

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 tlabfjd/priorr
```

```
docker run -p 8888:8888 -it --mount source=session,destination=/session -v path_to_local_annotation_dir:/home/app/resouces tlabfjd/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.

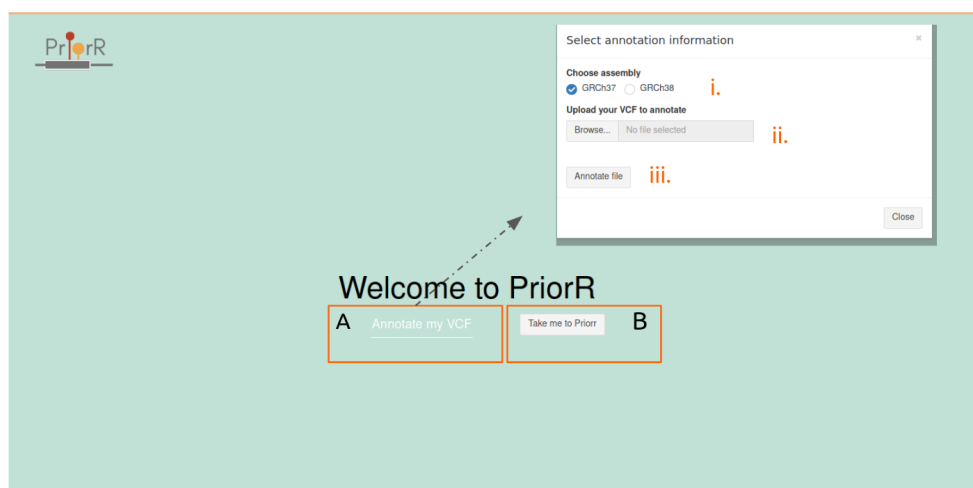


Figure 1. Annotation module of PriorR.

- 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:

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

The screenshot shows the PriorR 3.0 main interface. The top bar is green with the user name 'User: raquel'. Below it, there's a navigation bar with tabs for 'SNV' and 'CNV'. The left sidebar (C) contains various filters and functions like 'Upload analysis data', 'Columns', 'Filters', 'Frequencies', 'Pathogenicity', 'Inheritance', 'genes', 'Phenotypes / HPO', 'Family info / ROH', 'Commercial Pipeline', 'Classification', 'Prioritization', 'COLORING', 'PRIORITIZATION', and 'Save Data'. The main area (D) displays a table of variants with columns: CHROM, POS, REF, and a search bar. The table shows variants for chr13, chr16, chr2, chr10, chr8, chr19, chr6, and chr14. The variant pane (E) shows detailed information for a specific variant, including its coordinates, gene, and various frequency metrics. The session table (F) is at the bottom.

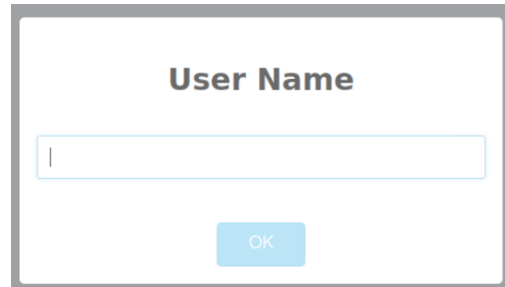
Figure 2: Main interface of PriorR (SNVs&INDELs)

The screenshot shows the PriorR 3.0 main interface for SVs. The top bar is green with the user name 'User: raquel'. Below it, there's a navigation bar with tabs for 'SNV' and 'CNV'. The left sidebar (C) contains various filters and functions like 'Upload analysis data', 'Columns', 'Filters', 'Frequencies', 'Pathogenicity', 'Inheritance', 'genes', 'Phenotypes / HPO', 'Family info / ROH', 'Commercial Pipeline', 'Classification', 'Prioritization', 'COLORING', 'PRIORITIZATION', and 'Save Data'. The main area (D) displays a table of variants with columns: ACMG_class, Samples_ID, SV_chrom, SV_start, SV_end, SV_length, SV_type, N_PROGRAMS, Annotation_mode, Gene_name, Gene_count, Tx, Frameshift, and Exon. The table shows variants for chr13, chr16, chr2, chr10, chr8, chr19, chr6, and chr14. The variant pane (E) shows detailed information for a specific variant, including its coordinates, gene, and various frequency metrics. The session table (F) is at the bottom.

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.

A simple login form with a title "User Name" in bold. Below the title is a text input field with a cursor inside. At the bottom of the form is a blue button labeled "OK".

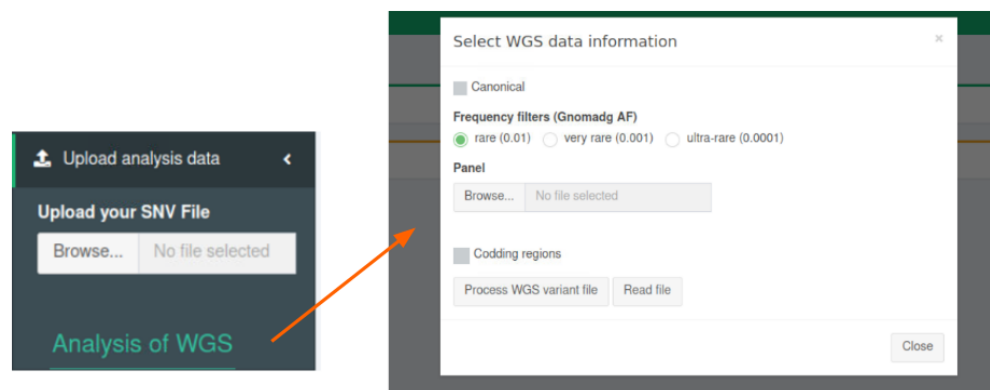
B. Analysis tabs (SNVs&INDELs/SVs).

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

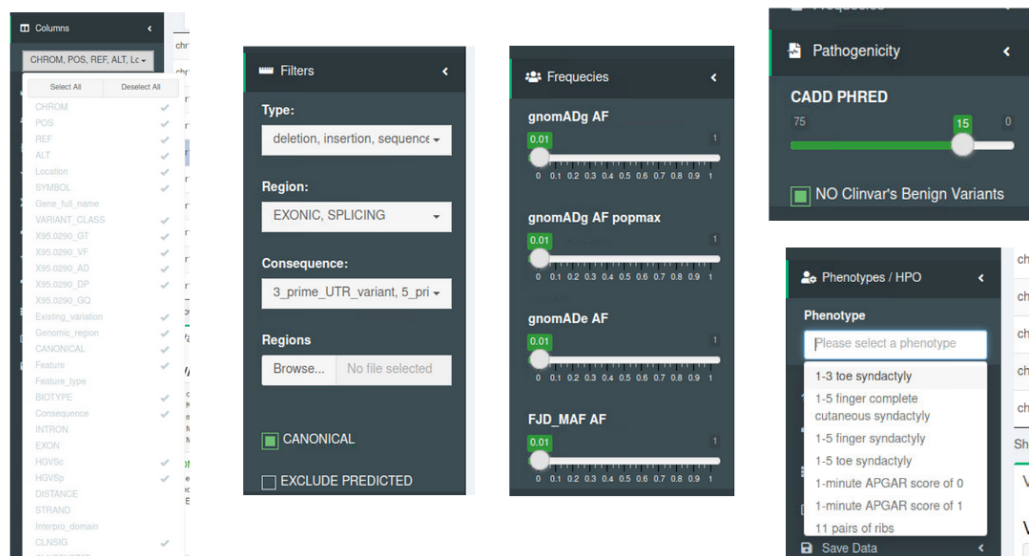
C. Filters and functions pane.

SNVs&INDELs

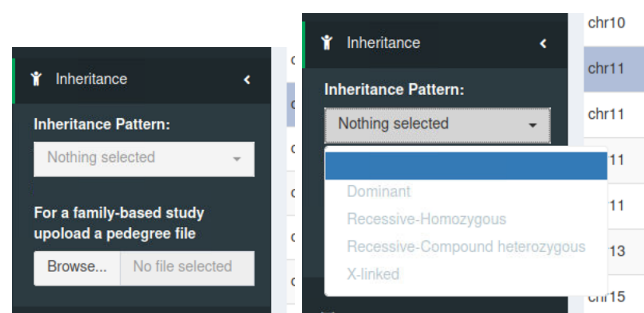
Upload analysis data. SNV files from WES or gene panels can be uploaded straight into PriorR, however if a WGS file is to be analysed, the user must select the option a "Analysis of WGS", which pops up a new windows in which the user can select how to pre-filter the file in order to speed up calculations.



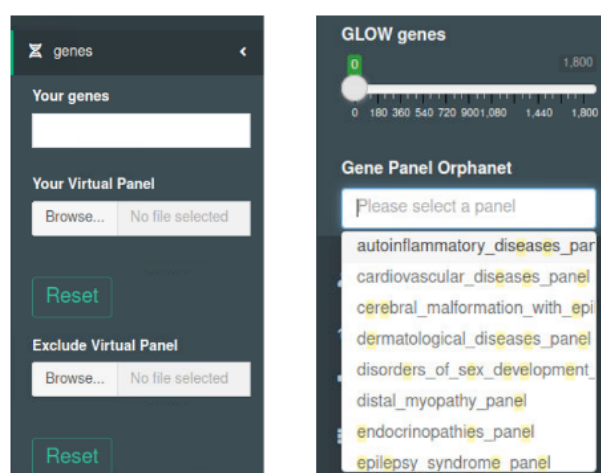
Columns and variant filters, frequency filters, pathogenicity and phenotype. Columns to display in the variant table can be selected from the menu. There is also a variety of variant filters such as variant type, consequence or region, or upload a region file to filter by those regions. Filtering by 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.

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User:

[Sign in](#)

[Upload analysis data](#)

[Columns](#)

[Filters](#)

[Frequencies](#)

[Pathogenicity](#)

[Inheritance](#)

[genes](#)

[Phenotypes / HPO](#)

[Family info / ROH](#)

Probands

Pedigree File

No file selected

☒ ROH

SNV CNV

Show 10 entries

CHROM	POS	REF	ALT	Location	SYMBOL	VARIANT_CLASS	X00.1064_GT
chr11	76905514	C	T	chr11:76905514	MYO7A	SNV	0/1
chr12	88496742	T	C	chr12:88496742	CEP290	SNV	0/1
chr5	89979568	G	A	chr5:89979568	ADGRV1	SNV	0/1
chrX	108708489	C	T	chrX:108708489	GUCY2F	SNV	1/1
chr7	39247082	T	C	chr7:39247082	POU6F2	SNV	0/1
chr14	6451861	C	T	chr14:6451861	SYNE2	SNV	0/1
chr9	2651922	G	A	chr9:2651922	VLDLR	SNV	0/1
chr1	94953334	C	T	chr1:94953334	ABCD3	SNV	0/1
chr2	228159736	C	T	chr2:228159736	COL4A3	SNV	0/1
chr1	220324715	T	C	chr1:220324715	RAB3GAP2	SNV	0/1

Showing 1 to 10 of 54 entries

Variant Information

VARIANT

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.

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User:

[Sign in](#)

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[Phenotypes / HPO](#)

[Family info / ROH](#)

[Commercial Pipeline](#)

☒ Classification

☐ Annotate Classified Variants

[Prioritization](#)

[Save Data](#)

Search:

nic_pred	N_Benign_pred	N_predictions	ada_score	rf_score	ExACpLI	CLASSIFICATION	COMMENTS	USER	Classification.Button
11	10	21			0				Classify
6	13	19			0				Classify
11	9	20			0	Benign	test curso	raquel	Classify
2	16	18			0				Classify
15	6	21			0				Classify
9	12	21			0				Classify
8	11	19			0				Classify
16	3	19			1				Classify
4	15	19			0				Classify
3	16	19			1				Classify

Previous 1 2 3 4 5 6 Next

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.



CHROM	POS	REF	ALT
chr2	27884248	C	T
chr2	3148371	G	A
chr20	10625853	ACTG	
chr22	4016857	G	A
chr3	7578046	C	T
chr3	11943233	C	T
chr4	5784542	A	C
chr5	14358810	A	T
chr5	33207314	C	T
chr6	162864358	T	

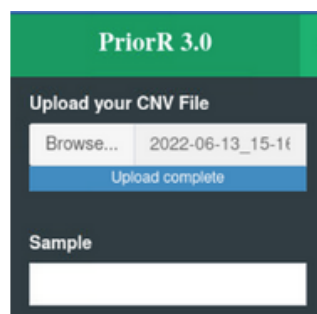
Showing 11 to 20 of 446 entries

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.



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Upload your CNV File

Browse... 2022-06-13_15-16

Upload complete

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.

Filters

Annotation Mode:

full

Type:

DEL, DUP

Region:

3'UTR, 5'UTR, 5'UTR-3'UTR

Number of programs

123

ACMG class:

531

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.

Show 10 entries

Search:

CHROM	POS	REF	ALT	Location	SYMBOL	VARIANT_CLASS	X95.0290_GT	X95.0290_VF	X95.0290_AD	X95.0290_DP	Existing_variation	
chr1	109805791	A	T	chr1:109805791	CELSR2	SNV	0/1	0.39 58_37		95	rs139406620,COSV99605153	E
chr1	186050402	A	G	chr1:186050402	HMCN1	SNV	0/1	0.35 15_8		23	rs141564494	E
chr1	237870323	G	A	chr1:237870323	RYR2	SNV	0/1	0.31 33_15		48	rs371147744,CM1411479,COSV63687970	E
chr10	112838337	G	A	chr10:112838337	ADRA2A	SNV	0/1	0.5 103_104		207	rs200592713,COSV54528144	E
chr11	47333332	C	T	chr11:47333332	MADD	SNV	0/1	0.25 55_18		73	rs759842048,COSV100212037	E
chr11	118896035	C	G	chr11:118896035	SLC37A4	SNV	0/1	0.31 18_8		26	rs782282206	E
chr11	121000636	A	G	chr11:121000636	TBC1E10	SNV	0/1	0.46 38_33		71	rs146175803,CM115245,COSV50713002	E
chr11	121000636	A	G	chr11:121000636	TECTA	SNV	0/1	0.46 38_33		71	rs146175803,CM115245,COSV50713002	E
chr13	32953896	G	A	chr13:32953896	BRCA2	SNV	0/1	0.42 7_5		12	rs778052683,COSV66464073	E
chr15	81225763	G	A	chr15:81225763	CEMP1	SNV	0/1	0.28 13_5		18	rs750368243,COSV99586282	E

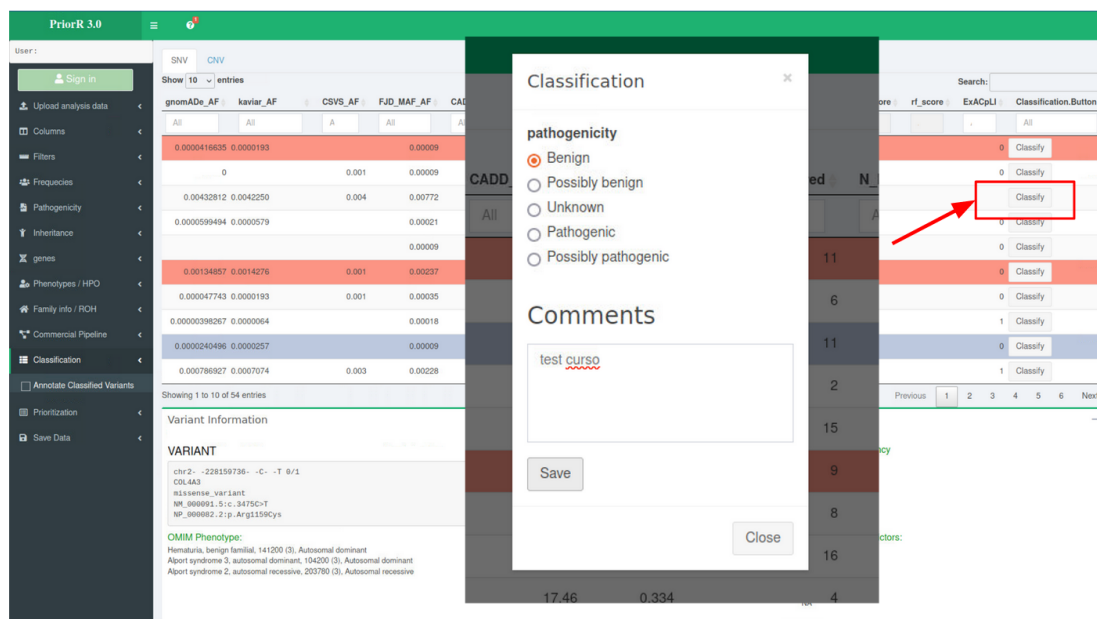
Showing 1 to 10 of 52 entries

Previous

123456

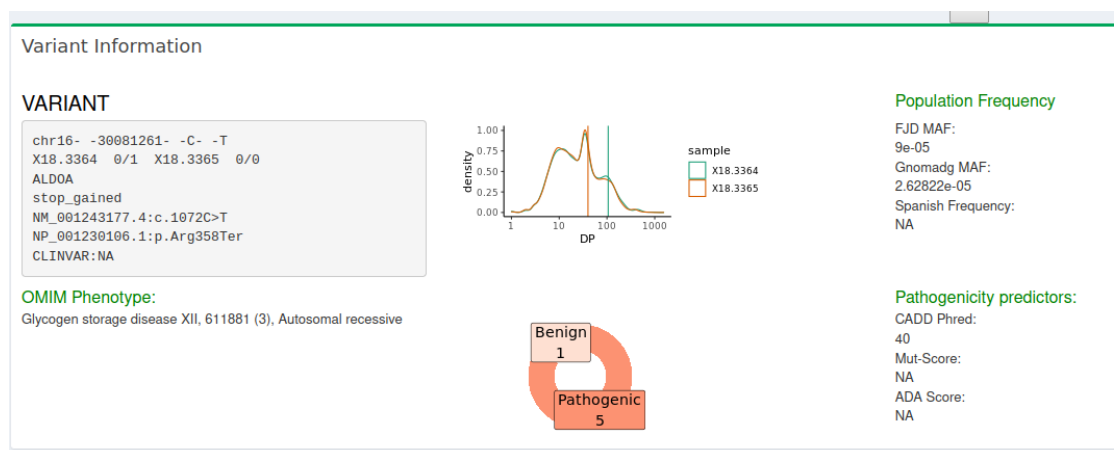
Next

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.

Saved Sessions

Bookmark description

Bookmark...

Show 10 entries

Search:

Description	URL	Timestamp	User
1	http://0.0.0.0:8888/?_state_id_=6598945f07af8512	2023-02-09T15:56:16Z	root

Showing 1 to 1 of 1 entries

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