REselect2 options)	X		X	X
<b>B_gain_source</b>	Origin of the benign gain genomic regions completely overlapping the SV to annotate:	X	X	X	X

	gnomAD, ClinVar (CLN), ClinGen (TS40), DGV (dgv, nsv or esv), DDD, 1000 genomes (1000g), Ira M. Hall's lab (IMH), Children's Mercy Research Institute (CMRI)				
<b>B_gain_coord</b>	Coordinates of the benign gain genomic regions completely overlapping the SV to annotate	X	X	X	X
<b>B_gain_AFmax</b>	Maximum allele frequency of the reported benign gain genomic regions (if available)	X	X	X	X
<b>B_loss_source</b>	Origin of the benign loss genomic regions completely overlapping the SV to annotate: gnomAD, ClinVar (CLN), ClinGen (HI40), DGV (dgv, nsv or esv), DDD, 1000 genomes (1000g), Ira M. Hall's lab (IMH), Children's Mercy Research Institute (CMRI)	X	X	X	X
<b>B_loss_coord</b>	Coordinates of the benign loss genomic regions completely overlapping the SV to annotate	X	X	X	X
<b>B_loss_AFmax</b>	Maximum allele frequency of the reported benign loss genomic regions (if available)	X	X	X	X
<b>B_ins_source</b>	Origin of the benign insertion genomic regions completely overlapping the SV to annotate: gnomAD, ClinVar (CLN), 1000 genomes (1000g), Ira M. Hall's lab (IMH), Children's Mercy Research Institute (CMRI)	X	X	X	X
<b>B_ins_coord</b>	Coordinates of the benign insertion genomic regions completely overlapping the SV to annotate	X	X	X	X
<b>B_ins_AFmax</b>	Maximum allele frequency of the reported benign insertion genomic regions (if available)	X	X	X	X
<b>B_inv_source</b>	Origin of the benign inversion genomic regions completely overlapping the SV to annotate: gnomAD, 1000 genomes (1000g), Ira M. Hall's lab (IMH), Children's Mercy Research Institute (CMRI)	X	X	X	X
<b>B_inv_coord</b>	Coordinates of the benign inversion genomic regions completely overlapping the SV to annotate	X	X	X	X
<b>B_inv_AFmax</b>	Maximum allele frequency of the reported benign inversion genomic regions (if available)	X	X	X	X
<b>P_gain_phen</b>	Phenotype of the pathogenic gain genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_gain_hpo</b>	HPO terms describing the pathogenic gain genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_gain_source</b>	Origin of the pathogenic gain genomic regions completely overlapped with the SV to annotate: dbVarNR (dbVar), ClinVar (CLN), ClinGen (TS3)	X	X	X	X
<b>P_gain_coord</b>	Coordinates of the pathogenic gain genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_loss_phen</b>	Phenotype of the pathogenic loss genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_loss_hpo</b>	HPO terms describing the pathogenic loss genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_loss_source</b>	Origin of the pathogenic loss genomic regions completely overlapped with the SV to annotate: dbVarNR (dbVar), ClinVar (CLN), ClinGen (HI3), morbid	X	X	X	X
<b>P_loss_coord</b>	Coordinates of the pathogenic loss genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_ins_phen</b>	Phenotype of the pathogenic insertion genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_ins_hpo</b>	HPO terms describing the pathogenic insertion genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_ins_source</b>	Origin of the pathogenic insertion genomic regions completely overlapped with the SV to annotate:	X	X	X	X

	dbVarNR (dbVar), ClinVar (CLN)				
<b>P_ins_coord</b>	Coordinates of the pathogenic insertion genomic regions completely overlapped with the SV to annotate	X	X	X	X
<b>P_snvindel_nb</b>	Number of pathogenic snv/indel from public databases completely overlapped with the SV to annotate	X	X	X	X
<b>P_snvindel_phen</b>	Phenotypes of pathogenic snv/indel from public databases completely overlapped with the SV to annotate	X	X	X	X
<b>TAD_coordinate<sup>3</sup></b>	Coordinates of the TAD whose boundaries overlapped with the annotated SV (boundaries included in the coordinates)	X		X	X
<b>ENCODE_experiment<sup>3</sup></b>	ENCODE experiments used to define the TAD	X		X	X
<b>Cosmic_ID</b>	COSMIC identifier	X	X	X	X
<b>Cosmic_mut_typ</b>	Defined as Gain or Loss	X	X	X	X
<b>CytoBand</b>	Cytogenic band annotation	X	X	X	X
<b>GC_content_left</b>	GC content around the left SV breakpoint (+/- 100bp)	X		X	X
<b>GC_content_right</b>	GC content around the right SV breakpoint (+/- 100bp)	X		X	X
<b>Repeat_coord_left</b>	Repeats coordinates around the left SV breakpoint (+/- 100bp)	X		X	X
<b>Repeat_type_left</b>	Repeats type around the left SV breakpoint (+/- 100bp) e.g. AluSp, L2b, L1PA2, LTR12C, SVA_D, ...	X		X	X
<b>Repeat_coord_right</b>	Repeats coordinates around the right SV breakpoint (+/- 100bp)	X		X	X
<b>Repeat_type_right</b>	Repeats type around the right SV breakpoint (+/- 100bp) e.g. AluSp, L2b, L1PA2, LTR12C, SVA_D, ...	X		X	X
<b>Gap_left</b>	Gap regions coordinates around the left SV breakpoint (+/- 100bp)	X		X	X
<b>Gap_right</b>	Gap regions coordinates around the right SV breakpoint (+/- 100bp)	X		X	X
<b>SegDup_left</b>	Segmental Duplication regions coordinates around the left SV breakpoint (+/- 100bp)	X		X	X
<b>SegDup_right</b>	Segmental Duplication regions coordinates around the right SV breakpoint (+/- 100bp)	X		X	X
<b>ENCODE_blacklist_left</b>	ENCODE blacklist regions coordinates around the left SV breakpoint (+/- 100bp)	X		X	X
<b>ENCODE_blacklist_characteristics_left</b>	ENCODE blacklist regions characteristics around the left SV breakpoint (+/- 100bp)	X		X	X
<b>ENCODE_blacklist_right</b>	ENCODE blacklist regions coordinates around the right SV breakpoint (+/- 100bp)	X		X	X
<b>ENCODE_blacklist_characteristics_right</b>	ENCODE blacklist regions characteristics around the right SV breakpoint (+/- 100bp)	X		X	X
<b>ACMG</b>	ACMG genes		X	X	X
<b>HI</b>	ClinGen Haploinsufficiency Score	X	X	X	X
<b>TS</b>	ClinGen Triplosensitivity Score	X	X	X	X
<b>DDD_HI_percent</b>	Haploinsufficiency ranks from DDD	X	X	X	X
<b>GenCC_disease</b>	GenCC disease name: e.g. Nizon-Isidor syndrome		X	X	X
<b>GenCC_moi</b>	GenCC mode of inheritance		X	X	X
<b>GenCC_classification</b>	GenCC classification (Definitive, Strong, Moderate, Limited, Disputed, Animal Model Only, Refuted or No known disease relationship)		X	X	X
<b>GenCC_pmid</b>	GenCC Pubmed Id		X	X	X
<b>ExAC_synZ</b>	Positive synZ_ExAC (Z score) from ExAC indicate gene intolerance to synonymous variation	X	X	X	X
<b>ExAC_misZ</b>	Positive misZ_ExAC (Z score) from ExAC indicate gene intolerance to missense variation	X	X	X	X

<b>ExAC_delZ</b>	Positive delZ_ExAC (Z score) from ExAC indicate gene intolerance to deletion	X	X	X	X
<b>ExAC_dupZ</b>	Positive dupZ_ExAC (Z score) from ExAC indicate gene intolerance to duplication	X	X	X	X
<b>ExAC_cnvZ</b>	Positive cnvZ_ExAC (Z score) from ExAC indicate gene intolerance to CNV	X	X	X	X
<b>OMIM_ID</b>	OMIM unique six-digit identifier	X	X	X	X
<b>OMIM_phenotype</b>	e.g. Charcot-Marie-Tooth disease		X	X	X
<b>OMIM_inheritance<sup>4</sup></b>	e.g. AD (= "Autosomal dominant")		X	X	X
<b>OMIM_morbid</b>	Set to "yes" if the SV overlaps an OMIM morbid gene	X	X	X	X
<b>OMIM_morbid_candidate</b>	Set to "yes" if the SV overlaps an OMIM morbid gene candidate	X	X	X	X
<b>LOEUF_bin</b>	Minimal "decile bin of LOEUF" for given transcripts of a gene (lower values indicate more constrained) Values = integer [0-9]	X	X	X	X
<b>GnomAD_pLI</b>	Score computed by gnomAD indicating the probability that a gene is intolerant to a loss of function variation (Nonsense, splice acceptor/donor variants due to SNV/indel).	X	X	X	X
<b>ExAC_pLI</b>	Score computed by ExAC indicating the probability that a gene is intolerant to a loss of function variation (Nonsense, splice acceptor/donor variants due to SNV/indel). ExAC considers pLI>=0.9 as an extremely LoF intolerant gene	X	X	X	X
<b>Exomiser_gene_pheno_score</b>	Exomiser score for how close each overlapped gene is to the phenotype	X	X	X	X
<b>Human_pheno_evidence</b>	Phenotypic evidence from Human model		X	X	X
<b>Mouse_pheno_evidence</b>	Phenotypic evidence from Mouse model		X	X	X
<b>Fish_pheno_evidence</b>	Phenotypic evidence from Fish model		X	X	X
<b>Compound_htz(sample)</b>	List of heterozygous SNV/indel (reported with "chrom_position") presents in the gene overlapped by the annotated SV	X	X		X
<b>Count_hom(sample)</b>	Number of homozygous SNV/indel (extracted from VCF input file) in the individual "sample" which are presents: - in the deletion SV ("full" annotation) - between intersectStart and intersectEnd ( "split" annotation)	X	X		X
<b>Count_htz(sample)</b>	Number of heterozygous SNV/indel (extracted from VCF input file) in the individual "sample" which are presents: - in the SV ("full" annotation) - between intersectStart and intersectEnd ( "split" annotation)	X	X		X
<b>Count_htz/allHom(sample)<sup>5</sup></b>	Ratio for QC filtering: #htz(sample)/#allHom(sample)	X	X		X
<b>Count_htz/total(cohort)</b>	Ratio for QC filtering: #htz(sample)/#total(cohort)	X	X		X
<b>Count_total(cohort)</b>	Total count of SNV/indel called from all the samples of the cohort and present in the interval of the deletion	X	X		X
<b>AnnotSV_ranking_score</b>	SV ranking score following the 2019 joint consensus recommendation of ACMG and ClinGen. Scoring: pathogenic ≥0.99, likely pathogenic [0.90;0.98], variant of uncertain significance [0.89;-0.89], likely benign [-0.90;-0.98], benign ≤-0.99.	X		X	X
<b>AnnotSV_ranking_criteria</b>	Decision criteria explaining the AnnotSV ranking score	X		X	X
<b>ACMG_class</b>	SV ranking class into 1 of 5: class 1 (benign) class 2 (likely benign) class 3 (variant of unknown significance) class 4 (likely pathogenic) class 5 (pathogenic)	X		X	X

<sup>1</sup>Given one gene, only a single transcript from all transcripts available is reported. The transcript selected by the user with the "-txFile" option is firstly reported. In case of transcripts with different CDS length (considering the overlapping region with the SV), the transcript with the longest CDS is reported. Otherwise, if there is no differences in CDS length, the longest transcript is reported.

<sup>2</sup>AnnotSV calculates the distance to the nearest splice site, upstream and downstream of exonic/intronic SV breakpoints. Then, if several distances were calculated, AnnotSV keeps the smallest one

<sup>3</sup>Very large SV (e.g. 30Mb) can sometime overlap too many features locations. It appears that depending on the visualisation program used (spreadsheet programs mostly) this annotation can be truncated. In order to avoid such embarrassing glitch and maybe also because overlapping so many features is already a problem, AnnotSV restrict the number of overlapping reported features to 20.

<sup>4</sup>Detailed in the FAQ

<sup>5</sup>allHom(sample): Count of homozygous SNV/indel called from the sample, including homozygous WT SNV/indel (extracted from VCF input file, GT=0/0), and present in the interval of the deletion

## e) User selection of the annotation columns

Users can modify the default order of the annotation columns provided by AnnotSV and only select a subset of them. This could especially help in reducing the size of the output file and the time of the annotation.

This setting can be easily done in a configfile (see INPUT section). There, the user can comment column names with a hash character («#»). An example of configfile is provided in the AnnotSV installation directory.

## 9. USAGE / OPTIONS

To run AnnotSV, the default command line is the following:

```
$ANNOTSV/bin/AnnotSV -SvinputFile '/Path/Of/Your/VCF/or/BED/Input/File' >& AnnotSV.log &
```

The command line can be completed by the list of options described below or modified in the configfile (see INPUT section). To show the options simply type:

```
$ANNOTSV/bin/AnnotSV -help
```

or

```
$ANNOTSV/bin/AnnotSV
```

OPTIONS:

-----

-annotationsDir:	Path of the annotations directory
-annotationMode:	Description of the types of lines produced by AnnotSV Values: both (default), full or split
-bcftools:	Path of the bcftools local installation
-bedtools:	Path of the bedtools local installation



-benignAF:	Allele frequency threshold to select the benign SV in the data sources Range values: [0.001-0.1], default = 0.01 (i.e. 1%)
-candidateGenesFile:	Path of a file containing the user defined candidate genes (gene names can be space-separated, tabulation-separated, or line-break-separated)
-candidateGenesFiltering:	To select only the SV annotations ("split" and "full") overlapping a gene from the "candidateGenesFile" Values: 0 (default) or 1
-candidateSnpIndelFiles:	Path of the filtered VCF input file(s) with SNV/indel coordinates for compound heterozygotes report (optional) Gzipped VCF files are supported as well as regular expression
-candidateSnpIndelSamples:	To specify the sample names from the VCF files defined from the -candidateSnpIndelFiles option Default: use all samples from the filtered VCF files
-genomeBuild:	Genome build used Values: GRCh38 (default) or GRCh37 or mm9 or mm10
-help:	More information on the arguments
-hpo:	HPO terms list describing the phenotype of the individual being investigated Values: use comma, semicolon or space separated class values Default = "" (e.g.: "HP:0001156,HP:0001363,HP:0011304")
-includeCI:	To expand the "start" and "end" SV positions with the VCF confidence intervals (CIPOS, CIEND) around the breakpoints Values: 1 (default) or 0
-metrics:	Changing numerical values from frequencies to us or fr metrics (e.g. 0.2 or 0,2) Values: us (default) or fr
-minTotalNumber:	Minimum number of individuals tested to consider a benign SV for the ranking Range values: [100;1000], default = 500
-outputDir:	Output path name
-outputFile:	Output path and file name
-overlap:	Minimum overlap (%) between user features (User BED) and the annotated SV to be reported Range values: [0;100], default = 100
-overwrite:	To overwrite existing output results Values: 1 (default) or 0
-promoterSize:	Number of bases upstream from the transcription start site Default = 500
-rankFiltering:	To select the SV of a user-defined specific class (from 1 to 5; or NA)

	<p>Values: use comma separated class values, or use a dash to denote a range of values (e.g.: "3,4,5" or "3-5"), default = "1-5,NA"</p>
-reciprocal:	<p>Use of a reciprocal overlap between SV and user features (only for annotations with features overlapping the SV) Values: 0 (default) or 1</p>
-REreport:	<p>Create a report to link the annotated SV and the overlapped regulatory elements (coordinates and sources) Values: 0 (default) or 1</p>
-REselect1:	<p>To report only the morbid, HI, TS, candidate and phenotype matched genes Values: 1 (default) or 0</p>
-REselect2:	<p>To report only the genes not present in "Gene_name" Values: 1 (default) or 0</p>
-samplesidBEDcol:	<p>Number of the column reporting the samples ID for which the SV was called (if the input SV file is a BED) Range values: [4-[, default = -1 (value not given) (Samples ID should be comma or space separated)</p>
-snvIndelFiles:	<p>Path of the VCF input file(s) with SNV/indel coordinates used for false positive discovery Use counts of the homozygous and heterozygous variants Gzipped VCF files are supported as well as regular expression</p>
-snvIndelPASS:	<p>Boolean. To only use variants from VCF input files that passed all filters during the calling (FILTER column value equal to PASS) Values: 0 (default) or 1</p>
-snvIndelSamples:	<p>To specify the sample names from the VCF files defined from the -snvIndelFiles option Default: use all samples from the VCF files</p>
-SVinputFile:	<p>Path of the input file (VCF or BED) with SV coordinates Gzipped VCF file is supported</p>
-SVinputInfo:	<p>To extract the additional SV input fields and insert the data in the outputfile Values: 1 (default) or 0</p>
-SVminSize:	<p>SV minimum size (in bp) Default = 50</p>
-svtBEDcol:	<p>Number of the column describing the SV type (DEL, DUP) if the input SV file is a BED Range values: [4-[, default = -1 (value not given)</p>
-tx:	<p>Origin of the transcripts (RefSeq or ENSEMBL) Values: RefSeq (default) or ENSEMBL</p>

-txFile: Path of a file containing a list of preferred genes transcripts to be used in priority during the annotation (Preferred genes transcripts names should be tab or space separated)

## 10. Test

In order to validate the AnnotSV installation and its functioning, an example is available in the “\$ANNOTSV/share/doc/AnnotSV/Example” directory. Command lines examples are available in the following file “\$ANNOTSV/share/doc/AnnotSV/commands.README”.

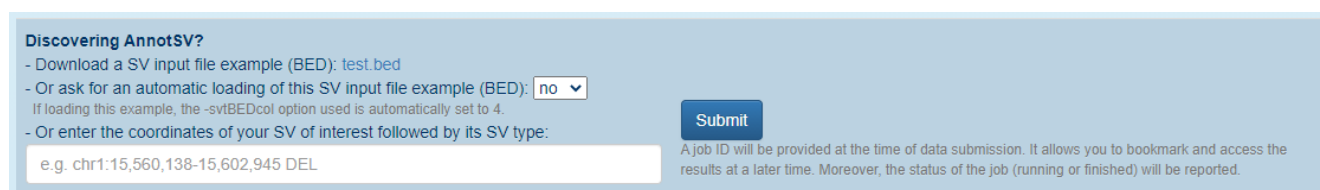
Moreover, an input/output example (the HG00096 individual from the 1000 Genomes project) is available on the [AnnotSV website](#).

## 11. WEB SERVER

### a) AnnotSV annotation and ranking

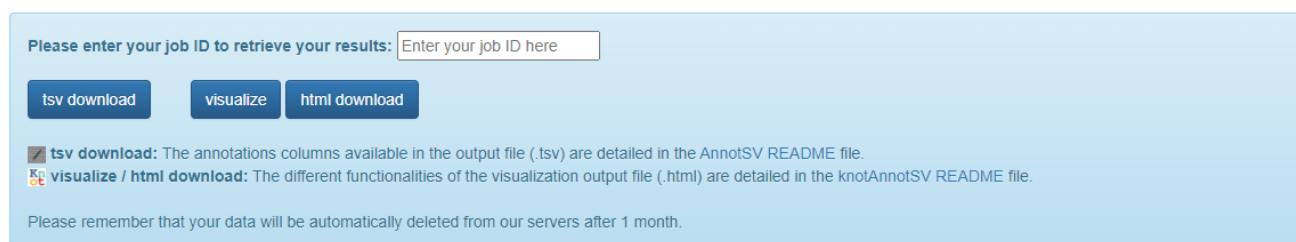
Annotation and ranking of your SV are freely available online at <https://lbgi.fr/AnnotSV/runjob>. User can operate through a web browser, which can be used to select the parameters, run the program, retrieve or visualize/analyze the results.

An SV input file example (BED) is available to easily evaluate AnnotSV online.



The screenshot shows the 'Discovering AnnotSV?' section of the web interface. It includes instructions for downloading a BED file example, asking for automatic loading of the example (with a 'no' dropdown), and entering coordinates and SV type. A 'Submit' button is present. Below the input fields, a text box contains the example coordinates: 'e.g. chr1:15,560,138-15,602,945 DEL'. To the right, a note states: 'A job ID will be provided at the time of data submission. It allows you to bookmark and access the results at a later time. Moreover, the status of the job (running or finished) will be reported.'

A job ID is provided at the time of data submission. It allows user to bookmark and access the results at a later time. The results are available at: <https://lbgi.fr/AnnotSV/retrievejob>



The screenshot shows the 'Please enter your job ID to retrieve your results:' section. It features a text input field for the job ID and three buttons: 'tsv download', 'visualize', and 'html download'. Below these buttons, there are two informational lines: one for 'tsv download' stating that annotations columns are detailed in the AnnotSV README file, and another for 'visualize / html download' stating that functionalities are detailed in the knotAnnotSV README file. At the bottom, a note says: 'Please remember that your data will be automatically deleted from our servers after 1 month.'

Moreover, this job ID will give access to the status of the job (running or finished).

User data are automatically deleted from our servers after 1 month.

## b) Visualization of the annotation data

AnnotSV results visualization are powered by [knotAnnotSV](#). The user can visualize, filter and analyze the annotation data thanks to different user-friendly functions (search/filtering box, tooltip, links to public databases, color coded information...)

COMPACT EXPANDED Show 50 lines												Search: <input type="text"/>	
AnnotSV_ID	ACMG_class	SV_type	Annotation_mode	Gene_name	Location	OMIM phenotype	Exomiser score	Regulatory elements	Benign SV sources	Pathogenic SV sources	Number of pathogenic SV/incl overlapped	ENCODE blacklist characteristics left	ENCODE blacklist characteristics right
Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search	Search
2-14214368-148245744-BND-1	-	BND	full	ARHGAP15, OTDC1, KYT5C, LINC01412, LOC101924336, ...	-	-	NA	ACTR23A, ARHGAP15, OTDC1, KYT5C (overlaid), LINC01412, ...	-	CLN3-448705	135	-	-
2-14214368-148245744-BND-1	-	BND	split	ARHGAP15	telchrnt-telEnd	Morvan-Willem syndrome, 257735 (O)	NA	-	-	CLN3-448705	129	-	-
2-14214368-148245744-BND-1	-	BND	split	KIT2G	telchrnt-telEnd	"epidermal, cardiac, renal, and limb defects [...]"	NA	-	-	-	6	-	-
2-14214368-148245744-BND-1	-	BND	split	ARHGAP15	telchrnt-introEnd	-	NA	-	-	-	-	-	-
2-14214368-148245744-BND-1	-	BND	split	ARHGAP15	telchrnt-telEnd	-	NA	-	-	-	-	-	-
2-14214368-148245744-BND-1	-	BND	split	OTDC1	telchrnt-telEnd	-	NA	-	-	-	-	-	-
2-14214368-148245744-BND-1	-	BND	split	ZEB2-AS1	telchrnt-telEnd	-	NA	-	-	-	-	-	-
2-14214368-148245744-BND-1	-	BND	split	LOC101924336	telchrnt-telEnd	-	NA	-	-	-	-	-	-
2-14214368-148245744-BND-1	-	BND	split	PABPC1P2	telchrnt-telEnd	Location : telchrnt-telEnd Location : 15778 Tx : NR_110257	-	-	-	-	-	-	-
2-14214368-148245744-BND-1	-	BND	split	LINC01412	telchrnt-telEnd	Long repeat Overlapped_CDS_length : 27089	-	-	-	-	-	-	-
2-14214368-148245744-BND-1	-	BND	split	TRXN1	telchrnt-telEnd	Overlapped_CDS_length : 0 Overlapped_CDS_percent : 0	-	-	-	-	-	-	-
2-14214368-148245744-BND-1	-	BND	split	LOC101374658	telchrnt-telEnd	Transcript ID : Repeat : 55 Repeat_Start : 144694653 Repeat_End : 144721722	-	-	-	D,D:DFZL, LRRK33	-	-	-
2-110274502-110478039-MANNA-1	-	MANNA	full	D,D:DFZL	intrchrnt-telEnd	-	NA	-	-	D,D:DFZL, LRRK33	-	-	-
2-110274502-110478039-DIV-1	-	DIV	full	D,D:DFZL	intrchrnt-telEnd	-	NA	-	-	D,D:DFZL, LRRK33	-	-	-
2-110274502-110478039-DIV-1	-	DIV	split	D,D:DFZL	intrchrnt-telEnd	-	NA	-	-	D,D:DFZL, LRRK33	-	-	-
2-110274502-110478039-CPX-1	-	CPX	full	D,D:DFZL	intrchrnt-telEnd	-	NA	-	-	D,D:DFZL, LRRK33	-	-	-
2-110274502-110478039-CPX-1	-	CPX	split	D,D:DFZL	intrchrnt-telEnd	-	NA	-	-	-	-	-	-
11-3247752-3273848-DIV-1	-	DIV	full	BGLT3, HBB, HBBP1, HBD, HBG1	-	-	NA	BGLT3, HBB (marked), HBBP1, HBD (marked), HBG1, ...	-	-	91	-	-
11-3247752-3273848-DIV-1	-	DIV	split	HBBG1	telchrnt-telEnd	Fetal hemoglobin quantitative trait locus [...]	NA	-	-	-	-	-	-
11-3247752-3273848-DIV-1	-	DIV	split	HBB	telchrnt-telEnd	Thalassemia, delta- Thalassemia, delta- Thalassemia, beta, ...	NA	-	-	-	-	-	-

Showing 1 to 40 of 40 lines

Previous 1 Next

The interface is well detailed in the knotAnnotSV README file:

[https://github.com/mobidic/knotAnnotSV/blob/master/README.knotAnnotSV latest.pdf](https://github.com/mobidic/knotAnnotSV/blob/master/README.knotAnnotSV%20latest.pdf)

At each stage of the analysis process, all the set-up filters are locally stored. The html file can thus be closed at any time by biologists and then re-opened on the same computer to continue the analysis.

The knotAnnotSV source code is available under the GNU GPL licence and is downloadable on [GitHub](#).

## 12. FAQ

### Q: What are Structural Variations (SV)?

SV are generally defined as variation in a DNA region that vary in length from ~50 base pairs to many megabases and include several classes such as translocations, inversions, insertions, deletions.

### Q: What are Copy Number Variations (CNV)?

CNV are deletions and duplications in the genome (unbalanced SV) that vary in length from ~50 base pairs to many megabases.

**Q: What are the differences between SV and CNV?**

CNV are unbalanced SV with gain or loss of genomic material. For example, a heterozygous duplication as a CNV will be characterized with the start and end coordinates and the number of copies that is 3.

**Q: Can AnnotSV annotate every format of SV?**

AnnotSV supports as well VCF or BED format in input.

- VCF format supports complex rearrangements with breakends, that can arbitrary be summarized as a set of novel adjacencies, as described in the Variant Call Format Specification [VCFv4.3](#) (Jun 2020).
- BED format does not allow inter-chromosomal feature definitions (e.g. inter-chromosomal translocation). A new file format (BEDPE) is proposed in order to concisely describe disjoint genome features but it is not yet supported by AnnotSV.

**Q: I would like to annotate my SV with new annotation sources but I do not know how to do that...**

No problem. AnnotSV is under active and continuous development. You can email me with a detailed request and I will answer as quickly as possible.

**Q: I have just updated AnnotSV or the annotations sources and the annotation process is longer than usual, is it normal?**

After an update of AnnotSV sources, some files will be reprocessed and thus taking several additional time. Further use of AnnotSV will be quicker!

**Q: How to cite AnnotSV in my work?**

We do appreciate citations very much.

So, if you are using AnnotSV, please cite our work using the following references:

**AnnotSV and knotAnnotSV: a web server for human structural variations annotations, ranking and analysis.**

Geoffroy V, Guignard T, Kress A, Gaillard JB, Solli-Nowlan T, Schalk A, Gatinois V, Dollfus H, Scheidecker S, Muller J. NAR. 2021 May 22. doi: [10.1093/nar/gkab402](#)

**AnnotSV: An integrated tool for Structural Variations annotation.** Geoffroy V, Herenger Y, Kress A, Stoetzel C, Piton A, Dollfus H, Muller J. Bioinformatics. 2018 Apr 14. doi: [10.1093/bioinformatics/bty304](#)

And if you use the phenotype-driven analysis in your work, please cite also the following articles:

- Next-generation diagnostics and disease-gene discovery with the Exomiser. Smedley D., *et al*, Nature Protocols (2015) [doi:10.1038/nprot.2015.124](#)
- Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources. Köhler S., *et al*, Nucleic Acids Research (2019) [doi: 10.1093/nar/gky1105](#)

**Q: What are the WARNINGS that AnnotSV mention while running?**

AnnotSV writes to the standard output progress of the analysis including warnings about issues or missing information that can be either blocking or simply informative.

**Q: Why are some values empty or set to -1 in the output files?**

When no information is available for a specific type of annotation, then the value is empty. Regarding the frequencies, the default is set to -1.

**Q: Why some SV have empty gene annotation in the output file?**

If a SV is located in an intergenic region and so does not cover a gene, then the SV is reported in the output file but without gene annotation.

**Q: Why can we have several gene annotations for one SV?**

In some cases, one SV overlaps a large portion of the genome including several genes. In these cases, the annotation of the SV is split on several lines.

Annotation example for the deletion 1:16892807-17087595

AnnotSV keep all gene annotations, with only one transcript annotation for each gene:

1	16892807	17087595	DEL CROCCP2	NR_026752	1	12652	txStart-txEnd
1	16892807	17087595	DEL ESPNP	NR_026567	1	28941	txStart-txEnd

1	16892807	17087595	DEL FAM231A	NM_001282321	511	511	txStart-txEnd
1	16892807	17087595	DEL FAM231C	NM_001310138	511	656	txStart-txEnd
1	16892807	17087595	DEL LOC102724562	NR_135824	1	2998	txStart-txEnd
1	16892807	17087595	DEL MIR3675	NR_037446	1	75	txStart-txEnd
1	16892807	17087595	DEL MST1L	NM_001271733	2015	6468	txStart-exon14
1	16892807	17087595	DEL MST1P2	NR_027504	1	4848	txStart-txEnd
1	16892807	17087595	DEL NBPF1	NM_017940	2912	47294	intron3-txEnd

**Q: I am confused by the difference between the 'full' and the 'split' Annotation\_mode. CNVs have been split into several lines, but each line gets different DB annotation (DGV, 1000g...). I thought that same region should have the same annotations (excluding gene/transcript)?**

AnnotSV builds 2 types of annotations, one based on the full-length SV (corresponding to the Annotation\_mode = "full") and one based on each gene within the SV (corresponding to the Annotation\_mode = "split"). Thus, you will have access to:

- all the overlapped genes information (ID, OMIM...)
- the SV location within each overlapped gene (e.g. "exon3-intron11", "txStart-intron19", ...)

Be careful: the first 3 columns (SV chrom, SV start and SV end) remains the same despite being in "full" or in "split" type.

Regarding these "split" lines,

- DGV and 1000g SV overlaps are examined with regards to these gene coordinates. So, each "split" line get different DB annotation (DGV, 1000g...).
- 2 more annotation columns (intersectStart and intersectEnd) providing the intersection coordinates between the SV and the gene transcript.

**Q: What do the OMIM and GenCC Inheritance annotations mean?**

AD = "Autosomal dominant"

AR = "Autosomal recessive"

XLD = "X-linked dominant"

XLR = "X-linked recessive"

YLD = "Y-linked dominant"

YLR = "Y-linked recessive"

XL = "X-linked"

YL = "Y-linked"

ADm = "Autosomal dominant with maternal imprinting"

ADp = "Autosomal dominant with paternal imprinting"

2G = "Digenic inheritance"

MT = "Mitochondrial"

sD = "Semidominant"

SOM = "Somatic mosaicism"

IPVE = "Incomplete Penetrance and/or Variable Expressivity" (according to [the recommendations from the French ACHRO-PUCE network](#))

**Q: Why do I get this error message: "Feature (10:134136286-134136486) beyond the length of 10 size (133797422 bp). Skipping."**

One possibility is that you are using the bad "-genomeBuild" option. For example, you are using a bedfile in input with the SV coordinates on GRCh37 but with the "-genomeBuild GRCh38" option.

**Q: Is AnnotSV available for other organisms?**

The main objective of AnnotSV is to annotate SV information from human data. By default, all the annotations are based on human specific databases. Nevertheless, some additional annotation files can be added for mouse. If you are interested, please see the specific mouse README file.

**Q: Is there an option to just generate SV “split” by gene?**

You can choose to keep only the split annotation lines thanks to the "-annotationMode" option.

**Q: I am unable to run the code on the input files provided. It crashes on the Repeat annotation step due to a bad\_alloc error. Do you have any ideas on why this is happening?**

AnnotSV needs to be run with an appropriate RAM (depending of the annotations used). Setting your system to allocate 10 Go should solve the problem.

**Q: I am getting the error: “ANNOTSV environment variable not specified. Please define it before running AnnotSV. Exit”. How can I fix this problem?**

ANNOTSV is the environment variable defining the installation path of the software.

- In csh, you can define it with the following command line:  
setenv ANNOTSV /path\_of\_AnnotSV\_installation/bin
- In bash, you can define it with the following command line:  
export ANNOTSV=/path\_of\_AnnotSV\_installation/bin

I advise you to save the good command in your .cshrc or .bashrc file.

**Q: My annotated SV is intersecting both a benign SV and a pathogenic SV. How can I explain that?**

Several possible explanations can be considered:

- The pathogenicity can concern a recessive disease. So the pathogenic SV can be present in the heterozygous state in the healthy population (with a DGV low frequency)
- The pathogenic region of the dbVar SV is not overlapping the DGV SV

**Q: I am getting the error: “-- max size for a Tcl value (2147483647 bytes) exceeded”. How can I fix this problem?**

You are probably using AnnotSV to annotate a very large SV input file (from a large cohort). Thus, you are facing a memory issue either caused by the current machine specification or the programming language used for AnnotSV (Tcl). To solve this, you can split your input file into smaller files, run AnnotSV and then later merge them into a single output file. This will be fixed in a future release.

**Q: For a VCF with only “BND” events, which refers to breakpoints, how are these being shown in the AnnotSV output when SVminSize is set to 50bp? Since a breakpoint start and stop positions only differ by 1bp, I am wondering why these are not filtered out by AnnotSV.**

AnnotSV is designed to annotate SV and not SNV/indel from a VCF, which is the aim of the "SVminSize" option. Actually, SV can be described in three different ways in a VCF file:

- Type1: ref="G" and alt="ACTGCTAACGATCCGTTTGCTGCTAACGATCTAACGATCGGGATTGCTAACGATCTCGGG" (length >SVminSize)
- Type2: alt="<INS>", "<DEL>", "<BND>"...
- Type3: complex rearrangements with breakends: alt="G]17:1584563]"

The “SVminSize” parameter is only used to exclude SNV/indel from the SV of Type1.

**Q: How is calculated the “SV length” annotation?**

- AnnotSV reports the “SVLEN” value if given in a VCF input file.
- Nevertheless, when it is not provided, AnnotSV calculates the SV length (with "alt length" - "ref length") depending on the description of it in a VCF input file: ref="G" and alt="ACTGCTAACGATCCGTTTGCTGCTAACGATCTAACGATCGGGATTGCTAATCTCGGG"

- Else, AnnotSV calculates the SV length only for deletion, duplication and inversion (with "SVend - SVstart", and with a negative value for deletion). Indeed, this calculation cannot be done for insertion, breakend, translocation...
- Else, the SV length is blank.

**Q: Why do I get negative values in the SV\_length column?**

It is to notice that deletions have negative values. Other SV types have positive values.

**Q: What does the candidateGenesFile parameter refer to?**

The candidateGenesFile contains the candidate genes of the user. This information is used to filter out the SV annotations that do not overlap a candidate gene (-candidateGenesFiltering 1).

**Q: My input bed file contains ~10000 SV, but only ~2000 SV are annotated. Why?**

AnnotSV does not annotate:

- The SNV/indel (size<50bp)
- The SV in a bad format
- The SV for which the "END" is not defined.

AnnotSV creates a report of unannotated variants ("unannotated.tsv" file).

If you want to annotate SNV/indel, please set the -SVminSize to 1.

**Q: How overlaps (%) are calculated?**

AnnotSV provides different types of annotations:

- An annotation with features **overlapping** the SV (DGV, 1000 genomes...):

$$\text{overlap (\%)} = \frac{(\text{length of overlap between the SV to annotate and the feature}) * 100}{(\text{SV to annotate length})}$$

- An annotation with features **overlapped** with the SV (pathogenic SV from dbVar, promoters, enhancers...):

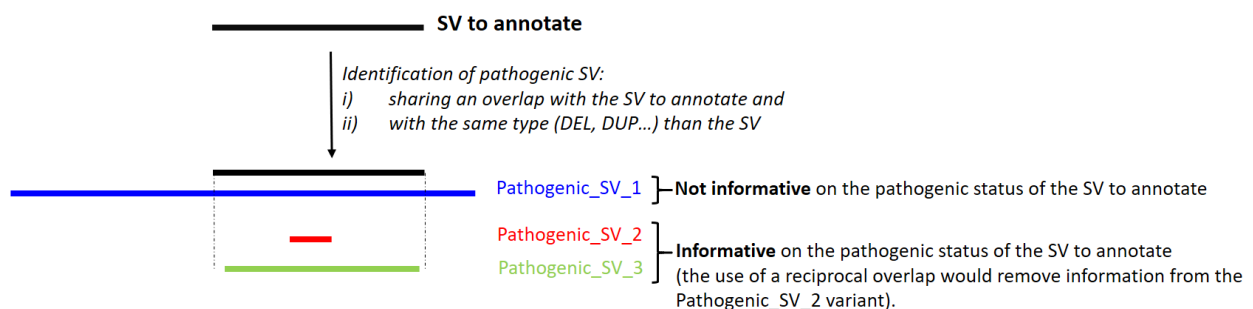
$$\text{overlap (\%)} = \frac{(\text{length of overlap between the SV to annotate and the feature}) * 100}{(\text{feature length})}$$

- A gene-based annotation

Each gene overlapped by the SV to annotate is reported (even with 1bp overlap).

**Q: Why not to use a reciprocal overlap with features overlapped with the SV to annotate?**

Let's take the example of pathogenic SV as features.



=> AnnotSV would lose some information if using a reciprocal overlap.

**Q: Concerning custom annotations, for example with benign genomic regions, where exactly should I put my bed file of interest between the "FtIncludedInSV", "SVincludedInFt" and "AnyOverlap" directories?**

This question refers to the "7 - d) Custom annotations: External BED annotation files (optional)" section of this README.



TheBED file should be placed in the "SVincludedInFt" directory. Indeed, the "benign SV" annotation might only be fully useful if overlapping 100% of your "SV to annotate". Otherwise, if the "SV to annotate" includes additional genomic material (compared to the benign SV), you cannot definitely conclude on its benign/pathogenic status.

**Q: I have a custom BED file with genomic regions and their AF identified from healthy population. How to use it for filtering my SV?**

Your BED file should be placed in the "SVincludedInFt" directory (please see the above question).

There are 2 different methods to use your BED file:

- Either, you can directly annotate your SV with your BED file to get the AF values associated with the overlapped SV. Then you can filter out your SV completely overlapped with a common SV (filtering based on the reported AF and a given threshold)
  - ⇒ This way, you will have to filter your data for each analysis (repetitive). But you keep the possibility to change the threshold (AF > 0.05 (i.e. 5%), AF > 0.01 (i.e. 1%)...) any time.
- Or, you can first filter your bed file to only keep common SV (can be considered as benign) (population AF > 0.01 (i.e. 1%)) and then just remove those SV which get annotated by the AF values.
  - ⇒ This way, you will only need to filter the BED file once. But you will not be able to modify the threshold.

Each method has its own pros and cons. If your threshold is fixed, the second method is more appropriate. Otherwise, use the first one.

**Q: What are the minimal info/headers needed in a VCF input file to run AnnotSV?**

AnnotSV is using the VCF format following official specification [VCF v4.3](#). Nevertheless, some flexibility is allowed:

- No meta-information line (prefixed with "##") is required

But the following is mandatory:

- A header line (prefixed with "#CHROM")

- The following INFO keys are required: GT, SVLEN, END and SVTYPE.

In order to be able to classify the SV, the "SVTYPE" values should be one of DEL, INS, DUP, INV, CNV, BND, LINE1, SVA, ALU. In addition, the <CN0>, <CN2>, <CN3>... angle-bracketed ID from the "ALT" column should be used in case of SVTYPE=CNV in the INFO column.

In order to use the "snvIndelPASS" option (using of the variants only if they passed all filters during the calling), the FILTER column value is mandatory.

**Q: I'm getting the error: "ERROR: chromosome sort ordering for file ... is inconsistent with other files". How can I fix this problem?**

The locale specified by your environment can affect the traditional "sort" order that uses native byte values.

Please, set LC\_ALL=C.

In csh, you can define it with the following command line:

```
setenv LC_ALL C
```

In bash:

```
export LC_ALL=C
```

**Q: I'm getting the error: « unexpected token "END" at position 0; expecting VALUE » while running Exomiser. How can I fix this problem?**

You are facing a memory issue. Please, try increasing RAM/MEM on your compute node.

**Q: I have some concerns about data sharing. Does AnnotSV connect somehow with the web version of Exomiser?**

AnnotSV doesn't connect with the web version of Exomiser.

All necessary Exomiser data (to score a gene by using the HPO terms) is installed locally in the \$ANNOVS/share/annotSV/Annotations\_Exomiser/ directory.

The code for the Exomiser module was extracted directly from Exomiser (thanks to developer Jules Jacobsen). A minimal Java 8 installation is required. Moreover, the Exomiser module writes in the /tmp/spring.log file that must, therefore, have write permissions.

Given the input (a gene name and HPO terms), this module returns a score. All locally.

#### Q: What is knotAnnotSV?

knotAnnotSV is a freely accessible web interface that allows you to explore your annotated SV dataset in a user-friendly way. This interface is well detailed in the "README.knotAnnotSV\_`version`.pdf" file available on Github: <https://github.com/mobidic/knotAnnotSV/>

#### Q: I have annotated a SV VCF file and some "Samples\_ID" are empty. What is happening?

Some VCF might contain multiple samples. Thus, each SV might have been called for only some or all of the samples (indicated with the GT feature). Since AnnotSV annotates all the SV from the input file, the reported "Samples\_ID" output column specifically lists the samples for which the SV was called.

As an example, from this SV VCF input file:

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	sample1	sample2	sample3
1	3085911	.	N	<DEL>	4752.09	PASS	SVTYPE=DEL;END=3095542	GT	0/0	0/1	1/1
5	25635	.	N	<DUP>	4256.36	PASS	SVTYPE=DUP;END=26358	GT	./.	./.	0/0
7	5085911	.	N	<DEL>	3752.09	PASS	SVTYPE=DEL;END=5095542	GT	0/1	0/1	0/0

AnnotSV will report the following "Samples\_ID" column:

AnnotSV_ID	Samples_ID
1_3085911_3095542_DEL_1	sample2,sample3
5_25635_26358_DUP_1	
7_5085911_5095542_DEL_1	sample1,sample2

## 13. REFERENCES

- Abel, H.J., Larson, D.E., Regier, A.A., Chiang, C., Das, I., Kanchi, K.L., Layer, R.M., Neale, B.M., Salerno, W.J., Reeves, C., Buyske, S., NHGRI Centers for Common Disease Genomics, Matise, T.C., Muzny, D.M., Zody, M.C., Lander, E.S., Dutcher, S.K., Stitzel, N.O., Hall, I.M., 2020. Mapping and characterization of structural variation in 17,795 human genomes. *Nature*. <https://doi.org/10.1038/s41586-020-2371-0>
- Collins, R.L., Brand, H., Karczewski, K.J., Zhao, X., Alföldi, J., Francioli, L.C., Khera, A.V., Lowther, C., Gauthier, L.D., Wang, H., Watts, N.A., Solomonson, M., O'Donnell-Luria, A., Baumann, A., Munshi, R., Walker, M., Whelan, C.W., Huang, Y., Brookings, T., Sharpe, T., Stone, M.R., Valkanas, E., Fu, J., Tiao, G., Laricchia, K.M., Ruano-Rubio, V., Stevens, C., Gupta, N., Cusick, C., Margolin, L., Taylor, K.D., Lin, H.J., Rich, S.S., Post, W.S., Chen, Y.-D.I., Rotter, J.I., Nusbaum, C., Philippakis, A., Lander, E., Gabriel, S., Neale, B.M., Kathiresan, S., Daly, M.J., Banks, E., MacArthur, D.G., Talkowski, M.E., 2020. A structural variation reference for medical and population genetics. *Nature* 581, 444–451. <https://doi.org/10.1038/s41586-020-2287-8>
- DiStefano, M.T., Goehringer, S., Babb, L., Alkuraya, F.S., Amberger, J., Amin, M., Austin-Tse, C., Balzotti, M., Berg, J.S., Birney, E., Bocchini, C., Bruford, E.A., Coffey, A.J., Collins, H., Cunningham, F., Daugherty, L.C., Einhorn, Y., Firth, H.V., Fitzpatrick, D.R., Foulger, R.E., Goldstein, J., Hamosh, A., Hurles, M.R., Leigh, S.E., Leong, I.U.S., Maddirevula, S., Martin, C.L., McDonagh, E.M., Olry, A., Puziakova, A., Radtke, K., Ramos, E.M., Rath, A., Riggs, E.R., Roberts, A.M., Rodwell, C., Snow, C., Stark, Z., Tahiliani, J., Tweedie, S., Ware, J.S., Weller, P., Williams, E., Wright, C.F., Yates, T.M., Rehm, H.L., 2022. The Gene Curation Coalition: A global effort to harmonize gene-disease evidence resources. *Genet Med* 24, 1732–1742. <https://doi.org/10.1016/j.gim.2022.04.017>
- Dittwald, P., Gambin, T., Szafranski, P., Li, J., Amato, S., Divon, M.Y., Rodríguez Rojas, L.X., Elton, L.E., Scott, D.A., Schaaf, C.P., Torres-Martinez, W., Stevens, A.K., Rosenfeld, J.A., Agadi, S., Francis, D., Kang, S.-H.L., Breman, A., Lalani, S.R., Bacino, C.A., Bi, W., Milosavljevic, A., Beaudet, A.L., Patel, A., Shaw, C.A., Lupski, J.R., Gambin, A., Cheung,

- S.W., Stankiewicz, P., 2013. NAHR-mediated copy-number variants in a clinical population: mechanistic insights into both genomic disorders and Mendelizing traits. *Genome Res.* 23, 1395–1409. <https://doi.org/10.1101/gr.152454.112>
- Firth, H.V., Richards, S.M., Bevan, A.P., Clayton, S., Corpas, M., Rajan, D., Vooren, S.V., Moreau, Y., Pettett, R.M., Carter, N.P., 2009. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. *The American Journal of Human Genetics* 84, 524–533. <https://doi.org/10.1016/j.ajhg.2009.03.010>
- Firth, H.V., Wright, C.F., DDD Study, 2011. The Deciphering Developmental Disorders (DDD) study. *Dev Med Child Neurol* 53, 702–703. <https://doi.org/10.1111/j.1469-8749.2011.04032.x>
- Fishilevich, S., Nudel, R., Rappaport, N., Hadar, R., Plaschkes, I., Iny Stein, T., Rosen, N., Kohn, A., Twik, M., Safran, M., Lancet, D., Cohen, D., 2017. GeneHancer: genome-wide integration of enhancers and target genes in GeneCards. Database (Oxford) 2017. <https://doi.org/10.1093/database/bax028>
- Gao, T., Qian, J., 2020. EnhancerAtlas 2.0: an updated resource with enhancer annotation in 586 tissue/cell types across nine species. *Nucleic Acids Research* 48, D58–D64. <https://doi.org/10.1093/nar/gkab980>
- Geoffroy, V., Guignard, T., Kress, A., Gaillard, J.-B., Solli-Nowlan, T., Schalk, A., Gatinois, V., Dollfus, H., Scheidecker, S., Muller, J., 2021. AnnotSV and knotAnnotSV: a web server for human structural variations annotations, ranking and analysis. *Nucleic Acids Research* 49, W21–W28. <https://doi.org/10.1093/nar/gkab402>
- Geoffroy, V., Herenger, Y., Kress, A., Stoetzel, C., Piton, A., Dollfus, H., Muller, J., 2018. AnnotSV: an integrated tool for structural variations annotation. *Bioinformatics* 34, 3572–3574. <https://doi.org/10.1093/bioinformatics/bty304>
- Hamosh, A., Scott, A.F., Amberger, J., Valle, D., McKusick, V.A., 2000. Online Mendelian Inheritance in Man (OMIM). *Hum. Mutat.* 15, 57–61. [https://doi.org/10.1002/\(SICI\)1098-1004\(200001\)15:1<57::AID-HUMU12>3.0.CO;2-G](https://doi.org/10.1002/(SICI)1098-1004(200001)15:1<57::AID-HUMU12>3.0.CO;2-G)
- Kern, F., Aparicio-Puerta, E., Li, Y., Fehlmann, T., Kehl, T., Wagner, V., Ray, K., Ludwig, N., Lenhof, H.-P., Meese, E., Keller, A., 2021. miRTargetLink 2.0—interactive miRNA target gene and target pathway networks. *Nucleic Acids Research* 49, W409–W416. <https://doi.org/10.1093/nar/gkab297>
- Köhler, S., Carmody, L., Vasilevsky, N., Jacobsen, J.O.B., Danis, D., Gouridine, J.-P., Gargano, M., Harris, N.L., Matentzoglou, N., McMurry, J.A., Osumi-Sutherland, D., Cipriani, V., Balhoff, J.P., Conlin, T., Blau, H., Baynam, G., Palmer, R., Gratian, D., Dawkins, H., Segal, M., Jansen, A.C., Muaz, A., Chang, W.H., Bergerson, J., Laulederkind, S.J.F., Yüksel, Z., Beltran, S., Freeman, A.F., Sergouniotis, P.I., Durkin, D., Storm, A.L., Hanauer, M., Brudno, M., Bello, S.M., Sincan, M., Rageth, K., Wheeler, M.T., Oegema, R., Loughi, H., Della Rocca, M.G., Thompson, R., Castellanos, F., Priest, J., Cunningham-Rundles, C., Hegde, A., Lovering, R.C., Hajek, C., Olry, A., Notarangelo, L., Similuk, M., Zhang, X.A., Gómez-Andrés, D., Lochmüller, H., Dollfus, H., Rosenzweig, S., Marwaha, S., Rath, A., Sullivan, K., Smith, C., Milner, J.D., Leroux, D., Boerkoel, C.F., Klion, A., Carter, M.C., Groza, T., Smedley, D., Haendel, M.A., Mungall, C., Robinson, P.N., 2019. Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources. *Nucleic Acids Res.* 47, D1018–D1027. <https://doi.org/10.1093/nar/gky1105>
- Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B., Tukiainen, T., Birnbaum, D.P., Kosmicki, J.A., Duncan, L.E., Estrada, K., Zhao, F., Zou, J., Pierce-Hoffman, E., Berghout, J., Cooper, D.N., Deflaux, N., DePristo, M., Do, R., Flannick, J., Fromer, M., Gauthier, L., Goldstein, J., Gupta, N., Howrigan, D., Kiezun, A., Kurki, M.I., Moonshine, A.L., Natarajan, P., Orozco, L., Peloso, G.M., Poplin, R., Rivas, M.A., Ruano-Rubio, V., Rose, S.A., Ruderfer, D.M., Shakir, K., Stenson, P.D., Stevens, C., Thomas, B.P., Tiao, G., Tusie-Luna, M.T., Weisburd, B., Won, H.-H., Yu, D., Altshuler, D.M., Ardissino, D., Boehnke, M., Danesh, J., Donnelly, S., Elosua, R., Florez, J.C., Gabriel, S.B., Getz, G., Glatt, S.J., Hultman, C.M., Kathiresan, S., Laakso, M., McCarroll, S., McCarthy, M.I., McGovern, D., McPherson, R., Neale, B.M., Palotie, A., Purcell, S.M., Saleheen, D., Scharf, J.M., Sklar, P., Sullivan, P.F., Tuomilehto, J., Tsuang, M.T., Watkins, H.C., Wilson, J.G., Daly, M.J., MacArthur, D.G., Exome Aggregation Consortium, 2016. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536, 285–291. <https://doi.org/10.1038/nature19057>
- Li, H., 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 27, 2987–2993. <https://doi.org/10.1093/bioinformatics/btr509>
- Lupiáñez, D.G., Spielmann, M., Mundlos, S., 2016. Breaking TADs: How Alterations of Chromatin Domains Result in Disease. *Trends Genet.* 32, 225–237. <https://doi.org/10.1016/j.tig.2016.01.003>
- MacDonald, J.R., Ziman, R., Yuen, R.K.C., Feuk, L., Scherer, S.W., 2014. The Database of Genomic Variants: a curated collection of structural variation in the human genome. *Nucleic Acids Res.* 42, D986–992. <https://doi.org/10.1093/nar/gkt958>
- Miller, D.T., Lee, K., Abul-Husn, N.S., Amendola, L.M., Brothers, K., Chung, W.K., Gollob, M.H., Gordon, A.S., Harrison, S.M., Hershberger, R.E., Klein, T.E., Richards, C.S., Stewart, D.R., Martin, C.L., 2022. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine* 24, 1407–1414. <https://doi.org/10.1016/j.gim.2022.04.006>

- Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H.L., ACMG Laboratory Quality Assurance Committee, 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* 17, 405–424. <https://doi.org/10.1038/gim.2015.30>
- Riggs, E.R., Andersen, E.F., Cherry, A.M., Kantarci, S., Kearney, H., Patel, A., Raca, G., Ritter, D.I., South, S.T., Thorland, E.C., Pineda-Alvarez, D., Aradhya, S., Martin, C.L., 2020. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genetics in Medicine* 22, 245–257. <https://doi.org/10.1038/s41436-019-0686-8>
- Smedley, D., Jacobsen, J.O.B., Jäger, M., Köhler, S., Holtgrewe, M., Schubach, M., Siragusa, E., Zemojtel, T., Buske, O.J., Washington, N.L., Bone, W.P., Haendel, M.A., Robinson, P.N., 2015. Next-generation diagnostics and disease-gene discovery with the Exomiser. *Nat Protoc* 10, 2004–2015. <https://doi.org/10.1038/nprot.2015.124>
- Sudmant, P.H., Rausch, T., Gardner, E.J., Handsaker, R.E., Abyzov, A., Huddleston, J., Zhang, Y., Ye, K., Jun, G., Fritz, M.H.-Y., Konkel, M.K., Malhotra, A., Stütz, A.M., Shi, X., Casale, F.P., Chen, J., Hormozdiari, F., Dayama, G., Chen, K., Malig, M., Chaisson, M.J.P., Walter, K., Meiers, S., Kashin, S., Garrison, E., Auton, A., Lam, H.Y.K., Mu, X.J., Alkan, C., Antaki, D., Bae, T., Cerveira, E., Chines, P., Chong, Z., Clarke, L., Dal, E., Ding, L., Emery, S., Fan, X., Gujral, M., Kahveci, F., Kidd, J.M., Kong, Y., Lammeijer, E.-W., McCarthy, S., Flicek, P., Gibbs, R.A., Marth, G., Mason, C.E., Menelaou, A., Muzny, D.M., Nelson, B.J., Noor, A., Parrish, N.F., Pendleton, M., Quitadamo, A., Raeder, B., Schadt, E.E., Romanovitch, M., Schlattl, A., Sebra, R., Shabalín, A.A., Untergasser, A., Walker, J.A., Wang, M., Yu, F., Zhang, C., Zhang, J., Zheng-Bradley, X., Zhou, W., Zichner, T., Sebat, J., Batzer, M.A., McCarroll, S.A., 1000 Genomes Project Consortium, Mills, R.E., Gerstein, M.B., Bashir, A., Stegle, O., Devine, S.E., Lee, C., Eichler, E.E., Korbel, J.O., 2015. An integrated map of structural variation in 2,504 human genomes. *Nature* 526, 75–81. <https://doi.org/10.1038/nature15394>
- Tate, J.G., Bamford, S., Jubb, H.C., Sondka, Z., Beare, D.M., Bindal, N., Boutselakis, H., Cole, C.G., Creatore, C., Dawson, E., Fish, P., Harsha, B., Hathaway, C., Jupe, S.C., Kok, C.Y., Noble, K., Ponting, L., Ramshaw, C.C., Rye, C.E., Speedy, H.E., Stefancsik, R., Thompson, S.L., Wang, S., Ward, S., Campbell, P.J., Forbes, S.A., 2019. COSMIC: the Catalogue Of Somatic Mutations In Cancer. *Nucleic Acids Res* 47, D941–D947. <https://doi.org/10.1093/nar/gky1015>