PriorR 3.0 User Guide

PriorR is a filtering and prioritization program of disease-linked genetic variants developed within the Genetics&Genomics Department of La Fundacion Jimenez Diaz University Hospital. PriorR performs annotation of VCFs and subsequent variant visualization in a GUI, where different filters and functions can be applied to perform the analysis. This program offers a number of useful functionalities for variant analysis such as: variant and gene prioritization, filtering by a virtual panel of genes. manual control of different population frequencies or pathogenicity predictors or filtering out variants that have been already found by another protocol.

SYSTEM REQUIREMENTS

PriorR requires the following system specifications:

- ➔ Operating system: Windows (64 bit processor), macOS (version 11 or newer), Linux (64 bit kernel).
- ➔ 8 GB RAM.
- ➔ 120 GB hard drive space for annotation resource download.

INSTALL PRIORR

PriorR is distributed in a Docker image.
Download Docker in https://docs.docker.com/get-docker/ for your operating system and follow the installation instructions.
Go to https://hub.docker.com/repository/docker/tblabfjd/priorr/general
Docker pull our docker image:

docker pull tblabfjd/priorr

Create the Session volume and copy the program data into it (only the first time).

docker volume create session

Download annotation files (only first time), specifying the assembly to use: GRCh37, GRCh38 or both. Specify the folder where the annotation files are to be downloaded and mount it as volume. In the following command, replace path_to_local_annotation_dir with the path to the said folder.

docker run -v path_to_local_annotation_dir:/home/app/resources -u $(id -u):$(id -g) tblabfjd/priorr bash /home/app/download_annotation.sh /home/app/resources [GRCh37 | GRCh38 | both]

Run the image

docker run -d -v session:/home tblabfjd/priorr

docker run -p 8888:8888 -it --mount source=session,destination=/session -v path_to_local_annotation_dir:/home/app/resouces tblabfjd/priorr

INPUT REQUIREMENTS

SNVs/INDELs

For SNPs and INDELs annotation PriorR requires variants reported in VCF v4.0, or later, file formats. For SNPs and INDELs analysis PriorR requires a tab separated file along with a config file specifying the equivalence between columns.

Structural Variants

PriorR does not perform annotation of structural variants. PriorR imports an annotated variant table from AnnotSV v3.0.

PRIORR GUI

I.   Annotation module.

Landing page: PriorR is initialised with a landing page (fig. 1) where users can choose whether to annotate a VCF file or to go straight to the analysis module, if variants have already been annotated.
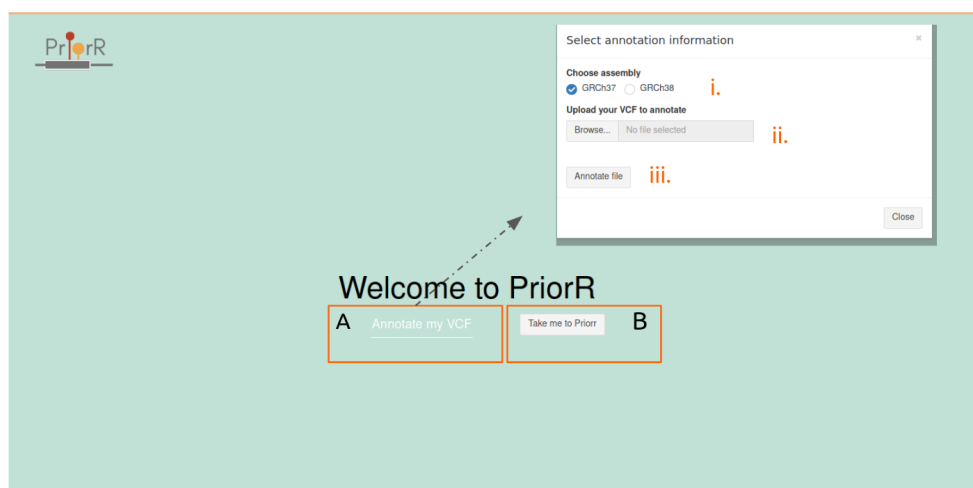
Figure 1. Annotation module of PriorR.

A. Annotate my VCF: If the option "annotate my VCF" is chosen a new window pops up where you can select in what assembly to annotate (i), upload your vcf file for annotation (ii) and launch the annotation (iii).

B. Go to PriorR: if the option "take me to PriorR" is chosen, the user is taken to the main GUI of PriorR.

II. Analysis module.

PriorR's main Interface:

A. User login.
B. Analysis tabs (SNV/CNV). The user can select what analysis to carry out, whether SNVs and INDELs or SVs.
C. Filters and functions pane. Provides options for filtering and transforming data using any combination of filters.
D. Table data. Displays variant table.
E. Variant pane. Displays a variant information summary.
F. Session table.

Figure 2: Main interface of PriorR (SNVs&INDELs)

Figure 3: Main interface of PriorR (SVs).

A. User login.

Users can login into PriorR using a predefined username. The login allows users to save sessions and keep their name when cataloguing a variant.


B. Analysis tabs (SNVs&INDELs/SVs).

Users can select what analysis to carry out whether SNV and INDELs or CNVs. Filters and functions are different for both options.

C. Filters and functions pane.

SNVs&INDELs

Upload analysis data. SNV files from WES or gene panels can be uploaded straight into PriorR, however if a WGS file is to be analysed, the user must select the option a "Analysis of WGS", which pops up a new windows in which the user can select how to pre-filter the file in order to speed up calculations.
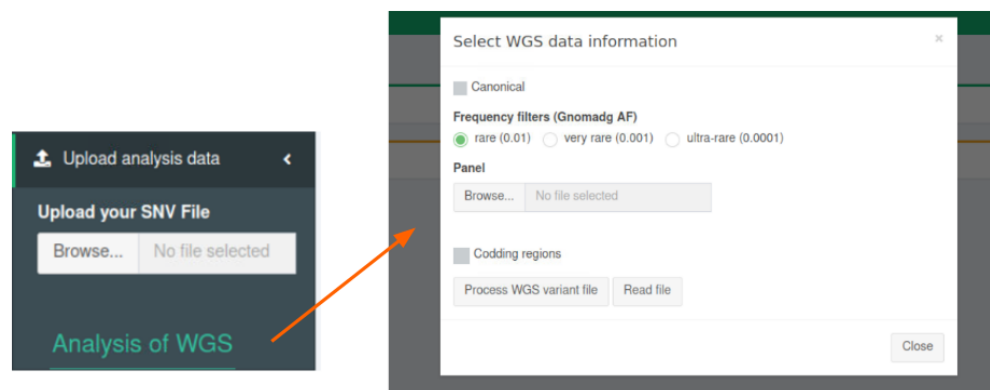

Columns and variant filters, frequency filters, pathogenicity and phenotype. Columns to display in the variant table can be selected from the menu. There is also a variety of variant filters such as variant type, consequence or region, or upload a region file to filter by those regions. Filtering by population frequency is also implemented. Furthermore, users can filter by the pathogenicity predictor CADD and also they can filter out those variants classified as 'benign' in Clinvar. Finally, the variant table can be filtered by HPO terms.
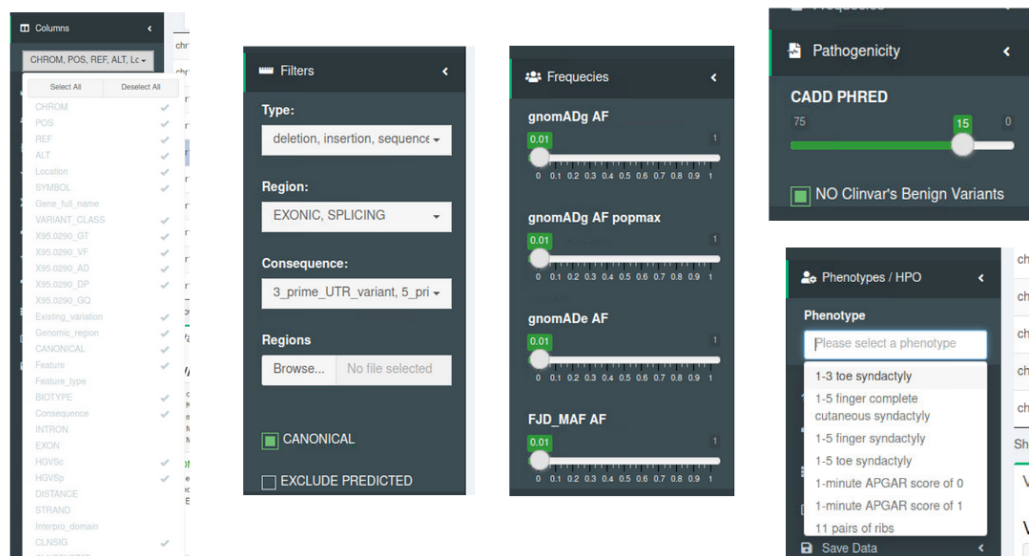
Inheritance filter: A filter by inheritance is also available within PriorR. If the filter is to be used on a family study, the user must upload a pedigree file.
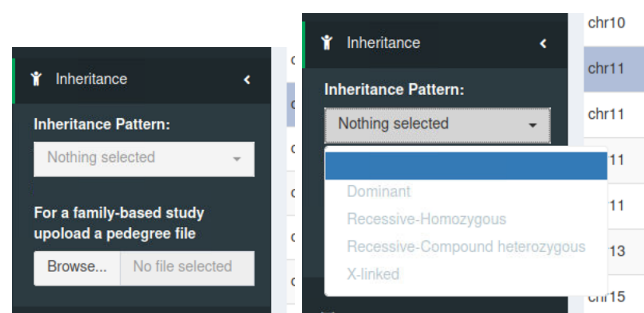
Gene filter: Variant table can be filtered by one gene or a virtual panel of genes, variants in the virtual panel can also be filtered out if the exclude panel of genes is chosen. PriorR also offers the user a number of virtual panels from Orphanet, by selecting one of those panels the table is prioritised according to GlowGenes score, which might also be modulated with a slide.
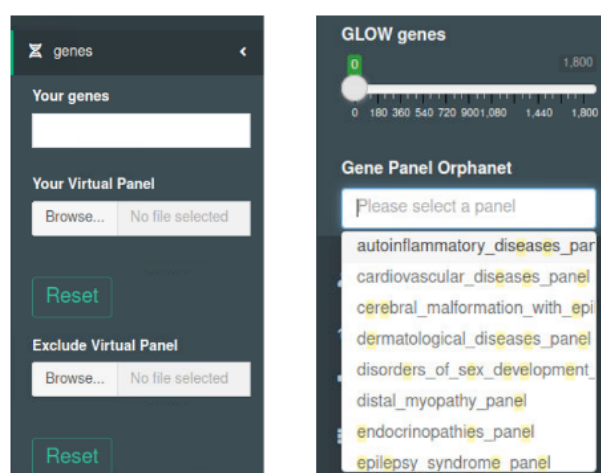
Regions of homozygosity (ROH). If users select the option ROH, variants that lie in ROH are coloured in salmon. This option is also available for family studies, users can upload a pedigree file and then variants that are in all affected individuals and lie in a ROH are coloured.

Filter by another vcf: PriorR has the option of filtering out all variants that have been already found in another vcf of the same sample.

Classification of variants: variants classified by users in the variant database (explained in section D) are annotated in the current table. When variant classification is selected three new columns appear at the end of the table ('Classification', 'comments' and 'User') that are filled for those variants that are in the database.

Prioritization: Pathogenic variants are coloured to red and likely pathogenic variants are coloured to orange if the option 'colouring' is selected. If the option 'prioritization' is also selected, variants will be prioritised by pathogenicity.
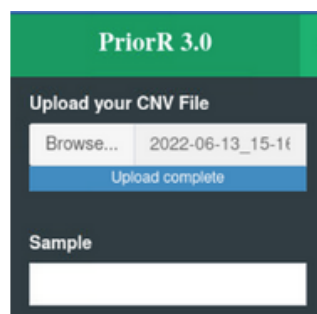

Download results: Users can download the filtered table as an excel file.

SVs

Filter and function pane in SV analysis is similar to the SNVs&INDELs analysis, with some exceptions.

Filter by sample: as most CNV callers are based on read depth and need to use references, a lot of SV files are multisample. PriorR offers the option of filtering the file by sample. The user must specify in the test box the sample.

General filters: General filters include annotation mode, "full" where the annotation by SV is shown or "split" where the annotation by each gene in the SV is displayed, type of SV, region where the SV lies or ACMG class catalogued by the annotation program AnnotSV.

D. Data table.

Variant table: Variant table is displayed in the body of the GUI after the user uploads the variant file. The number of variants to display may be modulated by the user. The table can also be filtered by each column using the headers.


Variant cataloguing: Users can catalogue the pathogenicity of the variants and make some comments on the variants by pressing the classification button in the final column of the table. When the button is pressed a new column pops up where the user can fill two fields: pathogenicity and comments.
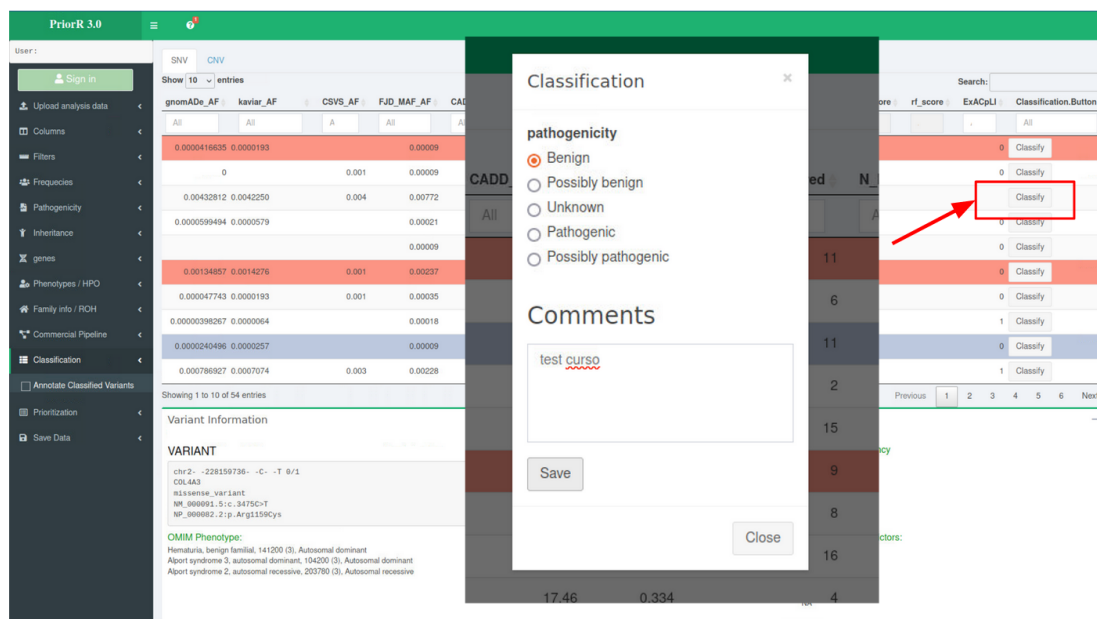
E. Variant pane

The variant pane is displayed in the body of the GUI right below the variant table. It shows a variant summary that includes information about the variant (chromosome, position, genotype, consequence or HGVS notation), associated phenotypes, population frequency and score of pathogenicity predictors. Two plots are also displayed: a density plot showing the depth of read in the variants and a doughnut plot that shows the number of pathogenicity predictors that predict the variant as benign, the number of predictors that predict it as pathogenic.
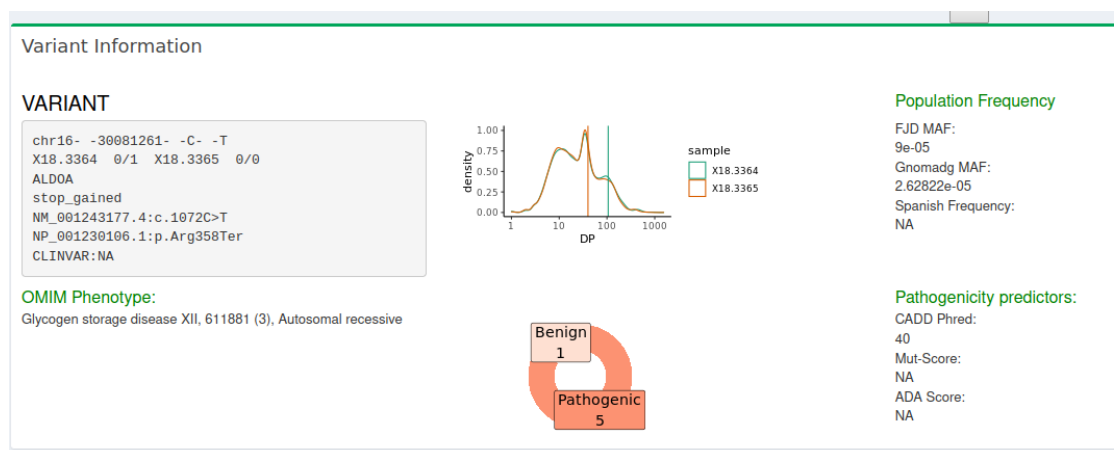
F. Session table

The session table is displayed in the body of the GUI right below the variant pane. When users press the button 'bookmark' all data of the session are stored and an URL link is generated, users may resume the session just by pressing the link generated.