Exome sequencing data analysis for diagnosing a genetic disease

Galaxy Training! tutorial

https://training.galaxyproject.org/training-material/topics/variant-analysis/tutorials/exome-seq/tutorial.html

Galaxy trainings!

- Calling variants on diploid organism :

  https://training.galaxyproject.org/training-material/topics/variant-analysis/tutorials/dip/tutorial.html

- Calling variants on non diploid system :

  https://training.galaxyproject.org/training-material/topics/variant-analysis/tutorials/non-dip/tutorial.html

- Microbial variants calling :

  https://training.galaxyproject.org/training-material/topics/variant-analysis/tutorials/microbial-variants/tutorial.html

- Genome annotations (eukaryotes, prokaryotes, other):

  https://training.galaxyproject.org/training-material/topics/genome-annotation/

Tutorial presentation

- Exome sequencing data from a family trio
- Boy child affected by a disease : osteopetrosis
- Parents unaffected but consanguineous

Goal : Identify the genetic variation responsible for the disease

Tutorial steps

1. Perform postprocessing from premapped reads

2. Variant calling

3. Variant annotation and reporting

Tutorial steps

1. Perform postprocessing from premapped reads

2. Variant calling

3. Variant annotation and reporting

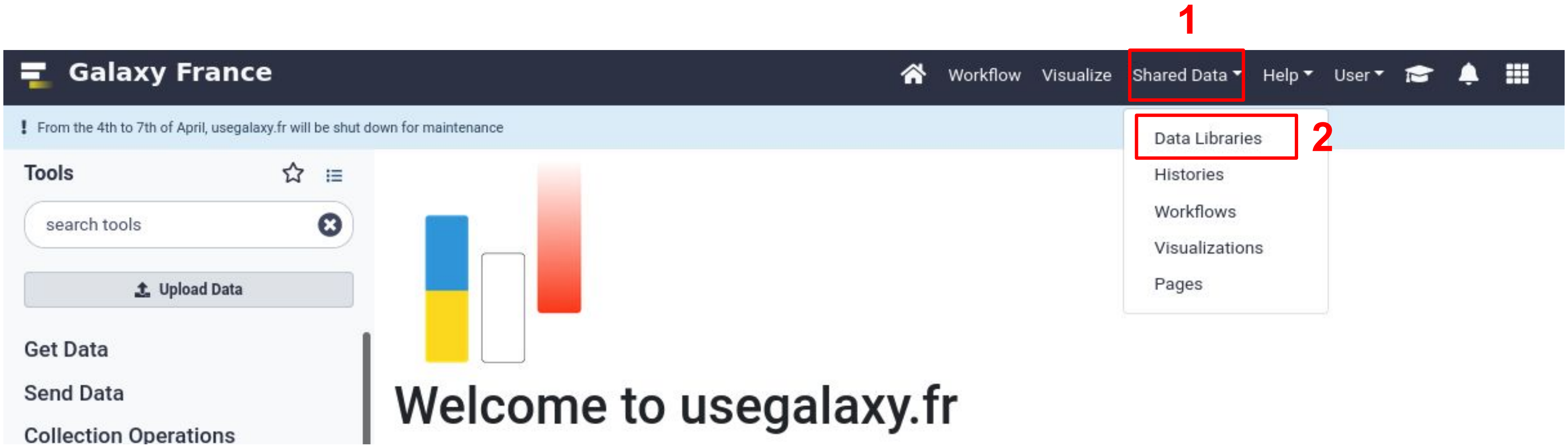
Premapped reads

- Data characteristics for the trio :
  - Whole exome sequencing
  - Paired-end reads

- Steps already performed :
  - Quality control (fastq)
  - Read mapping (Human Hg19 assembly)

- Format available : bam format

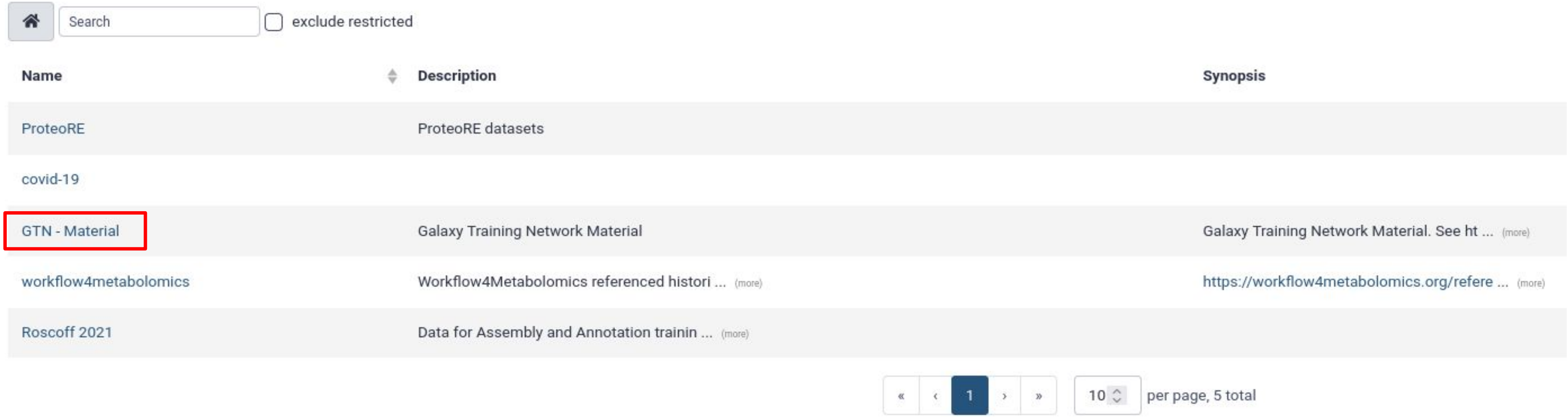
Premapped reads upload



From the 4th to 7th of April, usegalaxy.fr will be shut down for maintenance

Welcome to usegalaxy.fr

Premapped reads upload

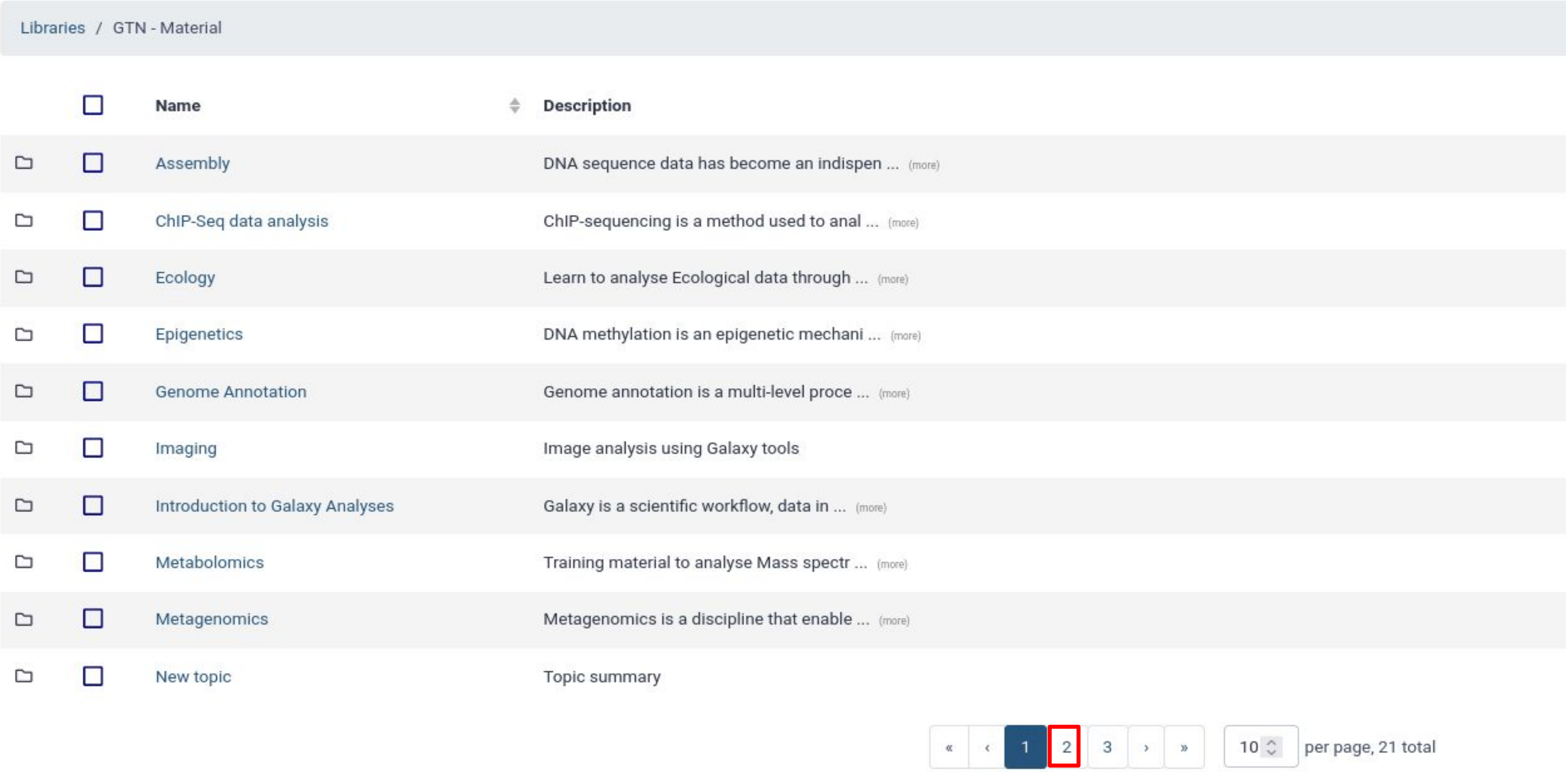


Search □ exclude restricted

Name	Description	Synopsis
ProteoRE	ProteoRE datasets	
covid-19		
GTN - Material	Galaxy Training Network Material	Galaxy Training Network Material. See ht ... (more)
workflow4metabolomics	Workflow4Metabolomics referenced histori ... (more)	https://workflow4metabolomics.org/refere ... (more)
Roscoff 2021	Data for Assembly and Annotation trainin ... (more)	

« ‹ 1 › » 10 per page, 5 total

Premapped reads upload

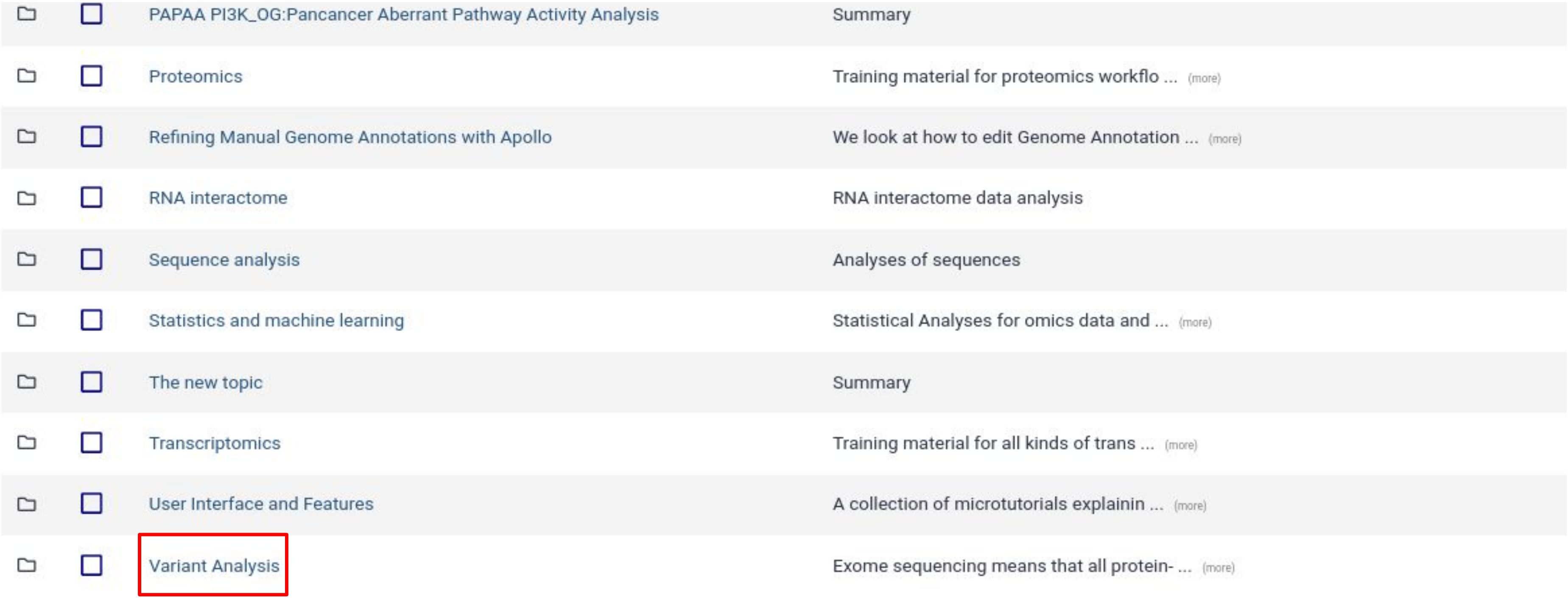


Libraries / GTN - Material

	□	Name	Description
🗀	□	Assembly	DNA sequence data has become an indispen ... (more)
🗀	□	ChIP-Seq data analysis	ChIP-sequencing is a method used to anal ... (more)
🗀	□	Ecology	Learn to analyse Ecological data through ... (more)
🗀	□	Epigenetics	DNA methylation is an epigenetic mechani ... (more)
🗀	□	Genome Annotation	Genome annotation is a multi-level proce ... (more)
🗀	□	Imaging	Image analysis using Galaxy tools
🗀	□	Introduction to Galaxy Analyses	Galaxy is a scientific workflow, data in ... (more)
🗀	□	Metabolomics	Training material to analyse Mass spectr ... (more)
🗀	□	Metagenomics	Metagenomics is a discipline that enable ... (more)
🗀	□	New topic	Topic summary

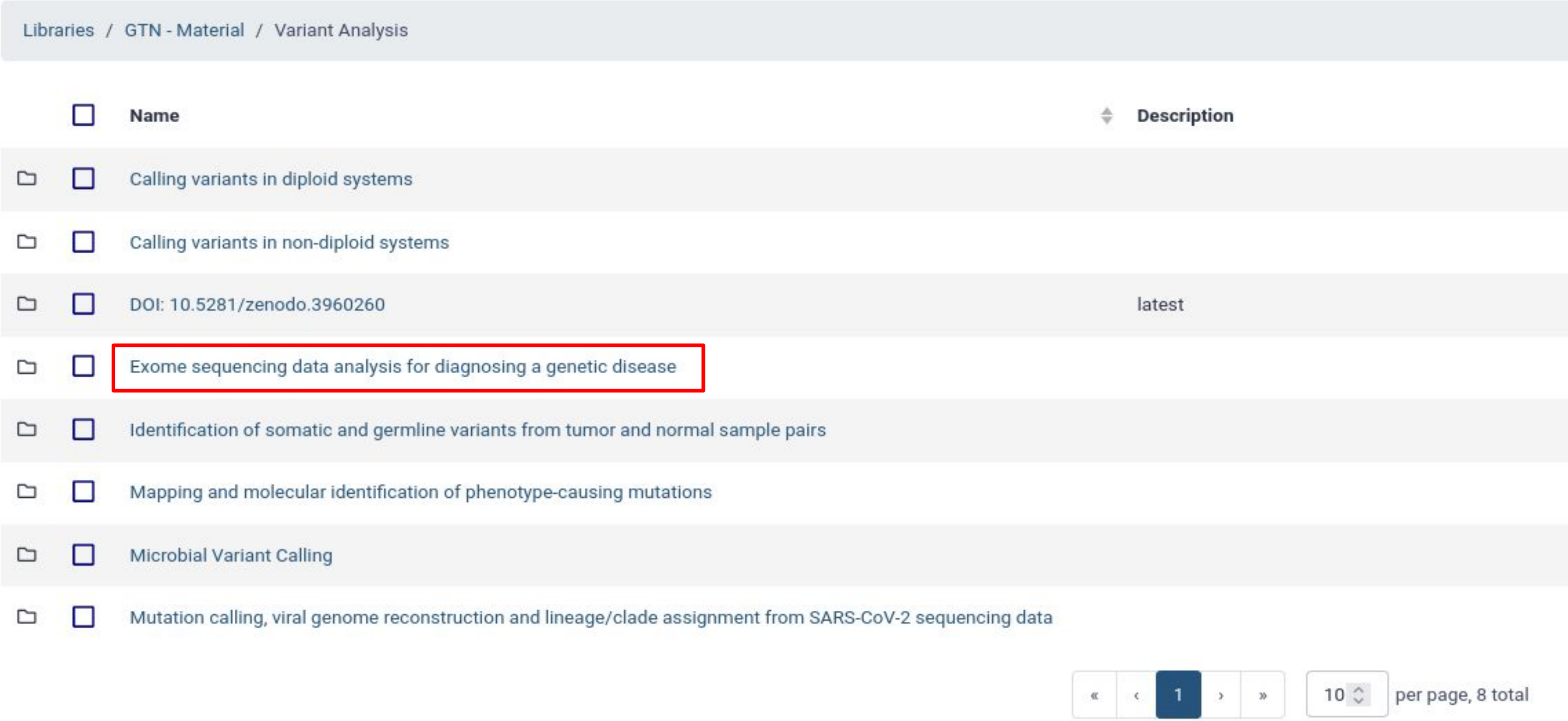
10 per page, 21 total

Premapped reads upload



🗀	□	PAPAA PI3K_OG:Pancancer Aberrant Pathway Activity Analysis	Summary
🗀	□	Proteomics	Training material for proteomics workflo ... (more)
🗀	□	Refining Manual Genome Annotations with Apollo	We look at how to edit Genome Annotation ... (more)
🗀	□	RNA interactome	RNA interactome data analysis
🗀	□	Sequence analysis	Analyses of sequences
🗀	□	Statistics and machine learning	Statistical Analyses for omics data and ... (more)
🗀	□	The new topic	Summary
🗀	□	Transcriptomics	Training material for all kinds of trans ... (more)
🗀	□	User Interface and Features	A collection of microtutorials explainin ... (more)
🗀	□	Variant Analysis	Exome sequencing means that all protein- ... (more)

Premapped reads upload

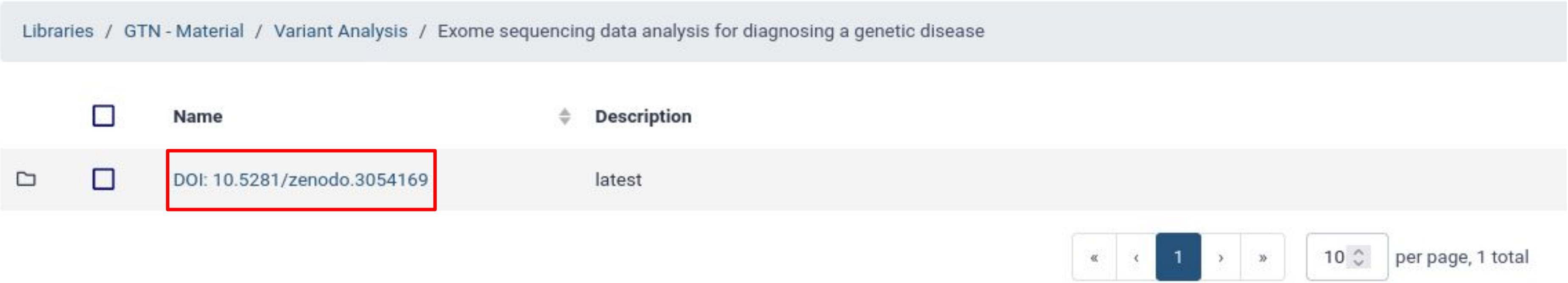


Libraries / GTN - Material / Variant Analysis

	□	Name	Description
🗀	□	Calling variants in diploid systems	
🗀	□	Calling variants in non-diploid systems	
🗀	□	DOI: 10.5281/zenodo.3960260	latest
🗀	□	Exome sequencing data analysis for diagnosing a genetic disease	
🗀	□	Identification of somatic and germline variants from tumor and normal sample pairs	
🗀	□	Mapping and molecular identification of phenotype-causing mutations	
🗀	□	Microbial Variant Calling	
🗀	□	Mutation calling, viral genome reconstruction and lineage/clade assignment from SARS-CoV-2 sequencing data	

10 per page, 8 total

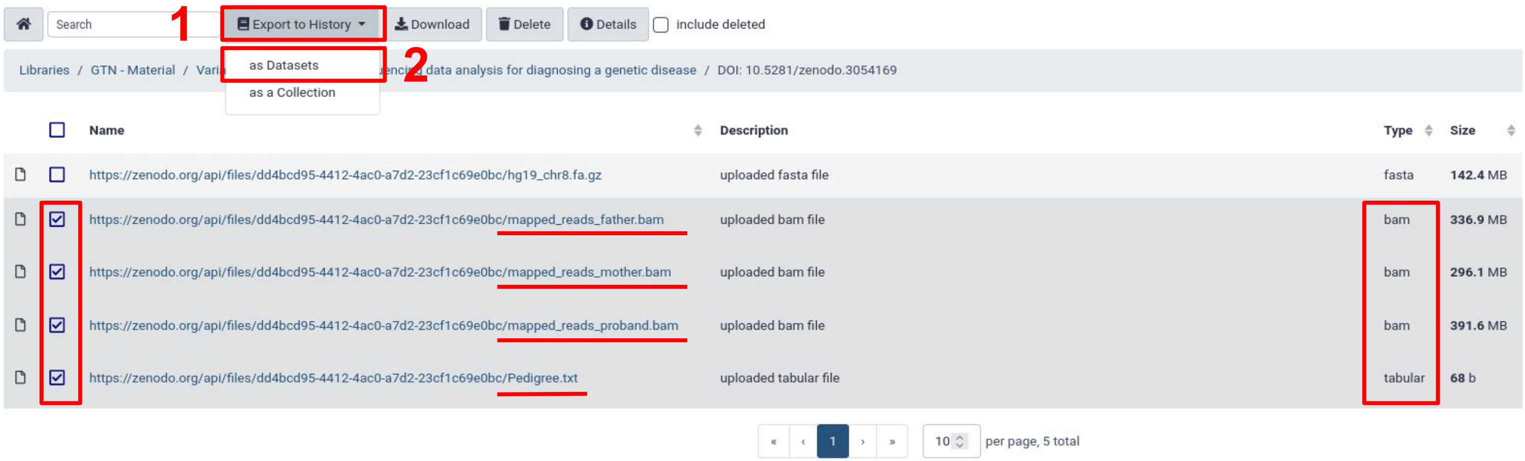
Premapped reads upload



Libraries / GTN - Material / Variant Analysis / Exome sequencing data analysis for diagnosing a genetic disease

	□	Name	Description
	□	DOI: 10.5281/zenodo.3054169	latest

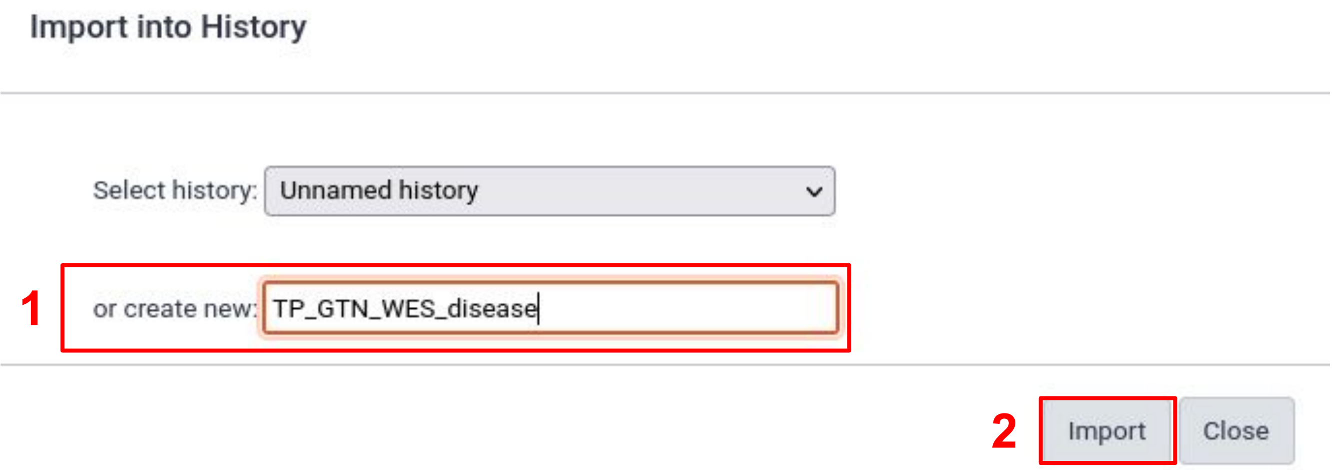
Premapped reads upload



Libraries / GTN - Material / Vari… …encing data analysis for diagnosing a genetic disease / DOI: 10.5281/zenodo.3054169

	□	Name	Description	Type	Size
🗋	□	https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/hg19_chr8.fa.gz	uploaded fasta file	fasta	142.4 MB
🗋	☑	https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_father.bam	uploaded bam file	bam	336.9 MB
🗋	☑	https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_mother.bam	uploaded bam file	bam	296.1 MB
🗋	☑	https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_proband.bam	uploaded bam file	bam	391.6 MB
🗋	☑	https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/Pedigree.txt	uploaded tabular file	tabular	68 b

Premapped reads upload

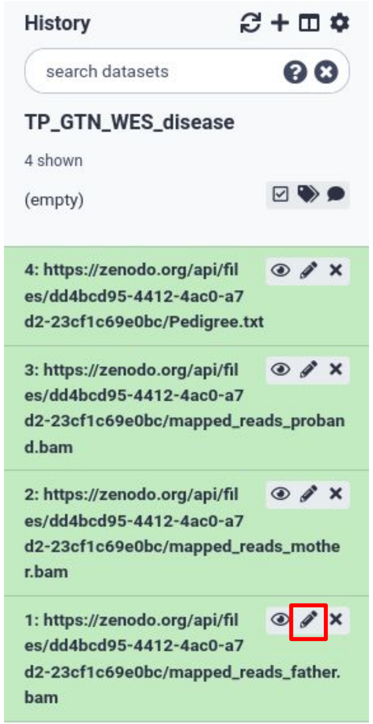


Import into History

Select history: Unnamed history

1 or create new: TP_GTN_WES_disease

Premapped reads upload



History

search datasets

TP_GTN_WES_disease

4 shown

(empty)

4: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/Pedigree.txt

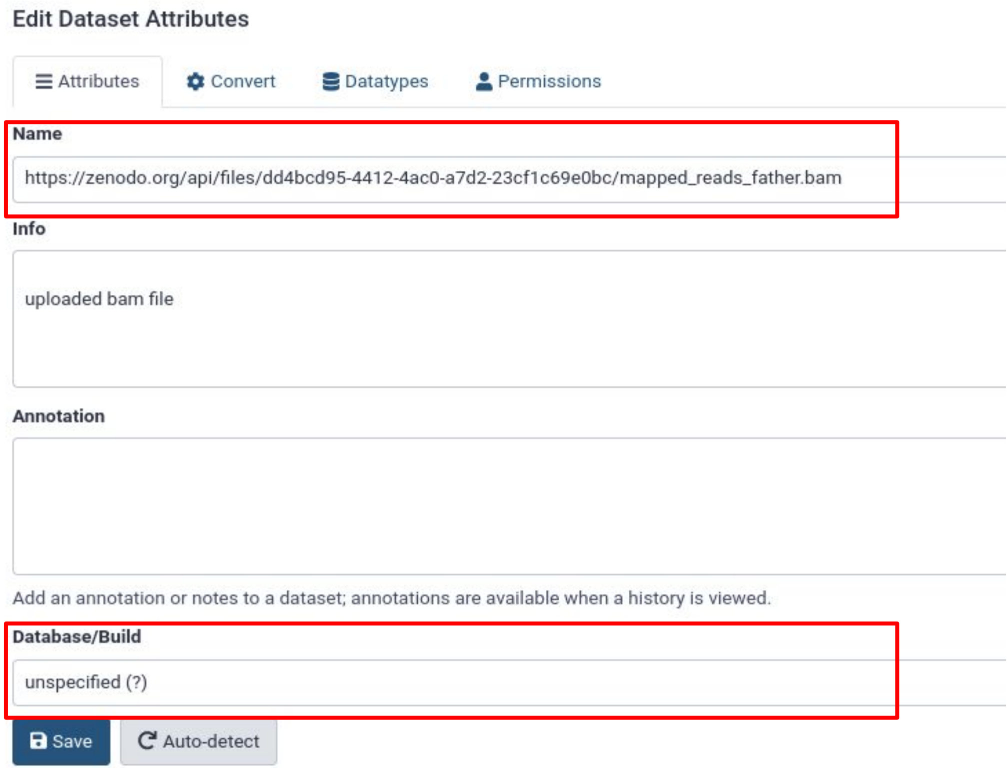
3: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_proband.bam

2: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_mother.bam

1: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_father.bam

1

Premapped reads upload



Edit Dataset Attributes

Name

https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_father.bam

Info

uploaded bam file

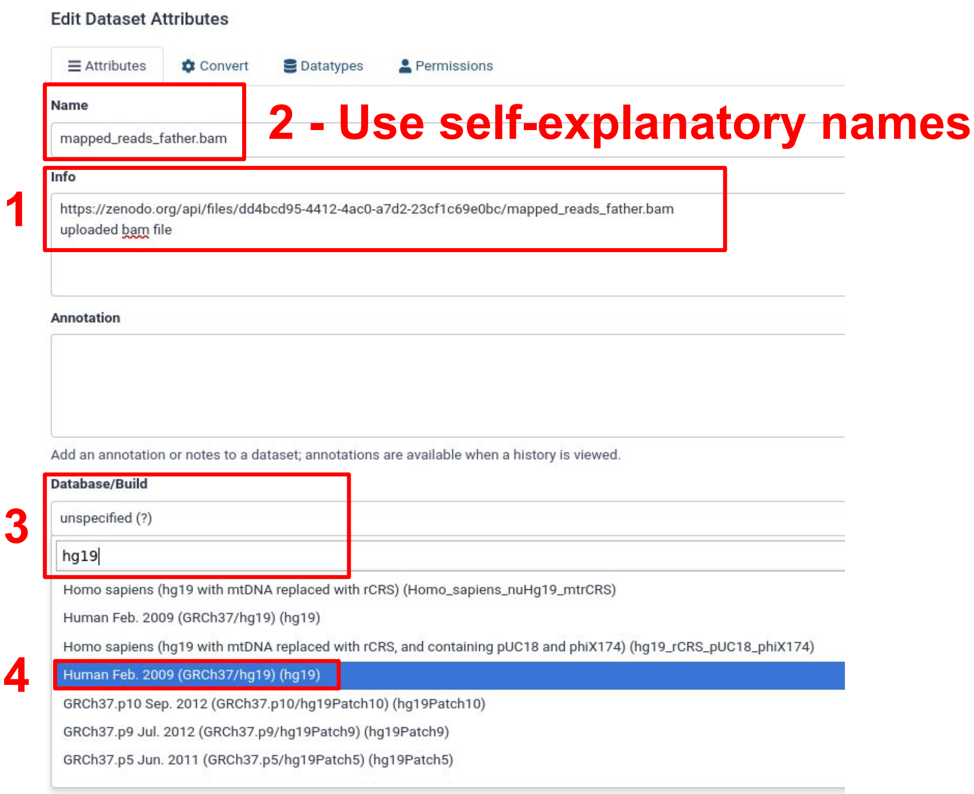
Annotation

Add an annotation or notes to a dataset; annotations are available when a history is viewed.

Database/Build

unspecified (?)

Premapped reads upload



Edit Dataset Attributes

Name

mapped_reads_father.bam

2 - Use self-explanatory names

1

Info

https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_father.bam
uploaded bam file

Annotation

Add an annotation or notes to a dataset; annotations are available when a history is viewed.

3

Database/Build

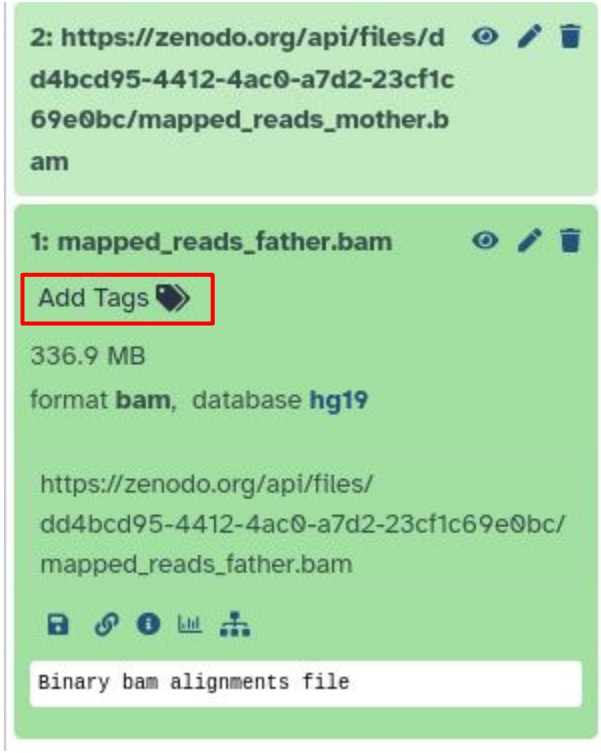
unspecified (?)

hg19

Homo sapiens (hg19 with mtDNA replaced with rCRS) (Homo_sapiens_nuHg19_mtrCRS)
Human Feb. 2009 (GRCh37/hg19) (hg19)
Homo sapiens (hg19 with mtDNA replaced with rCRS, and containing pUC18 and phiX174) (hg19_rCRS_pUC18_phiX174)
Human Feb. 2009 (GRCh37/hg19) (hg19)
GRCh37.p10 Sep. 2012 (GRCh37.p10/hg19Patch10) (hg19Patch10)
GRCh37.p9 Jul. 2012 (GRCh37.p9/hg19Patch9) (hg19Patch9)
GRCh37.p5 Jun. 2011 (GRCh37.p5/hg19Patch5) (hg19Patch5)

4

Premapped reads upload



2: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_mother.bam

1: mapped_reads_father.bam

1 Add Tags

336.9 MB

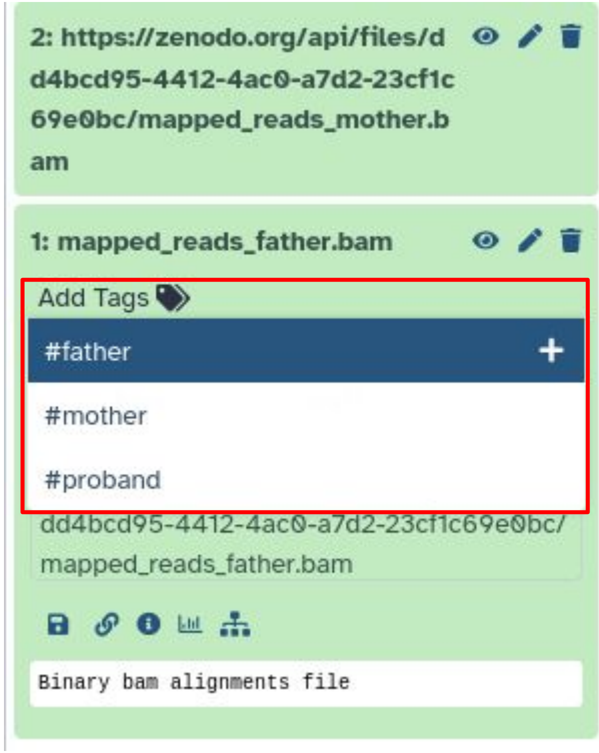
format **bam**, database **hg19**

https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_father.bam

```
Binary bam alignments file
```

Premapped reads upload

1



2: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_mother.bam

1: mapped_reads_father.bam

Add Tags

#father +

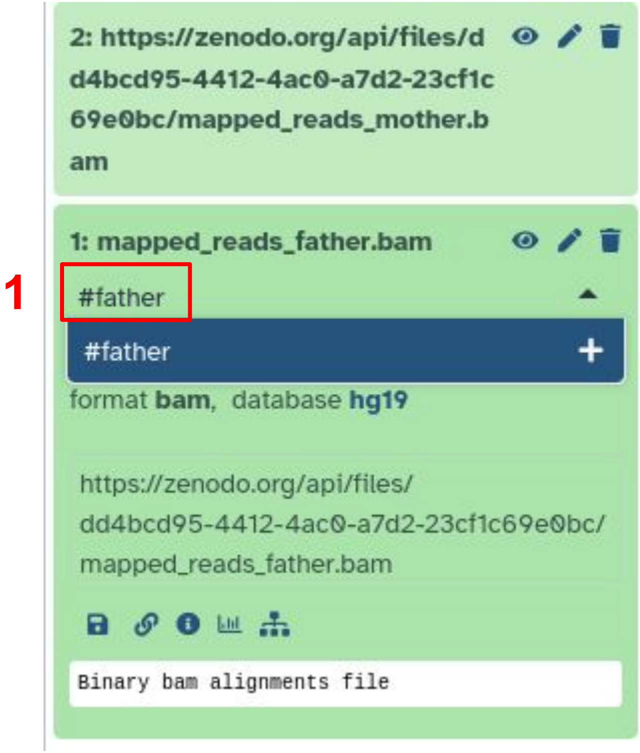
#mother

#proband

dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_father.bam

```
Binary bam alignments file
```

Premapped reads upload



2: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_mother.bam

1: mapped_reads_father.bam

1

#father

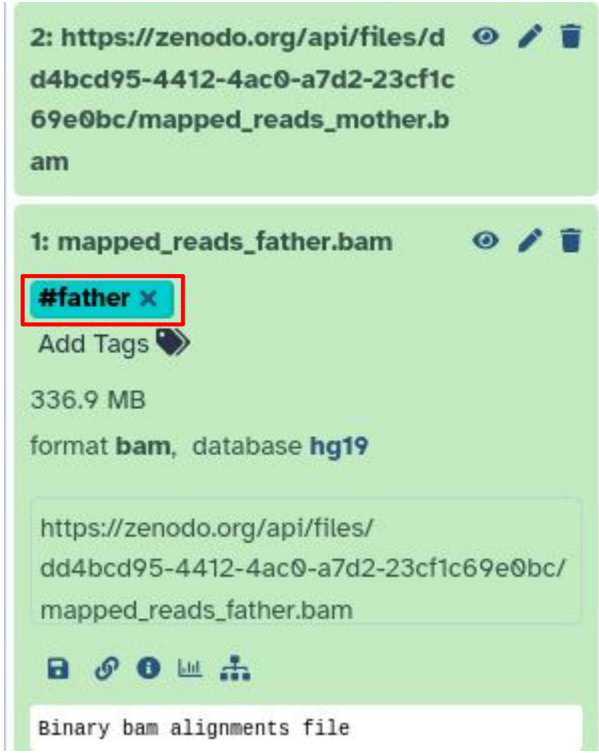
#father +

format **bam**, database **hg19**

https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_father.bam

```
Binary bam alignments file
```

Premapped reads upload



2: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_mother.bam

1: mapped_reads_father.bam

#father ×

Add Tags

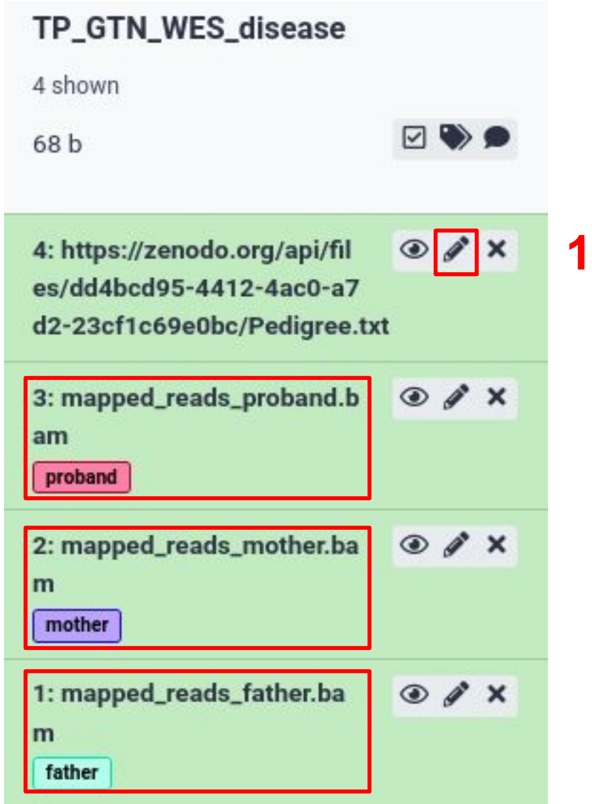
336.9 MB

format **bam**, database **hg19**

https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/mapped_reads_father.bam

```
Binary bam alignments file
```

Premapped reads upload



TP_GTN_WES_disease

4 shown

68 b

4: https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/Pedigree.txt

3: mapped_reads_proband.bam

proband

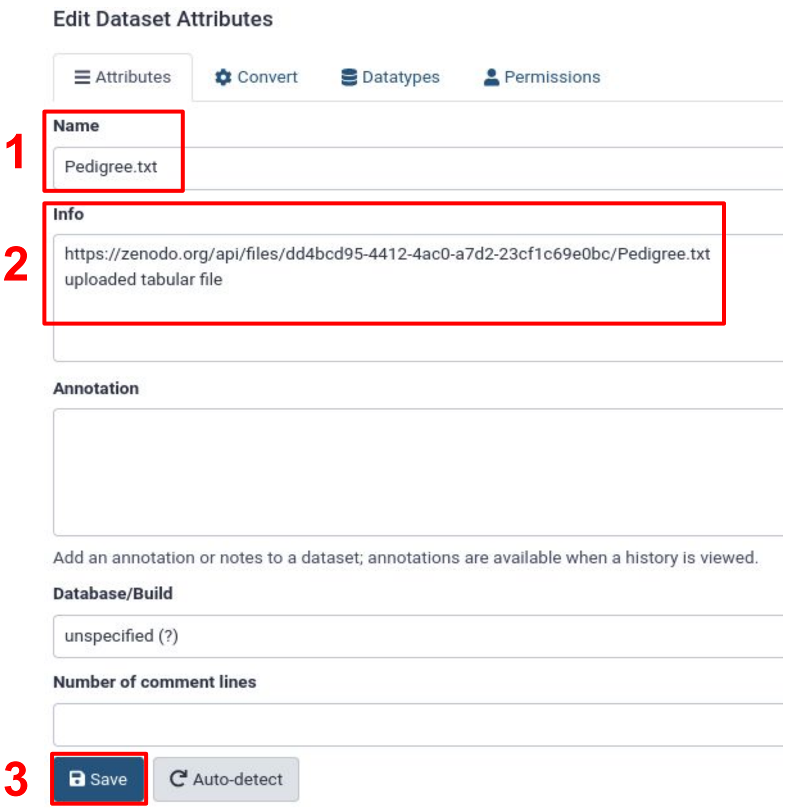
2: mapped_reads_mother.bam

mother

1: mapped_reads_father.bam

father

Premapped reads upload



Edit Dataset Attributes

1

Name

Pedigree.txt

2

Info

https://zenodo.org/api/files/dd4bcd95-4412-4ac0-a7d2-23cf1c69e0bc/Pedigree.txt
uploaded tabular file

Annotation

Add an annotation or notes to a dataset; annotations are available when a history is viewed.

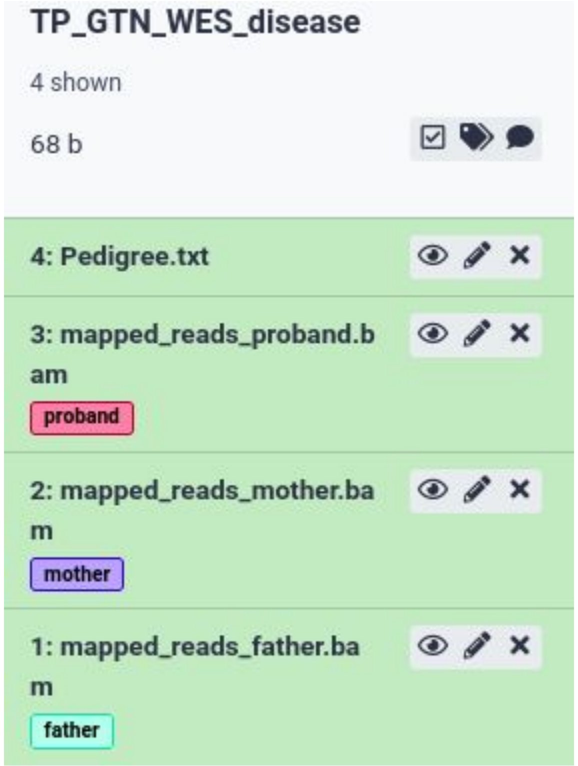
Database/Build

unspecified (?)

Number of comment lines

3

Premapped reads upload



TP_GTN_WES_disease

4 shown

68 b

4: Pedigree.txt

3: mapped_reads_proband.bam

proband

2: mapped_reads_mother.bam

mother

1: mapped_reads_father.bam

father

Mapped reads postprocessing

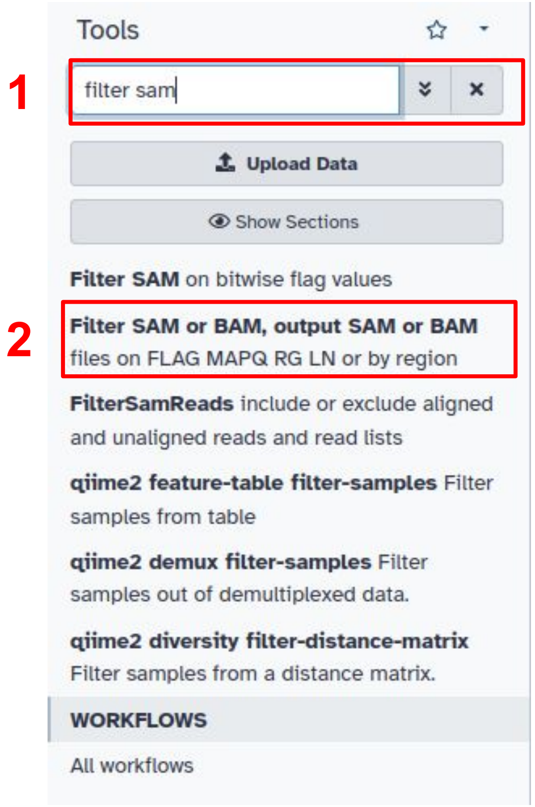
Warning :

- Depends on technology
- Depends on goal
- Depends on the pipeline used (steps, software, etc.)

1. Filter reads based on characteristics :
   - Retain only forward and reverse reads mapped successfully to the reference
   - Exclude possible contaminant DNA or sequencing artefact

2. Remove/Mark duplicate reads
   - PCR-overamplification of genomic fragment during sequencing library preparation

Mapped reads postprocessing - Filter reads



Tools

1 filter sam

Upload Data

Show Sections

Filter SAM on bitwise flag values

2 **Filter SAM or BAM, output SAM or BAM** files on FLAG MAPQ RG LN or by region

FilterSamReads include or exclude aligned and unaligned reads and read lists

qiime2 feature-table filter-samples Filter samples from table

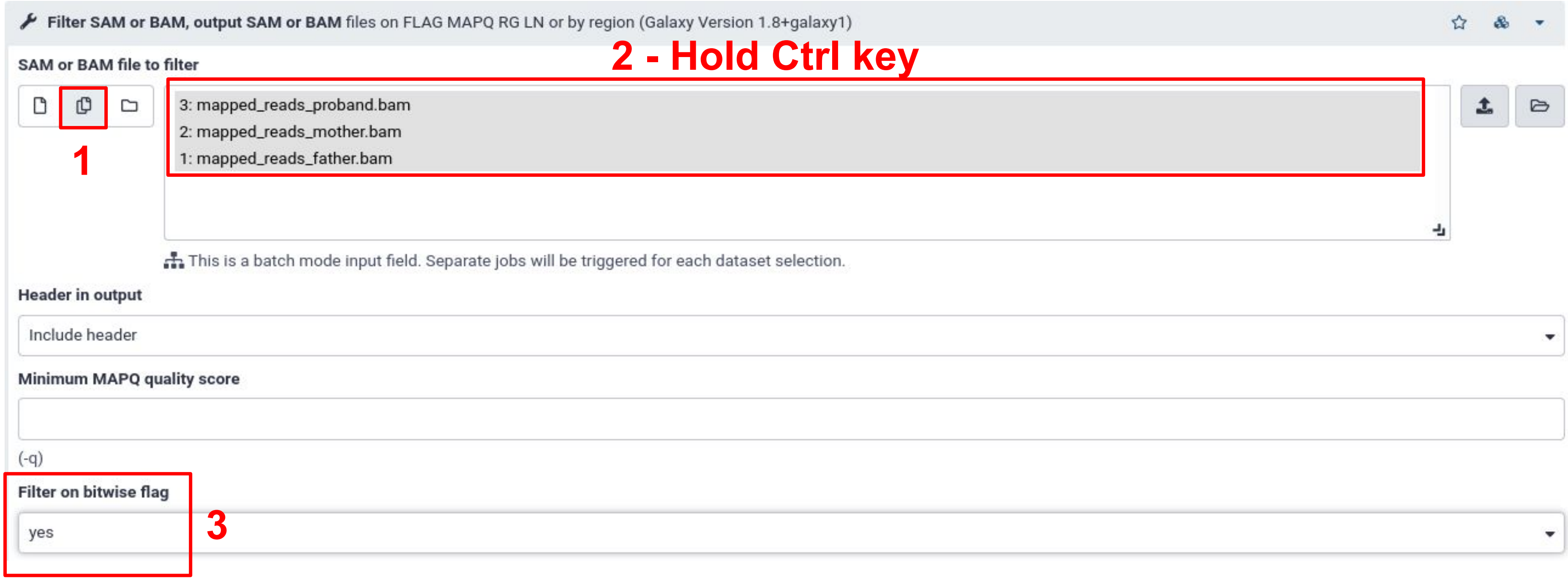
qiime2 demux filter-samples Filter samples out of demultiplexed data.

qiime2 diversity filter-distance-matrix Filter samples from a distance matrix.

WORKFLOWS

All workflows

Mapped reads processing - Filter reads



Filter SAM or BAM, output SAM or BAM files on FLAG MAPQ RG LN or by region (Galaxy Version 1.8+galaxy1)

SAM or BAM file to filter

3: mapped_reads_proband.bam
2: mapped_reads_mother.bam
1: mapped_reads_father.bam

This is a batch mode input field. Separate jobs will be triggered for each dataset selection.

Header in output

Include header

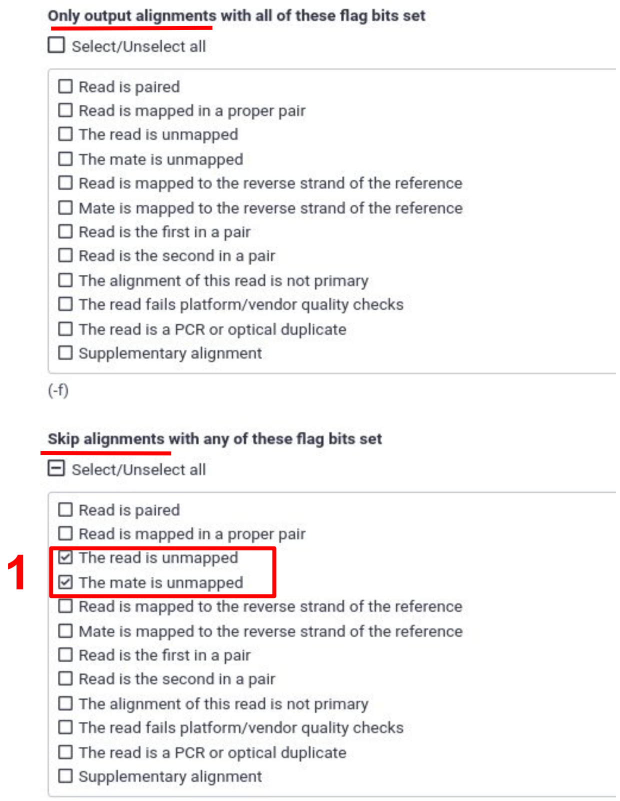
Minimum MAPQ quality score

(-q)

Filter on bitwise flag

yes

Mapped reads postprocessing - Filter reads



Only output alignments with all of these flag bits set

□ Select/Unselect all

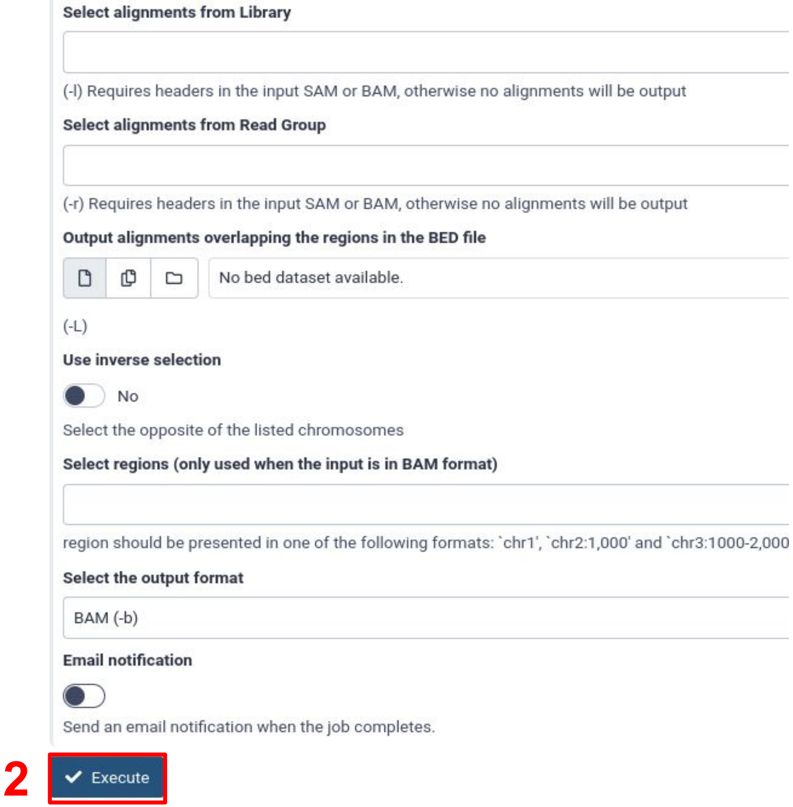
□ Read is paired
□ Read is mapped in a proper pair
□ The read is unmapped
□ The mate is unmapped
□ Read is mapped to the reverse strand of the reference
□ Mate is mapped to the reverse strand of the reference
□ Read is the first in a pair
□ Read is the second in a pair
□ The alignment of this read is not primary
□ The read fails platform/vendor quality checks
□ The read is a PCR or optical duplicate
□ Supplementary alignment

(-f)

Skip alignments with any of these flag bits set

⊟ Select/Unselect all

□ Read is paired
□ Read is mapped in a proper pair
☑ The read is unmapped
☑ The mate is unmapped
□ Read is mapped to the reverse strand of the reference
□ Mate is mapped to the reverse strand of the reference
□ Read is the first in a pair
□ Read is the second in a pair
□ The alignment of this read is not primary
□ The read fails platform/vendor quality checks
□ The read is a PCR or optical duplicate
□ Supplementary alignment



Select alignments from Library

(-l) Requires headers in the input SAM or BAM, otherwise no alignments will be output

Select alignments from Read Group

(-r) Requires headers in the input SAM or BAM, otherwise no alignments will be output

Output alignments overlapping the regions in the BED file

No bed dataset available.

(-L)

Use inverse selection

No

Select the opposite of the listed chromosomes

Select regions (only used when the input is in BAM format)

region should be presented in one of the following formats: `chr1', `chr2:1,000' and `chr3:1000-2,000

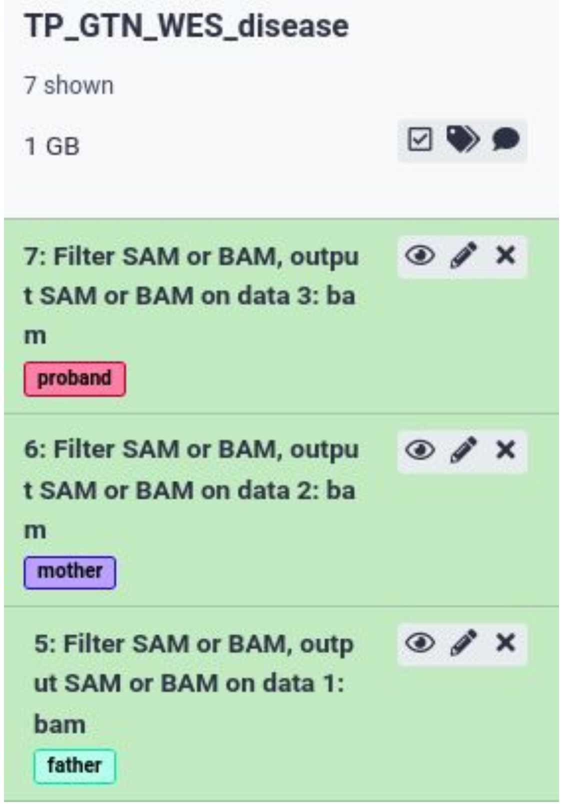
Select the output format

BAM (-b)

Email notification

Send an email notification when the job completes.

Mapped reads postprocessing - Filter reads



TP_GTN_WES_disease

7 shown

1 GB

7: Filter SAM or BAM, output SAM or BAM on data 3: bam

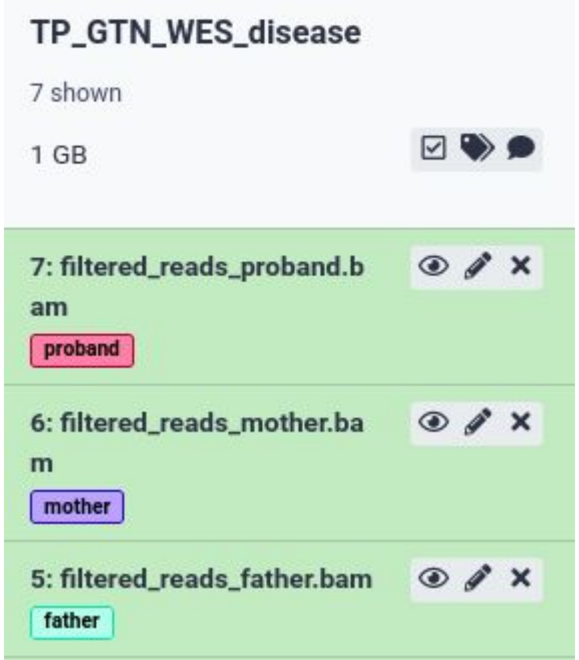
proband

6: Filter SAM or BAM, output SAM or BAM on data 2: bam

mother

5: Filter SAM or BAM, output SAM or BAM on data 1: bam

father



TP_GTN_WES_disease

7 shown

1 GB

7: filtered_reads_proband.bam

proband

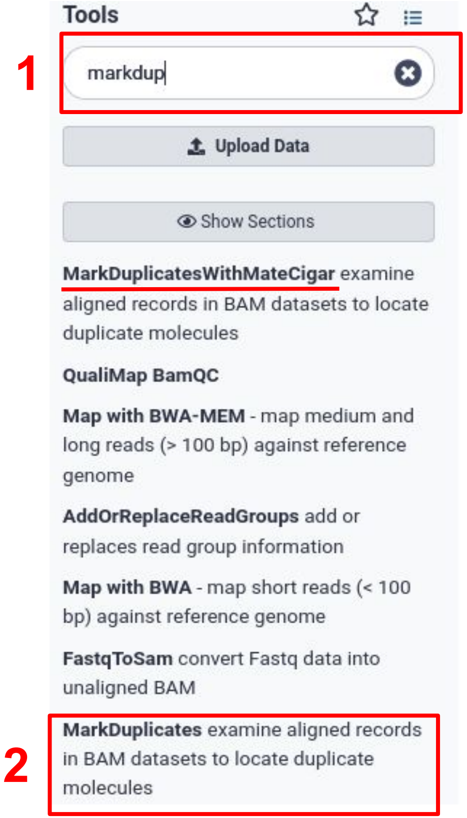
6: filtered_reads_mother.bam

mother

5: filtered_reads_father.bam

father

Mapped reads postprocessing - Duplicate reads



MarkDuplicatesWithMateCigar examine aligned records in BAM datasets to locate duplicate molecules

QualiMap BamQC

Map with BWA-MEM - map medium and long reads (> 100 bp) against reference genome

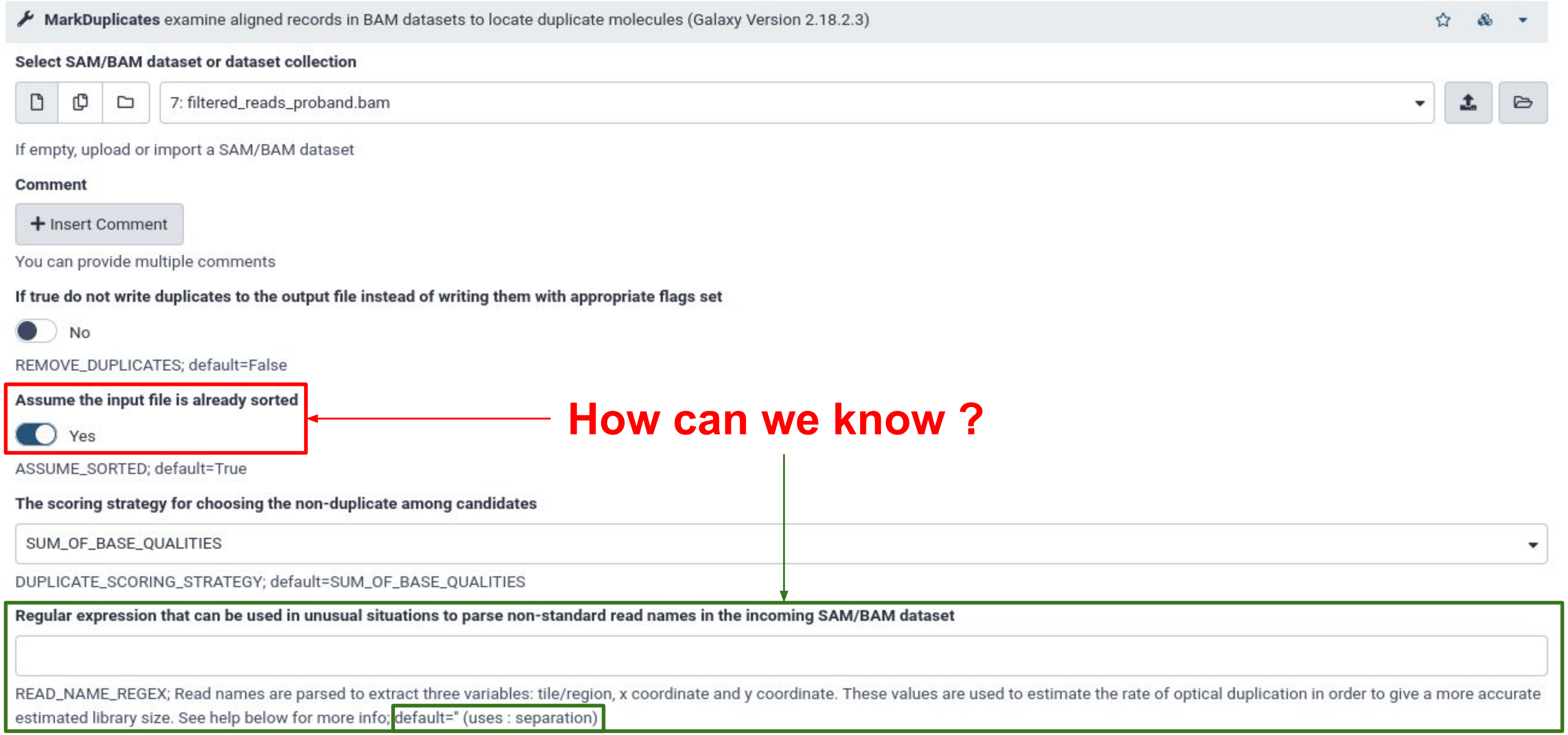
AddOrReplaceReadGroups add or replaces read group information

Map with BWA - map short reads (< 100 bp) against reference genome

FastqToSam convert Fastq data into unaligned BAM

MarkDuplicates examine aligned records in BAM datasets to locate duplicate molecules

Mapped reads postprocessing - Duplicate reads



MarkDuplicates examine aligned records in BAM datasets to locate duplicate molecules (Galaxy Version 2.18.2.3)

Select SAM/BAM dataset or dataset collection

7: filtered_reads_proband.bam

If empty, upload or import a SAM/BAM dataset

Comment

+ Insert Comment

You can provide multiple comments

If true do not write duplicates to the output file instead of writing them with appropriate flags set

No

REMOVE_DUPLICATES; default=False

Assume the input file is already sorted

Yes

ASSUME_SORTED; default=True

How can we know ?

The scoring strategy for choosing the non-duplicate among candidates

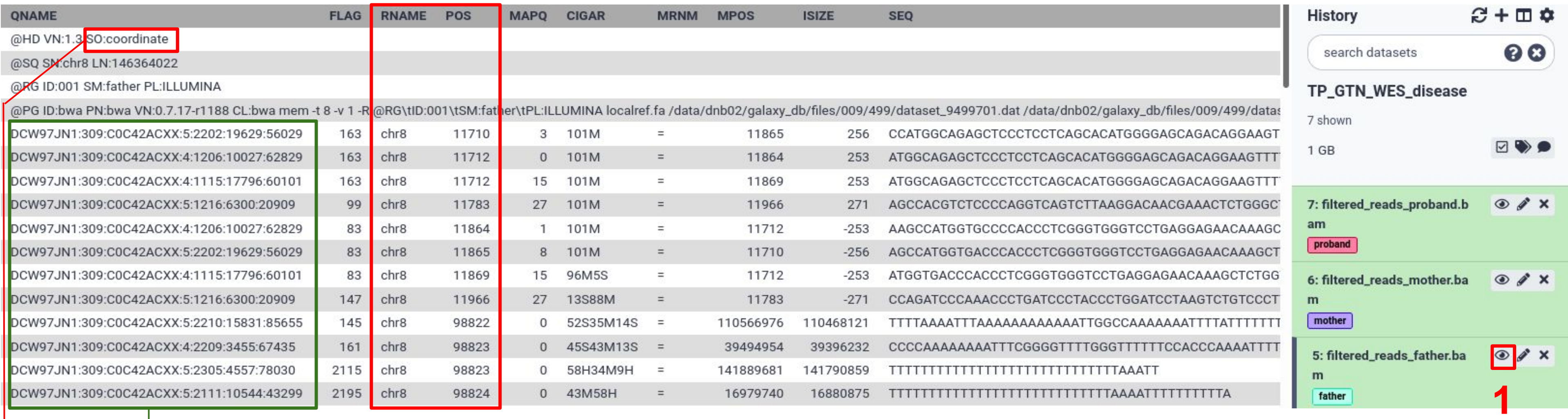
SUM_OF_BASE_QUALITIES

DUPLICATE_SCORING_STRATEGY; default=SUM_OF_BASE_QUALITIES

Regular expression that can be used in unusual situations to parse non-standard read names in the incoming SAM/BAM dataset

READ_NAME_REGEX; Read names are parsed to extract three variables: tile/region, x coordinate and y coordinate. These values are used to estimate the rate of optical duplication in order to give a more accurate estimated library size. See help below for more info; default='' (uses : separation)

Mapped reads postprocessing - Duplicate reads



QNAME	FLAG	RNAME	POS	MAPQ	CIGAR	MRNM	MPOS	ISIZE	SEQ
@HD VN:1.3 SO:coordinate									
@SQ SN:chr8 LN:146364022									
@RG ID:001 SM:father PL:ILLUMINA									
@PG ID:bwa PN:bwa VN:0.7.17-r1188 CL:bwa mem -t 8 -v 1 -R @RG\tID:001\tSM:father\tPL:ILLUMINA localref.fa /data/dnb02/galaxy_db/files/009/499/dataset_9499701.dat /data/dnb02/galaxy_db/files/009/499/datas									
DCW97JN1:309:C0C42ACXX:5:2202:19629:56029	163	chr8	11710	3	101M	=	11865	256	CCATGGCAGAGCTCCCTCCTCAGCACATGGGGAGCAGACAGGAAGT
DCW97JN1:309:C0C42ACXX:4:1206:10027:62829	163	chr8	11712	0	101M	=	11864	253	ATGGCAGAGCTCCCTCCTCAGCACATGGGGAGCAGACAGGAAGTTT
DCW97JN1:309:C0C42ACXX:4:1115:17796:60101	163	chr8	11712	15	101M	=	11869	253	ATGGCAGAGCTCCCTCCTCAGCACATGGGGAGCAGACAGGAAGTTT
DCW97JN1:309:C0C42ACXX:5:1216:6300:20909	99	chr8	11783	27	101M	=	11966	271	AGCCACGTCTCCCCAGGTCAGTCTTAAGGACAACGAAACTCTGGGC
DCW97JN1:309:C0C42ACXX:4:1206:10027:62829	83	chr8	11864	1	101M	=	11712	-253	AAGCCATGGTGCCCCACCCTCGGGTGGGTCCTGAGGAGAACAAAGC
DCW97JN1:309:C0C42ACXX:5:2202:19629:56029	83	chr8	11865	8	101M	=	11710	-256	AGCCATGGTGACCCACCCTCGGGTGGGTCCTGAGGAGAACAAAGCT
DCW97JN1:309:C0C42ACXX:4:1115:17796:60101	83	chr8	11869	15	96M5S	=	11712	-253	ATGGTGACCCACCCTCGGGTGGGTCCTGAGGAGAACAAAGCTCTGG
DCW97JN1:309:C0C42ACXX:5:1216:6300:20909	147	chr8	11966	27	13S88M	=	11783	-271	CCAGATCCCAAACCCTGATCCCTACCCTGGATCCTAAGTCTGTCCCT
DCW97JN1:309:C0C42ACXX:5:2210:15831:85655	145	chr8	98822	0	52S35M14S	=	110566976	110468121	TTTTAAAATTTAAAAAAAAAAAAATTGGCCAAAAAAATTTTATTTTTTT
DCW97JN1:309:C0C42ACXX:4:2209:3455:67435	161	chr8	98823	0	45S43M13S	=	39494954	39396232	CCCCAAAAAAAAATTTCGGGGTTTTGGGTTTTTTCCACCCAAAATTTT
DCW97JN1:309:C0C42ACXX:5:2305:4557:78030	2115	chr8	98823	0	58H34M9H	=	141889681	141790859	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAATT
DCW97JN1:309:C0C42ACXX:5:2111:10544:43299	2195	chr8	98824	0	43M58H	=	16979740	16880875	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAAAATTTTTTTTTTA

History

search datasets

TP_GTN_WES_disease

7 shown

1 GB

7: filtered_reads_proband.bam

proband

6: filtered_reads_mother.bam

mother

5: filtered_reads_father.bam

father

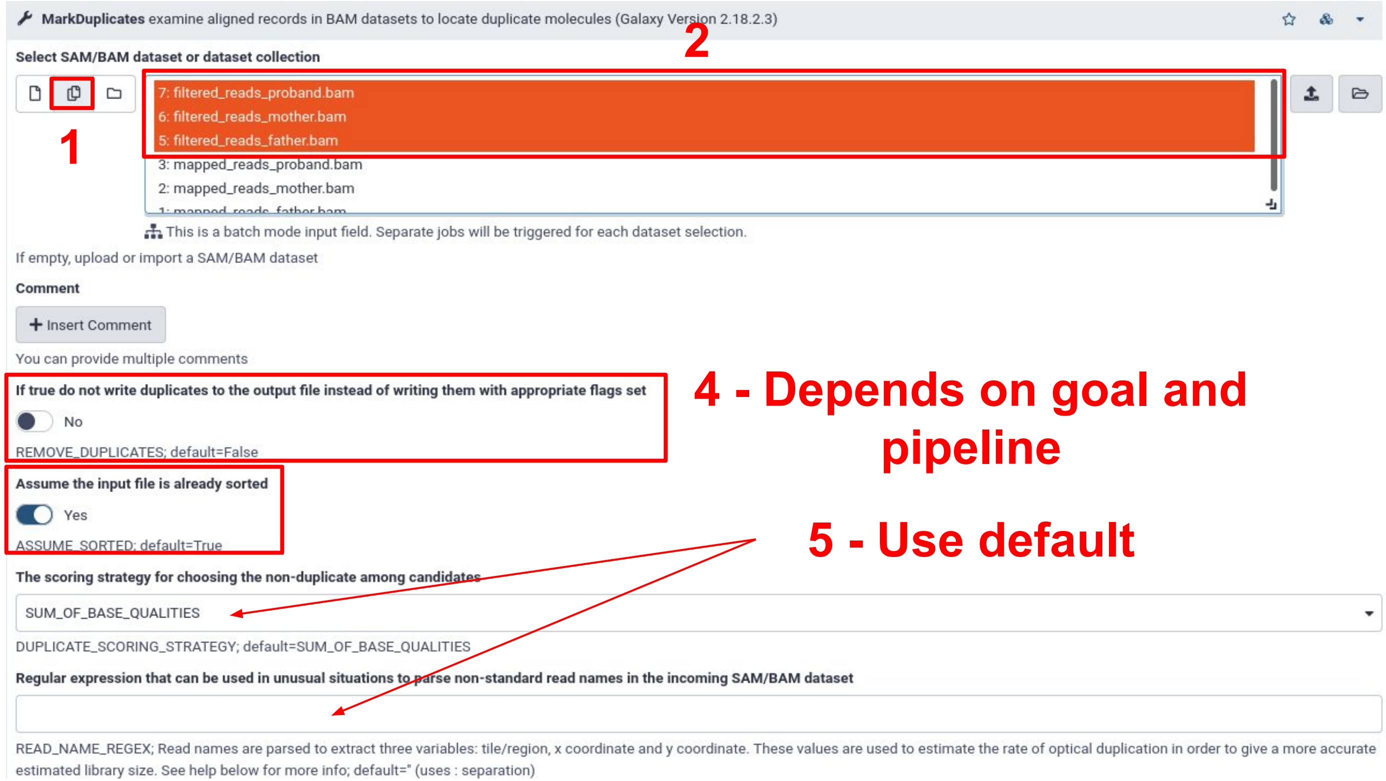
- <instrument>:<run_number>:<flowcell_ID>:<lane>:<tile>:<x-pos>:<y-pos>

SO tag :

- Sorting order of alignments
- Unknown, unsorted, queryname (QNAME) or coordinate (RNAME/POS)

https://support.basespace.illumina.com/articles/descriptive/fastq-files/

Mapped reads postprocessing - Duplicate reads



MarkDuplicates examine aligned records in BAM datasets to locate duplicate molecules (Galaxy Version 2.18.2.3)

Select SAM/BAM dataset or dataset collection

- 7: filtered_reads_proband.bam
- 6: filtered_reads_mother.bam
- 5: filtered_reads_father.bam
- 3: mapped_reads_proband.bam
- 2: mapped_reads_mother.bam
- 1: mapped_reads_father.bam

This is a batch mode input field. Separate jobs will be triggered for each dataset selection.

If empty, upload or import a SAM/BAM dataset

Comment

+ Insert Comment

You can provide multiple comments

If true do not write duplicates to the output file instead of writing them with appropriate flags set

No

REMOVE_DUPLICATES; default=False

Assume the input file is already sorted

Yes

ASSUME_SORTED; default=True

The scoring strategy for choosing the non-duplicate among candidates

SUM_OF_BASE_QUALITIES

DUPLICATE_SCORING_STRATEGY; default=SUM_OF_BASE_QUALITIES

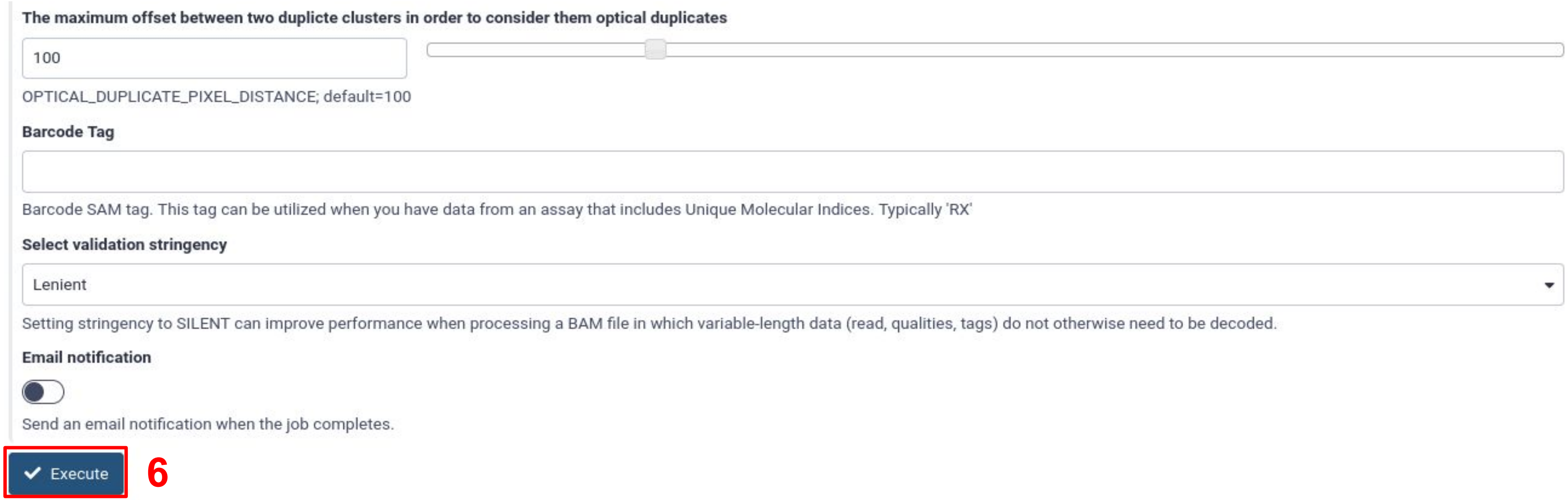
Regular expression that can be used in unusual situations to parse non-standard read names in the incoming SAM/BAM dataset

READ_NAME_REGEX; Read names are parsed to extract three variables: tile/region, x coordinate and y coordinate. These values are used to estimate the rate of optical duplication in order to give a more accurate estimated library size. See help below for more info; default='' (uses : separation)

4 - Depends on goal and pipeline

5 - Use default

Mapped reads postprocessing - Duplicate reads



The maximum offset between two duplicte clusters in order to consider them optical duplicates

100

OPTICAL_DUPLICATE_PIXEL_DISTANCE; default=100

Barcode Tag

Barcode SAM tag. This tag can be utilized when you have data from an assay that includes Unique Molecular Indices. Typically 'RX'

Select validation stringency

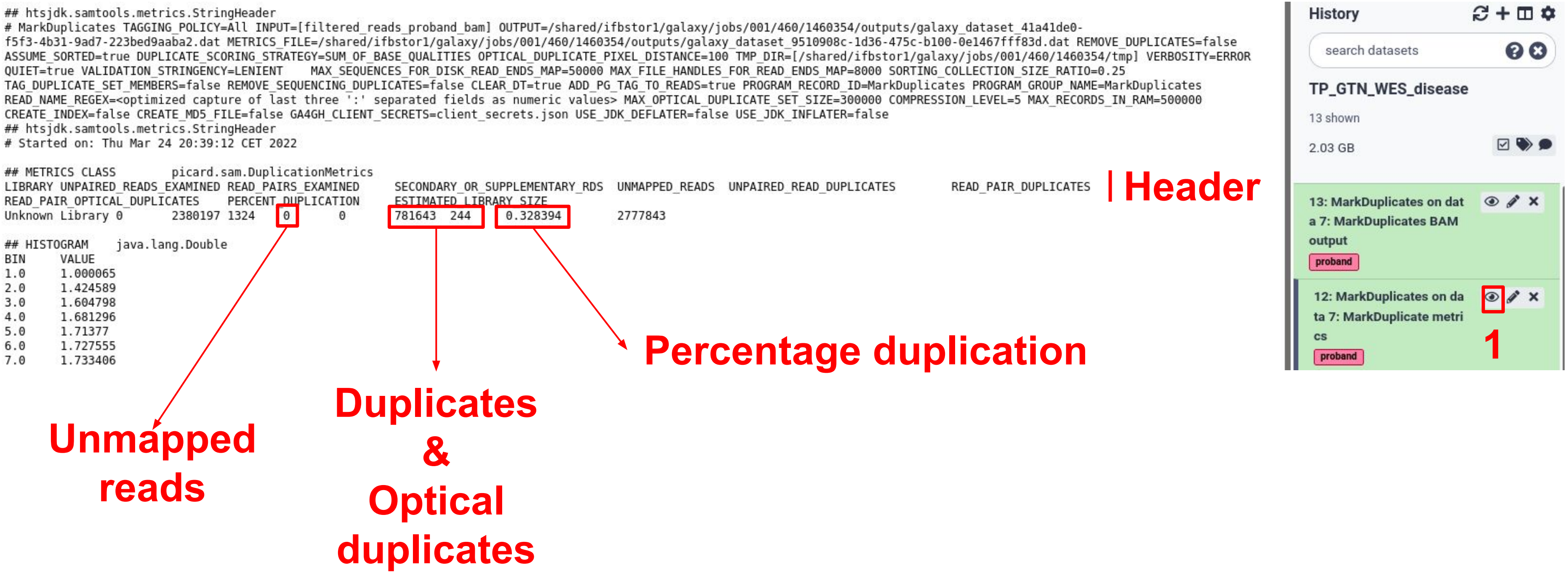
Lenient

Setting stringency to SILENT can improve performance when processing a BAM file in which variable-length data (read, qualities, tags) do not otherwise need to be decoded.

Email notification

Send an email notification when the job completes.

Mapped reads postprocessing - Duplicate reads



```
## htsjdk.samtools.metrics.StringHeader
# MarkDuplicates TAGGING_POLICY=All INPUT=[filtered_reads_proband_bam] OUTPUT=/shared/ifbstor1/galaxy/jobs/001/460/1460354/outputs/galaxy_dataset_41a41de0-
f5f3-4b31-9ad7-223bed9aaba2.dat METRICS_FILE=/shared/ifbstor1/galaxy/jobs/001/460/1460354/outputs/galaxy_dataset_9510908c-1d36-475c-b100-0e1467fff83d.dat REMOVE_DUPLICATES=false
ASSUME_SORTED=true DUPLICATE_SCORING_STRATEGY=SUM_OF_BASE_QUALITIES OPTICAL_DUPLICATE_PIXEL_DISTANCE=100 TMP_DIR=[/shared/ifbstor1/galaxy/jobs/001/460/1460354/tmp] VERBOSITY=ERROR
QUIET=true VALIDATION_STRINGENCY=LENIENT    MAX_SEQUENCES_FOR_DISK_READ_ENDS_MAP=50000 MAX_FILE_HANDLES_FOR_READ_ENDS_MAP=8000 SORTING_COLLECTION_SIZE_RATIO=0.25
TAG_DUPLICATE_SET_MEMBERS=false REMOVE_SEQUENCING_DUPLICATES=false CLEAR_DT=true ADD_PG_TAG_TO_READS=true PROGRAM_RECORD_ID=MarkDuplicates PROGRAM_GROUP_NAME=MarkDuplicates
READ_NAME_REGEX=<optimized capture of last three ':' separated fields as numeric values> MAX_OPTICAL_DUPLICATE_SET_SIZE=300000 COMPRESSION_LEVEL=5 MAX_RECORDS_IN_RAM=500000
CREATE_INDEX=false CREATE_MD5_FILE=false GA4GH_CLIENT_SECRETS=client_secrets.json USE_JDK_DEFLATER=false USE_JDK_INFLATER=false
## htsjdk.samtools.metrics.StringHeader
# Started on: Thu Mar 24 20:39:12 CET 2022

## METRICS CLASS        picard.sam.DuplicationMetrics
LIBRARY UNPAIRED_READS_EXAMINED READ_PAIRS_EXAMINED     SECONDARY_OR_SUPPLEMENTARY_RDS  UNMAPPED_READS  UNPAIRED_READ_DUPLICATES        READ_PAIR_DUPLICATES
READ_PAIR_OPTICAL_DUPLICATES    PERCENT_DUPLICATION     ESTIMATED_LIBRARY_SIZE
Unknown Library 0       2380197 1324    0       0       781643  244     0.328394        2777843

## HISTOGRAM    java.lang.Double
BIN     VALUE
1.0     1.000065
2.0     1.424589
3.0     1.604798
4.0     1.681296
5.0     1.71377
6.0     1.727555
7.0     1.733406
```

History
search datasets
TP_GTN_WES_disease
13 shown
2.03 GB
13: MarkDuplicates on data 7: MarkDuplicates BAM output
proband
12: MarkDuplicates on data 7: MarkDuplicate metrics
proband
1

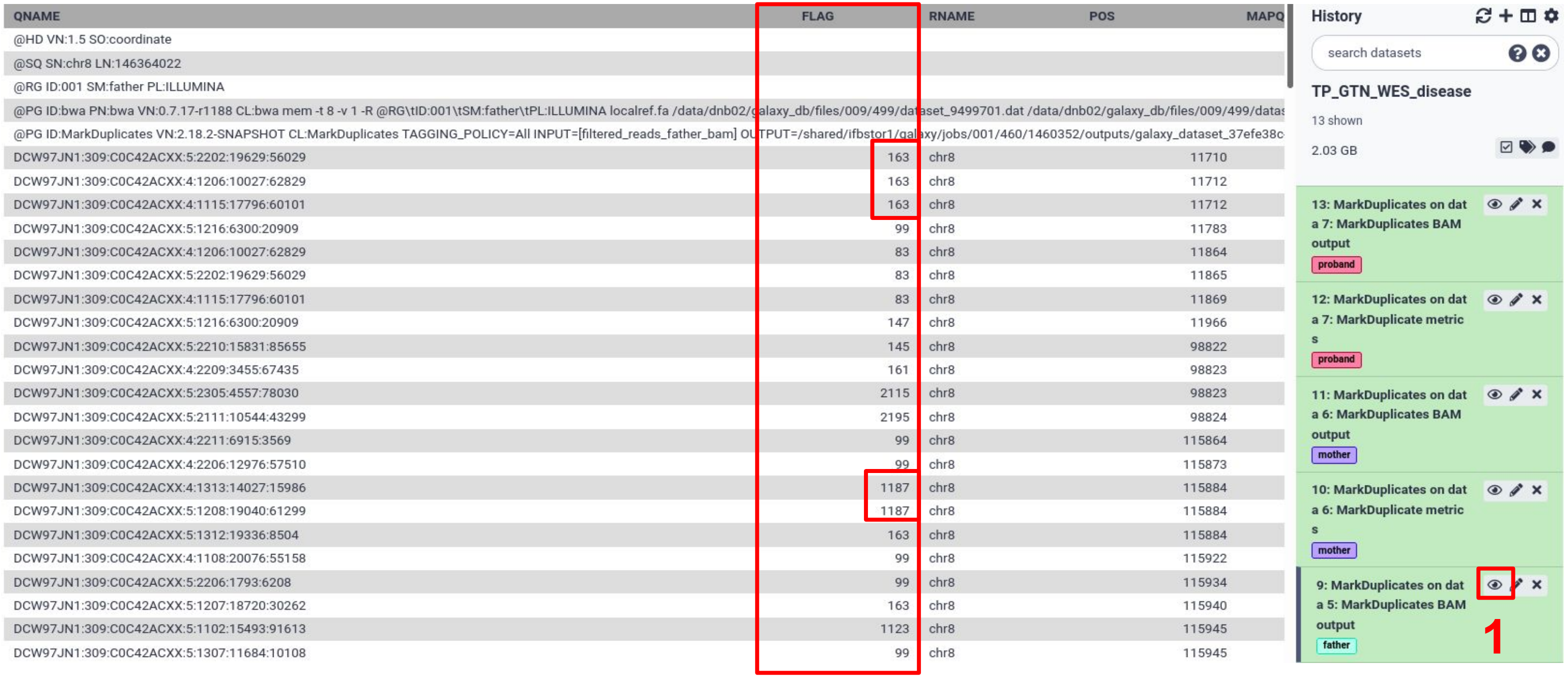
| Header

Unmapped reads

Duplicates & Optical duplicates

Percentage duplication

Mapped reads postprocessing - Duplicate reads



QNAME	FLAG	RNAME	POS	MAPQ
@HD VN:1.5 SO:coordinate				
@SQ SN:chr8 LN:146364022				
@RG ID:001 SM:father PL:ILLUMINA				
@PG ID:bwa PN:bwa VN:0.7.17-r1188 CL:bwa mem -t 8 -v 1 -R @RG\tID:001\tSM:father\tPL:ILLUMINA localref.fa /data/dnb02/galaxy_db/files/009/499/dataset_9499701.dat /data/dnb02/galaxy_db/files/009/499/datas				
@PG ID:MarkDuplicates VN:2.18.2-SNAPSHOT CL:MarkDuplicates TAGGING_POLICY=All INPUT=[filtered_reads_father_bam] OUTPUT=/shared/ifbstor1/galaxy/jobs/001/460/1460352/outputs/galaxy_dataset_37efe38c				
DCW97JN1:309:C0C42ACXX:5:2202:19629:56029	163	chr8	11710	
DCW97JN1:309:C0C42ACXX:4:1206:10027:62829	163	chr8	11712	
DCW97JN1:309:C0C42ACXX:4:1115:17796:60101	163	chr8	11712	
DCW97JN1:309:C0C42ACXX:5:1216:6300:20909	99	chr8	11783	
DCW97JN1:309:C0C42ACXX:4:1206:10027:62829	83	chr8	11864	
DCW97JN1:309:C0C42ACXX:5:2202:19629:56029	83	chr8	11865	
DCW97JN1:309:C0C42ACXX:4:1115:17796:60101	83	chr8	11869	
DCW97JN1:309:C0C42ACXX:5:1216:6300:20909	147	chr8	11966	
DCW97JN1:309:C0C42ACXX:5:2210:15831:85655	145	chr8	98822	
DCW97JN1:309:C0C42ACXX:4:2209:3455:67435	161	chr8	98823	
DCW97JN1:309:C0C42ACXX:5:2305:4557:78030	2115	chr8	98823	
DCW97JN1:309:C0C42ACXX:5:2111:10544:43299	2195	chr8	98824	
DCW97JN1:309:C0C42ACXX:4:2211:6915:3569	99	chr8	115864	
DCW97JN1:309:C0C42ACXX:4:2206:12976:57510	99	chr8	115873	
DCW97JN1:309:C0C42ACXX:4:1313:14027:15986	1187	chr8	115884	
DCW97JN1:309:C0C42ACXX:5:1208:19040:61299	1187	chr8	115884	
DCW97JN1:309:C0C42ACXX:5:1312:19336:8504	163	chr8	115884	
DCW97JN1:309:C0C42ACXX:4:1108:20076:55158	99	chr8	115922	
DCW97JN1:309:C0C42ACXX:5:2206:1793:6208	99	chr8	115934	
DCW97JN1:309:C0C42ACXX:5:1207:18720:30262	163	chr8	115940	
DCW97JN1:309:C0C42ACXX:5:1102:15493:91613	1123	chr8	115945	
DCW97JN1:309:C0C42ACXX:5:1307:11684:10108	99	chr8	115945	

History

search datasets

TP_GTN_WES_disease

13 shown

2.03 GB

13: MarkDuplicates on data 7: MarkDuplicates BAM output
proband

12: MarkDuplicates on data 7: MarkDuplicate metrics
proband

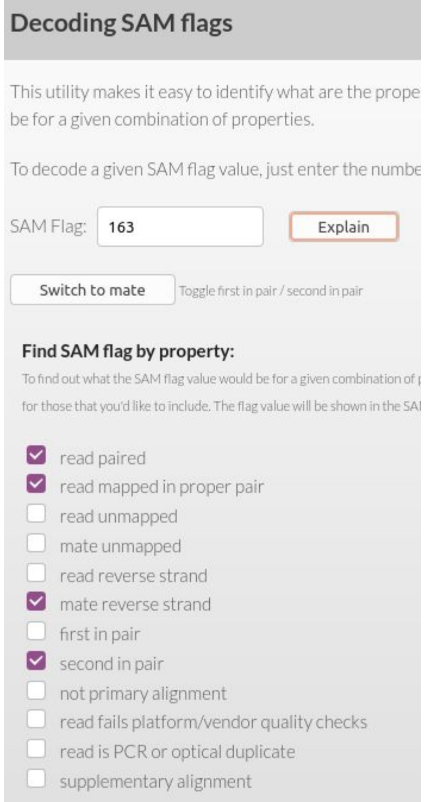
11: MarkDuplicates on data 6: MarkDuplicates BAM output
mother

10: MarkDuplicates on data 6: MarkDuplicate metrics
mother

9: MarkDuplicates on data 5: MarkDuplicates BAM output
father

1

Mapped reads postprocessing - Duplicate reads



Decoding SAM flags

This utility makes it easy to identify what are the prope be for a given combination of properties.

To decode a given SAM flag value, just enter the numbe

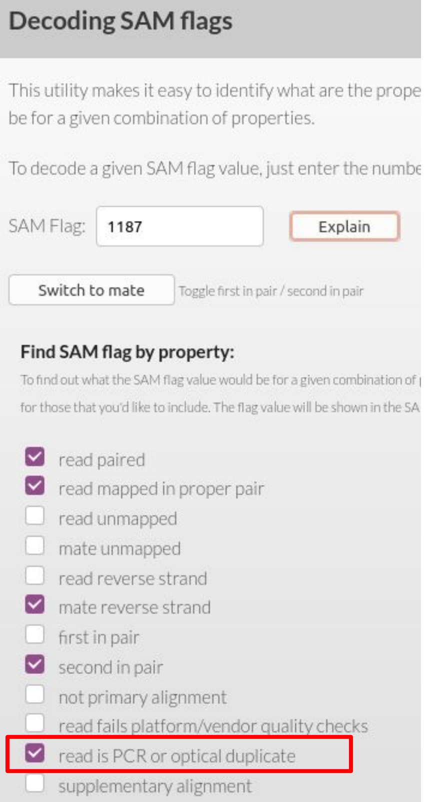
SAM Flag: 163 Explain

Switch to mate Toggle first in pair / second in pair

Find SAM flag by property:

To find out what the SAM flag value would be for a given combination of for those that you'd like to include. The flag value will be shown in the SAI

- ☑ read paired
- ☑ read mapped in proper pair
- □ read unmapped
- □ mate unmapped
- □ read reverse strand
- ☑ mate reverse strand
- □ first in pair
- ☑ second in pair
- □ not primary alignment
- □ read fails platform/vendor quality checks
- □ read is PCR or optical duplicate
- □ supplementary alignment



Decoding SAM flags

This utility makes it easy to identify what are the prope be for a given combination of properties.

To decode a given SAM flag value, just enter the numbe

SAM Flag: 1187 Explain

Switch to mate Toggle first in pair / second in pair

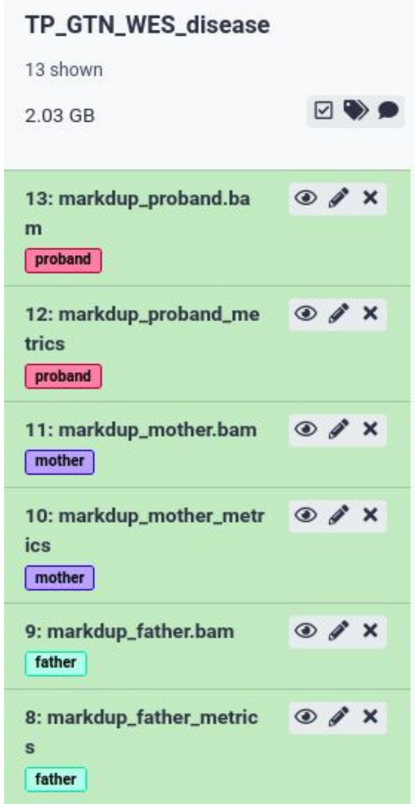
Find SAM flag by property:

To find out what the SAM flag value would be for a given combination of for those that you'd like to include. The flag value will be shown in the SA

- ☑ read paired
- ☑ read mapped in proper pair
- □ read unmapped
- □ mate unmapped
- □ read reverse strand
- ☑ mate reverse strand
- □ first in pair
- ☑ second in pair
- □ not primary alignment
- □ read fails platform/vendor quality checks
- ☑ read is PCR or optical duplicate
- □ supplementary alignment

http://broadinstitute.github.io/picard/explain-flags

Mapped reads postprocessing - Duplicate reads



TP_GTN_WES_disease

13 shown

2.03 GB

13: markdup_proband.bam
proband

12: markdup_proband_metrics
proband

11: markdup_mother.bam
mother

10: markdup_mother_metrics
mother

9: markdup_father.bam
father

8: markdup_father_metrics
father

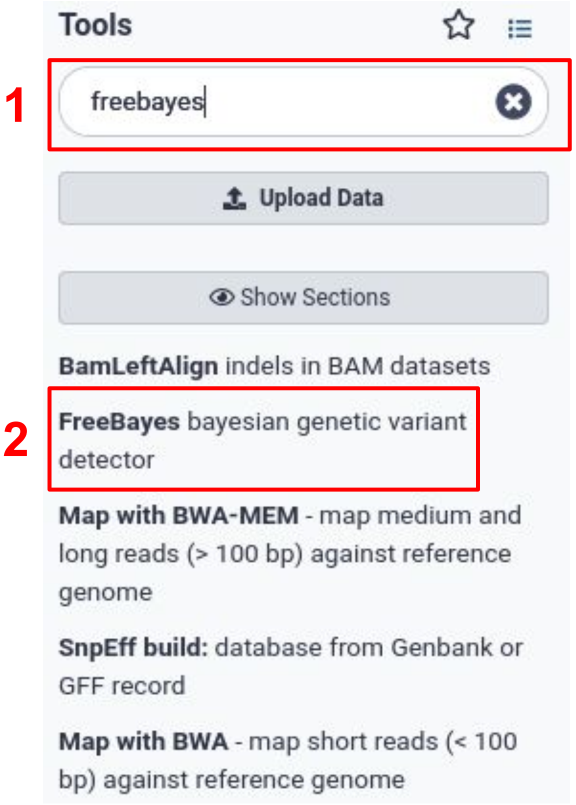
Tutorial steps

1. Perform postprocessing from premapped reads

2. Variant calling

3. Variant annotation and reporting

Variant calling



BamLeftAlign indels in BAM datasets

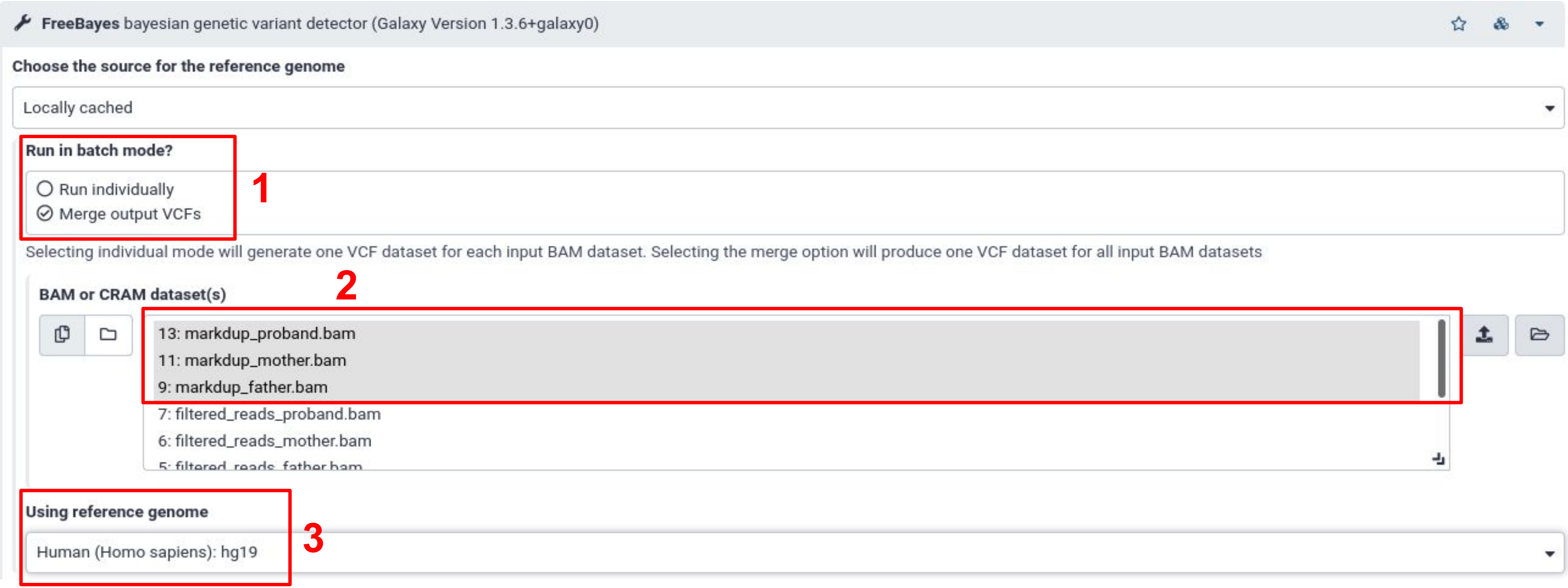
FreeBayes bayesian genetic variant detector

Map with BWA-MEM - map medium and long reads (> 100 bp) against reference genome

SnpEff build: database from Genbank or GFF record

Map with BWA - map short reads (< 100 bp) against reference genome

Variant calling



FreeBayes bayesian genetic variant detector (Galaxy Version 1.3.6+galaxy0)

Choose the source for the reference genome

Locally cached

1 **Run in batch mode?**

○ Run individually
☑ Merge output VCFs

Selecting individual mode will generate one VCF dataset for each input BAM dataset. Selecting the merge option will produce one VCF dataset for all input BAM datasets

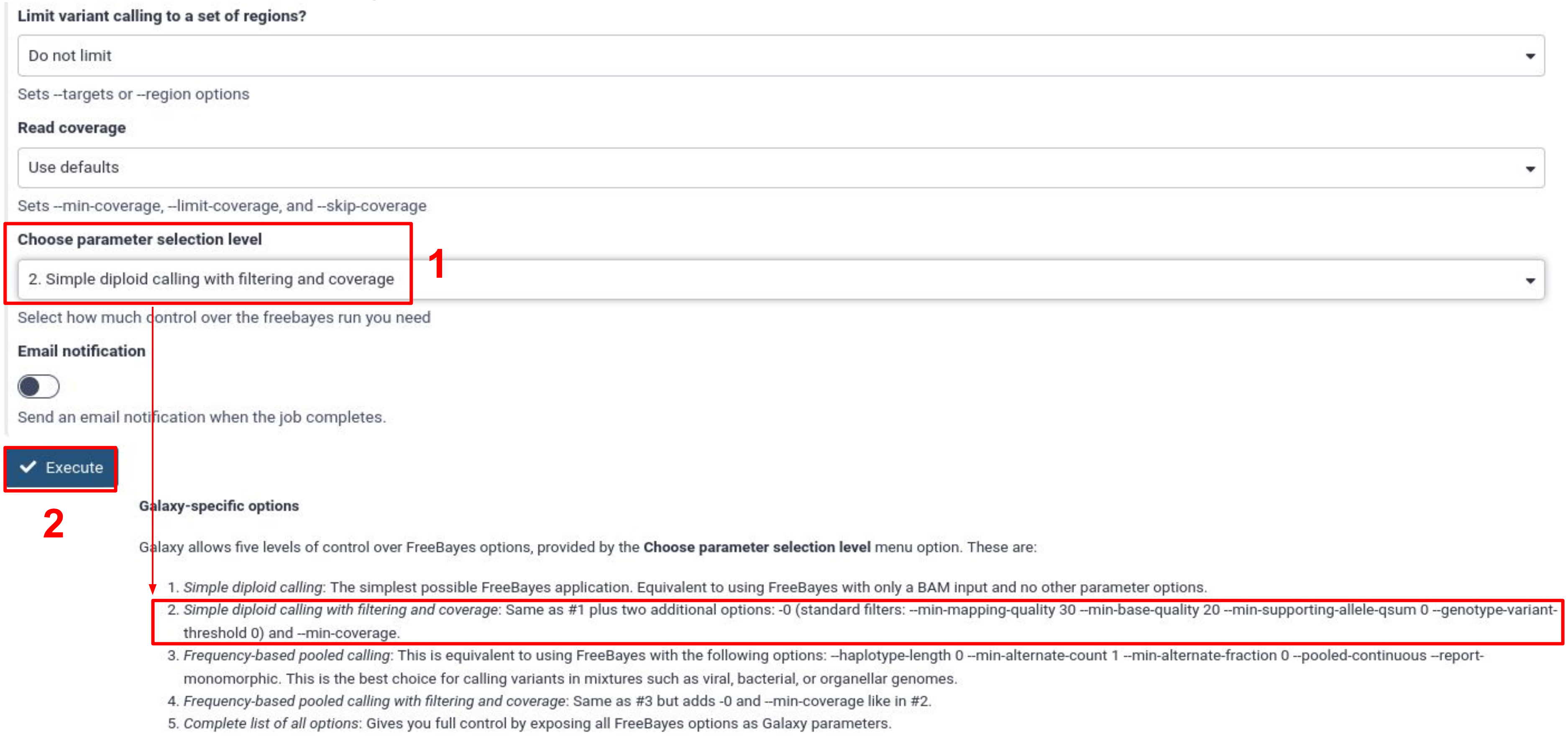
2 **BAM or CRAM dataset(s)**

- 13: markdup_proband.bam
- 11: markdup_mother.bam
- 9: markdup_father.bam
- 7: filtered_reads_proband.bam
- 6: filtered_reads_mother.bam
- 5: filtered_reads_father.bam

3 **Using reference genome**

Human (Homo sapiens): hg19

Variant calling



Limit variant calling to a set of regions?

Do not limit

Sets --targets or --region options

Read coverage

Use defaults

Sets --min-coverage, --limit-coverage, and --skip-coverage

Choose parameter selection level

2. Simple diploid calling with filtering and coverage

Select how much control over the freebayes run you need

Email notification

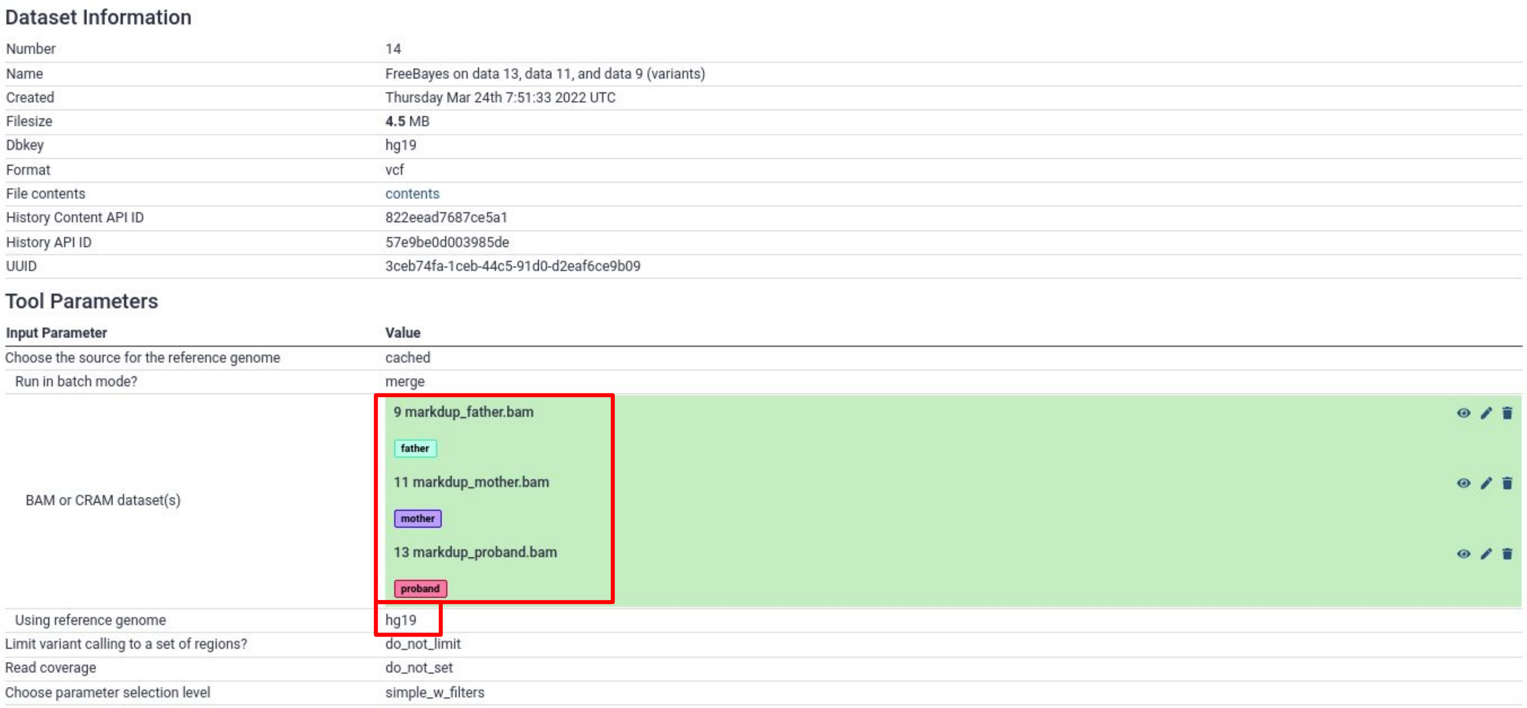
Send an email notification when the job completes.

Galaxy-specific options

Galaxy allows five levels of control over FreeBayes options, provided by the **Choose parameter selection level** menu option. These are:

1. *Simple diploid calling*: The simplest possible FreeBayes application. Equivalent to using FreeBayes with only a BAM input and no other parameter options.
2. *Simple diploid calling with filtering and coverage*: Same as #1 plus two additional options: -0 (standard filters: --min-mapping-quality 30 --min-base-quality 20 --min-supporting-allele-qsum 0 --genotype-variant-threshold 0) and --min-coverage.
3. *Frequency-based pooled calling*: This is equivalent to using FreeBayes with the following options: –haplotype-length 0 --min-alternate-count 1 --min-alternate-fraction 0 --pooled-continuous --report-monomorphic. This is the best choice for calling variants in mixtures such as viral, bacterial, or organellar genomes.
4. *Frequency-based pooled calling with filtering and coverage*: Same as #3 but adds -0 and --min-coverage like in #2.
5. *Complete list of all options*: Gives you full control by exposing all FreeBayes options as Galaxy parameters.

Variant calling

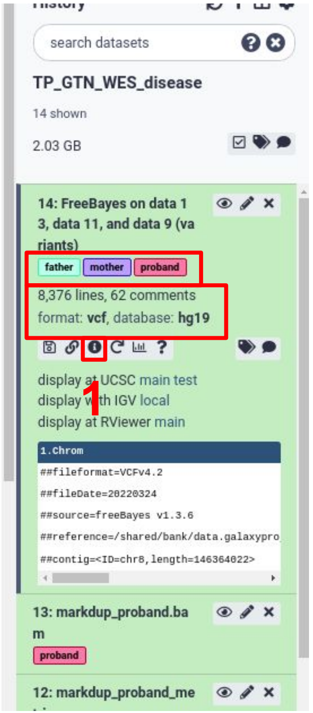


Dataset Information

Number	14
Name	FreeBayes on data 13, data 11, and data 9 (variants)
Created	Thursday Mar 24th 7:51:33 2022 UTC
Filesize	**4.5** MB
Dbkey	hg19
Format	vcf
File contents	contents
History Content API ID	822eead7687ce5a1
History API ID	57e9be0d003985de
UUID	3ceb74fa-1ceb-44c5-91d0-d2eaf6ce9b09

Tool Parameters

Input Parameter	Value
Choose the source for the reference genome	cached
Run in batch mode?	merge
BAM or CRAM dataset(s)	9 markdup_father.bam father 11 markdup_mother.bam mother 13 markdup_proband.bam proband
Using reference genome	hg19
Limit variant calling to a set of regions?	do_not_limit
Read coverage	do_not_set
Choose parameter selection level	simple_w_filters



History

search datasets

TP_GTN_WES_disease

14 shown

2.03 GB

14: FreeBayes on data 13, data 11, and data 9 (variants)

father mother proband

8,376 lines, 62 comments

format: **vcf**, database: **hg19**

display at UCSC main test

display with IGV local

display at RViewer main

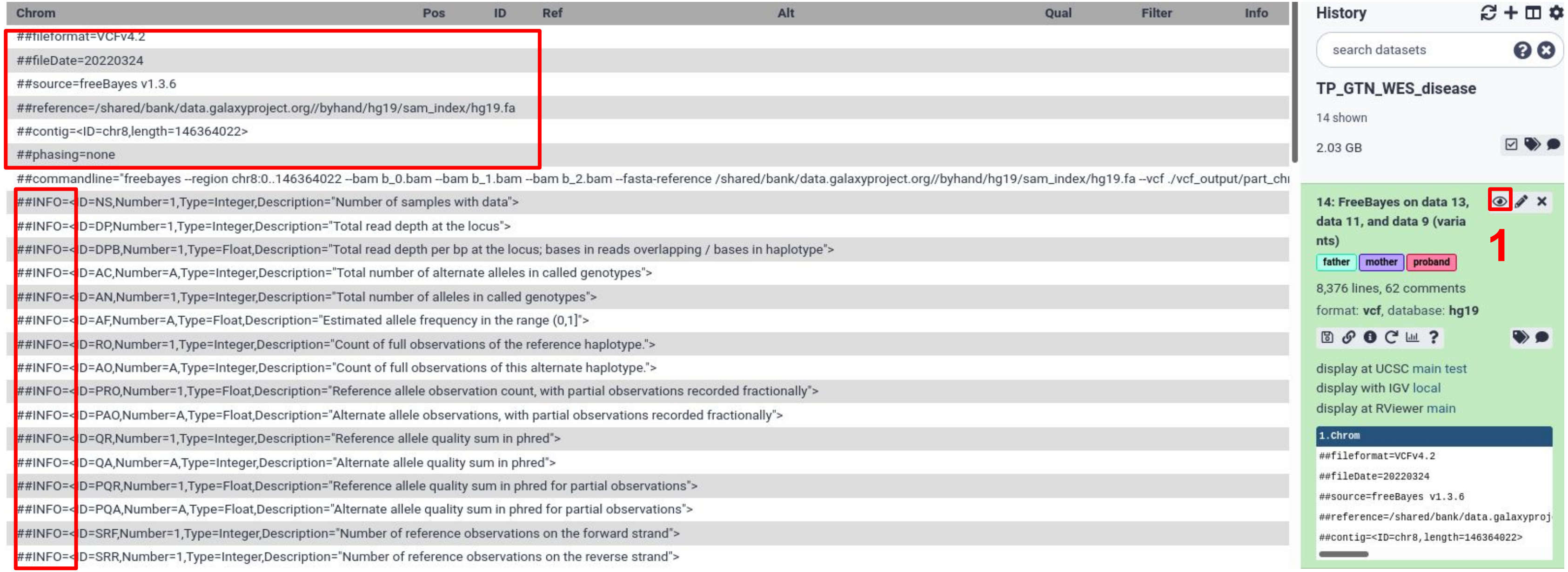
```
1.Chrom
##fileformat=VCFv4.2
##fileDate=20220324
##source=freeBayes v1.3.6
##reference=/shared/bank/data.galaxypro
##contig=<ID=chr8,length=146364022>
```

13: markdup_proband.bam

proband

12: markdup_proband_me

Variant calling - VCF



```
Chrom	Pos	ID	Ref	Alt	Qual	Filter	Info
##fileformat=VCFv4.2
##fileDate=20220324
##source=freeBayes v1.3.6
##reference=/shared/bank/data.galaxyproject.org//byhand/hg19/sam_index/hg19.fa
##contig=<ID=chr8,length=146364022>
##phasing=none
##commandline="freebayes --region chr8:0..146364022 --bam b_0.bam --bam b_1.bam --bam b_2.bam --fasta-reference /shared/bank/data.galaxyproject.org//byhand/hg19/sam_index/hg19.fa --vcf ./vcf_output/part_ch
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of samples with data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total read depth at the locus">
##INFO=<ID=DPB,Number=1,Type=Float,Description="Total read depth per bp at the locus; bases in reads overlapping / bases in haplotype">
##INFO=<ID=AC,Number=A,Type=Integer,Description="Total number of alternate alleles in called genotypes">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##INFO=<ID=AF,Number=A,Type=Float,Description="Estimated allele frequency in the range (0,1]">
##INFO=<ID=RO,Number=1,Type=Integer,Description="Count of full observations of the reference haplotype.">
##INFO=<ID=AO,Number=A,Type=Integer,Description="Count of full observations of this alternate haplotype.">
##INFO=<ID=PRO,Number=1,Type=Float,Description="Reference allele observation count, with partial observations recorded fractionally">
##INFO=<ID=PAO,Number=A,Type=Float,Description="Alternate allele observations, with partial observations recorded fractionally">
##INFO=<ID=QR,Number=1,Type=Integer,Description="Reference allele quality sum in phred">
##INFO=<ID=QA,Number=A,Type=Integer,Description="Alternate allele quality sum in phred">
##INFO=<ID=PQR,Number=1,Type=Float,Description="Reference allele quality sum in phred for partial observations">
##INFO=<ID=PQA,Number=A,Type=Float,Description="Alternate allele quality sum in phred for partial observations">
##INFO=<ID=SRF,Number=1,Type=Integer,Description="Number of reference observations on the forward strand">
##INFO=<ID=SRR,Number=1,Type=Integer,Description="Number of reference observations on the reverse strand">
```

TP_GTN_WES_disease

14 shown

2.03 GB

14: FreeBayes on data 13, data 11, and data 9 (variants)

8,376 lines, 62 comments

format: **vcf**, database: **hg19**

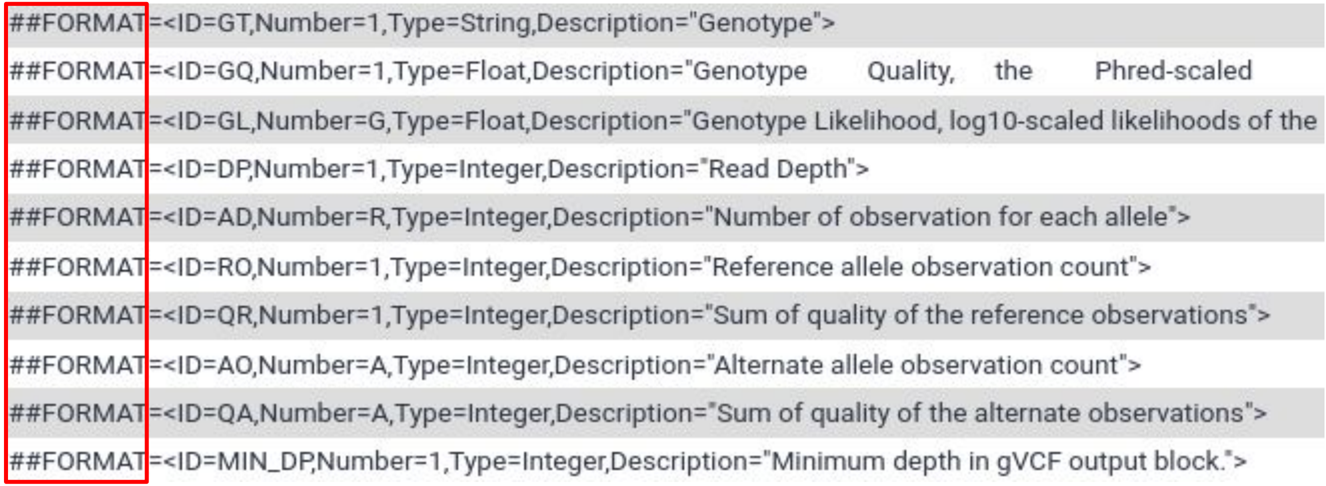
display at UCSC main test

display with IGV local

display at RViewer main

```
1.Chrom
##fileformat=VCFv4.2
##fileDate=20220324
##source=freeBayes v1.3.6
##reference=/shared/bank/data.galaxyproj
##contig=<ID=chr8,length=146364022>
```

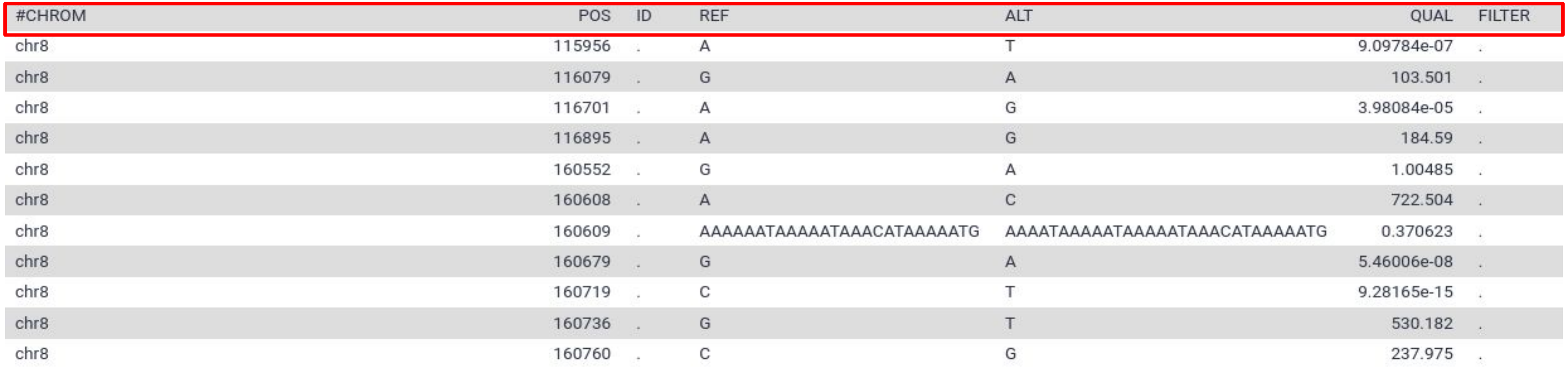
Variant calling - VCF



```
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Float,Description="Genotype Quality, the Phred-scaled
##FORMAT=<ID=GL,Number=G,Type=Float,Description="Genotype Likelihood, log10-scaled likelihoods of the
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Number of observation for each allele">
##FORMAT=<ID=RO,Number=1,Type=Integer,Description="Reference allele observation count">
##FORMAT=<ID=QR,Number=1,Type=Integer,Description="Sum of quality of the reference observations">
##FORMAT=<ID=AO,Number=A,Type=Integer,Description="Alternate allele observation count">
##FORMAT=<ID=QA,Number=A,Type=Integer,Description="Sum of quality of the alternate observations">
##FORMAT=<ID=MIN_DP,Number=1,Type=Integer,Description="Minimum depth in gVCF output block.">
```

Variant calling - VCF

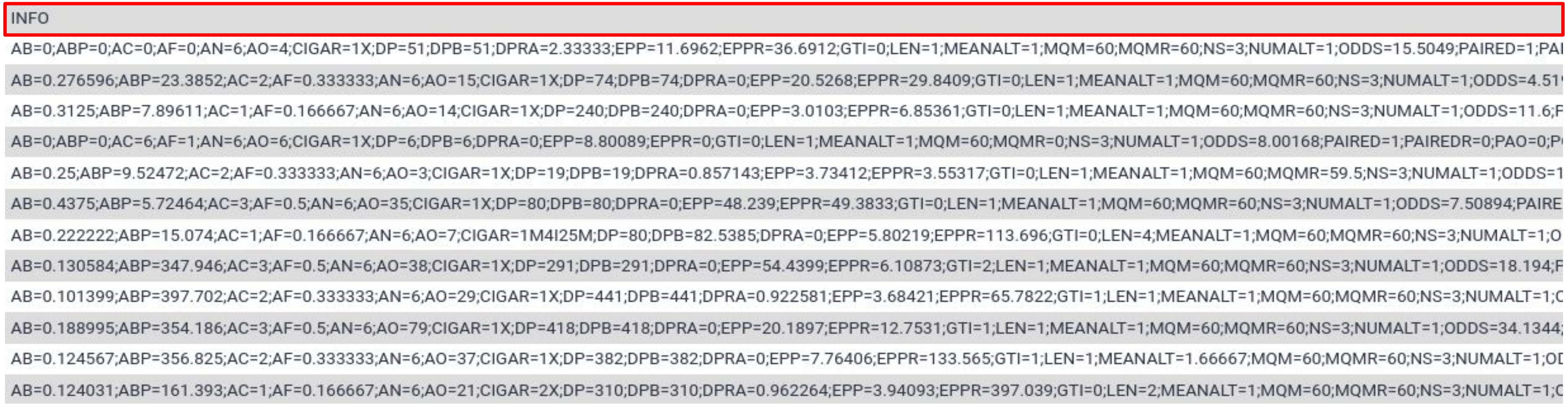
Mandatory columns



#CHROM	POS	ID	REF	ALT	QUAL	FILTER
chr8	115956	.	A	T	9.09784e-07	.
chr8	116079	.	G	A	103.501	.
chr8	116701	.	A	G	3.98084e-05	.
chr8	116895	.	A	G	184.59	.
chr8	160552	.	G	A	1.00485	.
chr8	160608	.	A	C	722.504	.
chr8	160609	.	AAAAAATAAAAATAAACATAAAAATG	AAAATAAAAATAAAAATAAACATAAAAATG	0.370623	.
chr8	160679	.	G	A	5.46006e-08	.
chr8	160719	.	C	T	9.28165e-15	.
chr8	160736	.	G	T	530.182	.
chr8	160760	.	C	G	237.975	.

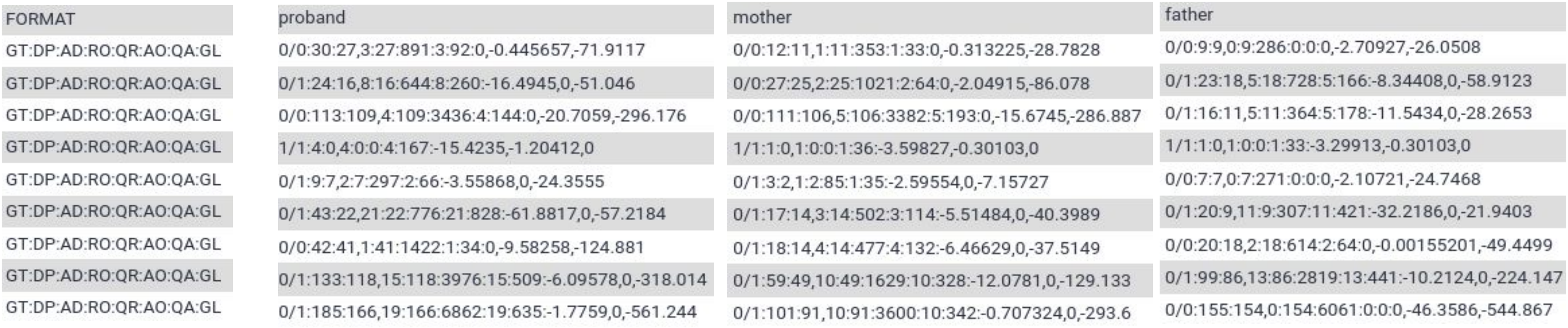
Variant calling - VCF

Mandatory column



INFO
AB=0;ABP=0;AC=0;AF=0;AN=6;AO=4;CIGAR=1X;DP=51;DPB=51;DPRA=2.33333;EPP=11.6962;EPPR=36.6912;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;ODDS=15.5049;PAIRED=1;PAI
AB=0.276596;ABP=23.3852;AC=2;AF=0.333333;AN=6;AO=15;CIGAR=1X;DP=74;DPB=74;DPRA=0;EPP=20.5268;EPPR=29.8409;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;ODDS=4.51
AB=0.3125;ABP=7.89611;AC=1;AF=0.166667;AN=6;AO=14;CIGAR=1X;DP=240;DPB=240;DPRA=0;EPP=3.0103;EPPR=6.85361;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;ODDS=11.6;P
AB=0;ABP=0;AC=6;AF=1;AN=6;AO=6;CIGAR=1X;DP=6;DPB=6;DPRA=0;EPP=8.80089;EPPR=0;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=0;NS=3;NUMALT=1;ODDS=8.00168;PAIRED=1;PAIREDR=0;PAO=0;P
AB=0.25;ABP=9.52472;AC=2;AF=0.333333;AN=6;AO=3;CIGAR=1X;DP=19;DPB=19;DPRA=0.857143;EPP=3.73412;EPPR=3.55317;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=59.5;NS=3;NUMALT=1;ODDS=1
AB=0.4375;ABP=5.72464;AC=3;AF=0.5;AN=6;AO=35;CIGAR=1X;DP=80;DPB=80;DPRA=0;EPP=48.239;EPPR=49.3833;GTI=0;LEN=1;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;ODDS=7.50894;PAIRE
AB=0.222222;ABP=15.074;AC=1;AF=0.166667;AN=6;AO=7;CIGAR=1M4I25M;DP=80;DPB=82.5385;DPRA=0;EPP=5.80219;EPPR=113.696;GTI=0;LEN=4;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;O
AB=0.130584;ABP=347.946;AC=3;AF=0.5;AN=6;AO=38;CIGAR=1X;DP=291;DPB=291;DPRA=0;EPP=54.4399;EPPR=6.10873;GTI=2;LEN=1;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;ODDS=18.194;P
AB=0.101399;ABP=397.702;AC=2;AF=0.333333;AN=6;AO=29;CIGAR=1X;DP=441;DPB=441;DPRA=0.922581;EPP=3.68421;EPPR=65.7822;GTI=1;LEN=1;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;O
AB=0.188995;ABP=354.186;AC=3;AF=0.5;AN=6;AO=79;CIGAR=1X;DP=418;DPB=418;DPRA=0;EPP=20.1897;EPPR=12.7531;GTI=1;LEN=1;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;ODDS=34.1344;
AB=0.124567;ABP=356.825;AC=2;AF=0.333333;AN=6;AO=37;CIGAR=1X;DP=382;DPB=382;DPRA=0;EPP=7.76406;EPPR=133.565;GTI=1;LEN=1;MEANALT=1.66667;MQM=60;MQMR=60;NS=3;NUMALT=1;OD
AB=0.124031;ABP=161.393;AC=1;AF=0.166667;AN=6;AO=21;CIGAR=2X;DP=310;DPB=310;DPRA=0.962264;EPP=3.94093;EPPR=397.039;GTI=0;LEN=2;MEANALT=1;MQM=60;MQMR=60;NS=3;NUMALT=1;O

Variant calling - VCF



FORMAT	proband	mother	father
GT:DP:AD:RO:QR:AO:QA:GL	0/0:30:27,3:27:891:3:92:0,-0.445657,-71.9117	0/0:12:11,1:11:353:1:33:0,-0.313225,-28.7828	0/0:9:9,0:9:286:0:0:0,-2.70927,-26.0508
GT:DP:AD:RO:QR:AO:QA:GL	0/1:24:16,8:16:644:8:260:-16.4945,0,-51.046	0/0:27:25,2:25:1021:2:64:0,-2.04915,-86.078	0/1:23:18,5:18:728:5:166:-8.34408,0,-58.9123
GT:DP:AD:RO:QR:AO:QA:GL	0/0:113:109,4:109:3436:4:144:0,-20.7059,-296.176	0/0:111:106,5:106:3382:5:193:0,-15.6745,-286.887	0/1:16:11,5:11:364:5:178:-11.5434,0,-28.2653
GT:DP:AD:RO:QR:AO:QA:GL	1/1:4:0,4:0:0:4:167:-15.4235,-1.20412,0	1/1:1:0,1:0:0:1:36:-3.59827,-0.30103,0	1/1:1:0,1:0:0:1:33:-3.29913,-0.30103,0
GT:DP:AD:RO:QR:AO:QA:GL	0/1:9:7,2:7:297:2:66:-3.55868,0,-24.3555	0/1:3:2,1:2:85:1:35:-2.59554,0,-7.15727	0/0:7:7,0:7:271:0:0:0,-2.10721,-24.7468
GT:DP:AD:RO:QR:AO:QA:GL	0/1:43:22,21:22:776:21:828:-61.8817,0,-57.2184	0/1:17:14,3:14:502:3:114:-5.51484,0,-40.3989	0/1:20:9,11:9:307:11:421:-32.2186,0,-21.9403
GT:DP:AD:RO:QR:AO:QA:GL	0/0:42:41,1:41:1422:1:34:0,-9.58258,-124.881	0/1:18:14,4:14:477:4:132:-6.46629,0,-37.5149	0/0:20:18,2:18:614:2:64:0,-0.00155201,-49.4499
GT:DP:AD:RO:QR:AO:QA:GL	0/1:133:118,15:118:3976:15:509:-6.09578,0,-318.014	0/1:59:49,10:49:1629:10:328:-12.0781,0,-129.133	0/1:99:86,13:86:2819:13:441:-10.2124,0,-224.147
GT:DP:AD:RO:QR:AO:QA:GL	0/1:185:166,19:166:6862:19:635:-1.7759,0,-561.244	0/1:101:91,10:91:3600:10:342:-0.707324,0,-293.6	0/0:155:154,0:154:6061:0:0:0,-46.3586,-544.867

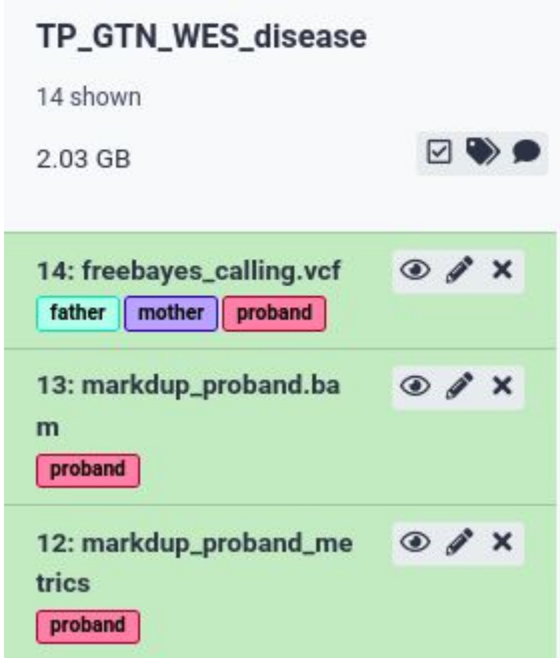
Genotypes format

Proband genotypes information

Mother genotypes information

Father genotypes information

Variant calling



TP_GTN_WES_disease

14 shown

2.03 GB

14: freebayes_calling.vcf

father mother proband

13: markdup_proband.bam

proband

12: markdup_proband_metrics

proband

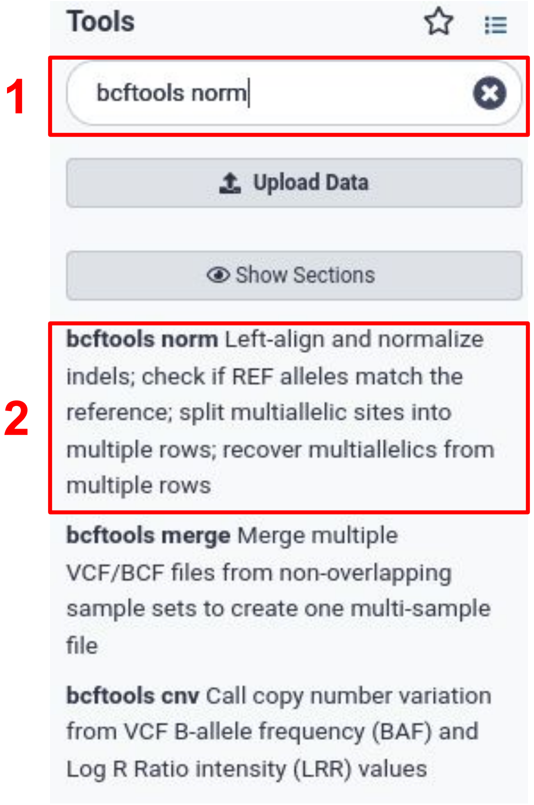
Tutorial steps

1. Perform postprocessing from premapped reads

2. Variant calling

3. Variant annotation and reporting

Variant normalization

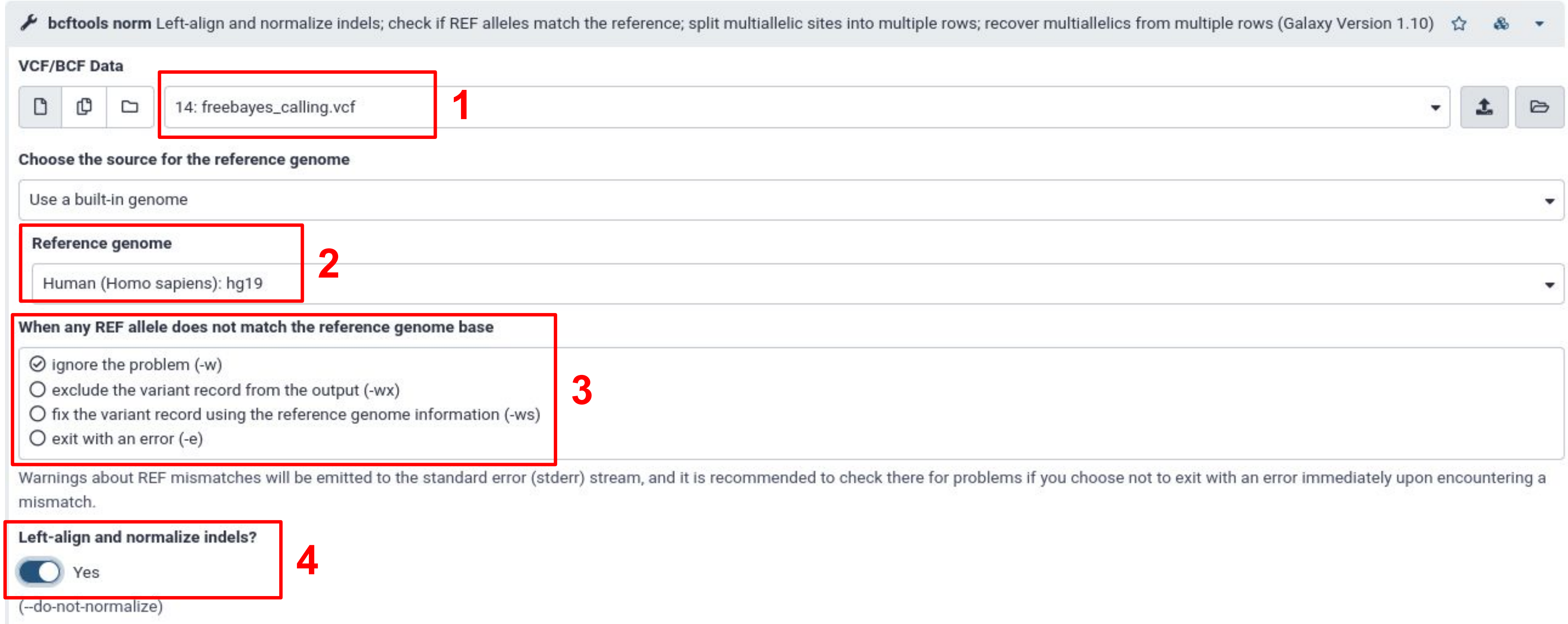


bcftools norm Left-align and normalize indels; check if REF alleles match the reference; split multiallelic sites into multiple rows; recover multiallelics from multiple rows

bcftools merge Merge multiple VCF/BCF files from non-overlapping sample sets to create one multi-sample file

bcftools cnv Call copy number variation from VCF B-allele frequency (BAF) and Log R Ratio intensity (LRR) values

Variant normalization



bcftools norm Left-align and normalize indels; check if REF alleles match the reference; split multiallelic sites into multiple rows; recover multiallelics from multiple rows (Galaxy Version 1.10)

VCF/BCF Data

14: freebayes_calling.vcf

Choose the source for the reference genome

Use a built-in genome

Reference genome

Human (Homo sapiens): hg19

When any REF allele does not match the reference genome base

- ● ignore the problem (-w)
- ○ exclude the variant record from the output (-wx)
- ○ fix the variant record using the reference genome information (-ws)
- ○ exit with an error (-e)

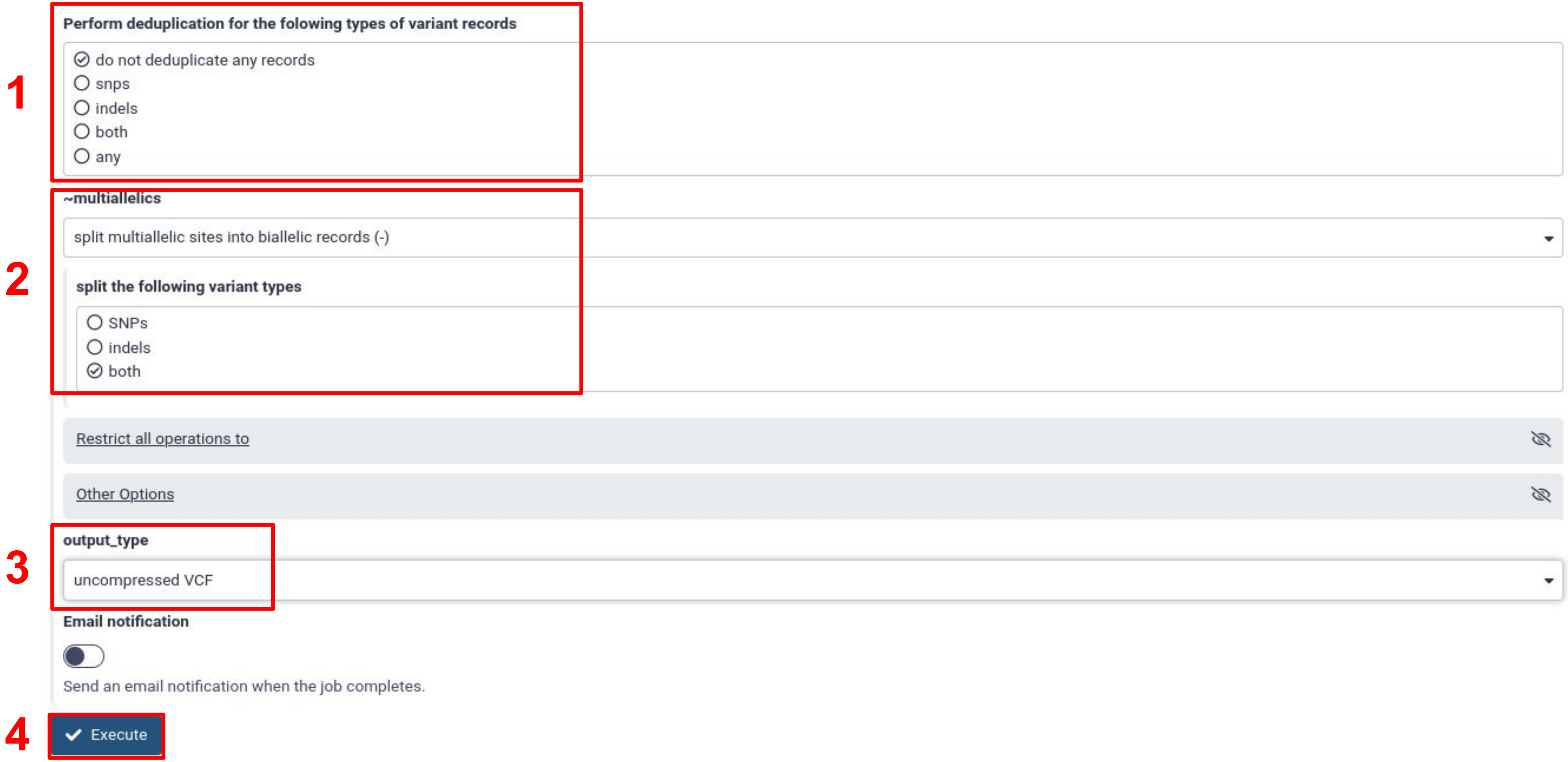
Warnings about REF mismatches will be emitted to the standard error (stderr) stream, and it is recommended to check there for problems if you choose not to exit with an error immediately upon encountering a mismatch.

Left-align and normalize indels?

● Yes

(--do-not-normalize)

Variant normalization



1

Perform deduplication for the folowing types of variant records

● do not deduplicate any records
○ snps
○ indels
○ both
○ any

2

~multiallelics

split multiallelic sites into biallelic records (-)

split the following variant types

○ SNPs
○ indels
● both

3

output_type

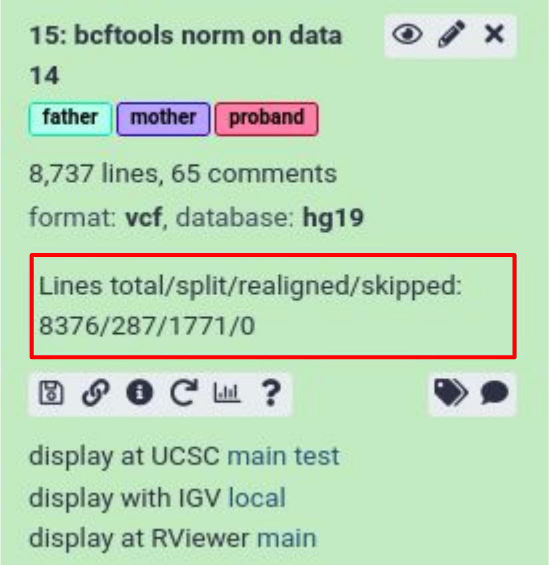
uncompressed VCF

Email notification

Send an email notification when the job completes.

4

Variant normalization



15: bcftools norm on data 14

8,737 lines, 65 comments

format: **vcf**, database: **hg19**

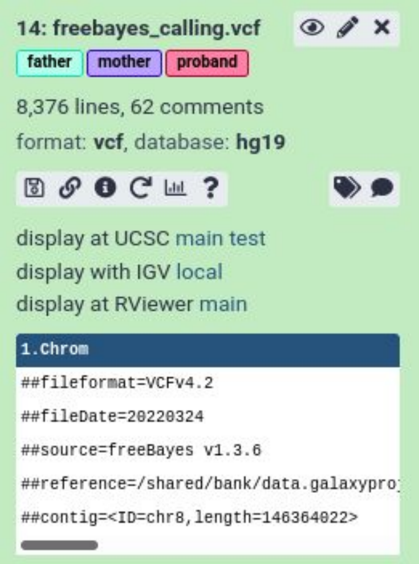
Lines total/split/realigned/skipped:
8376/287/1771/0

display at UCSC main test

display with IGV local

display at RViewer main

Variant normalization - Alleles



14: freebayes_calling.vcf

father mother proband

8,376 lines, 62 comments

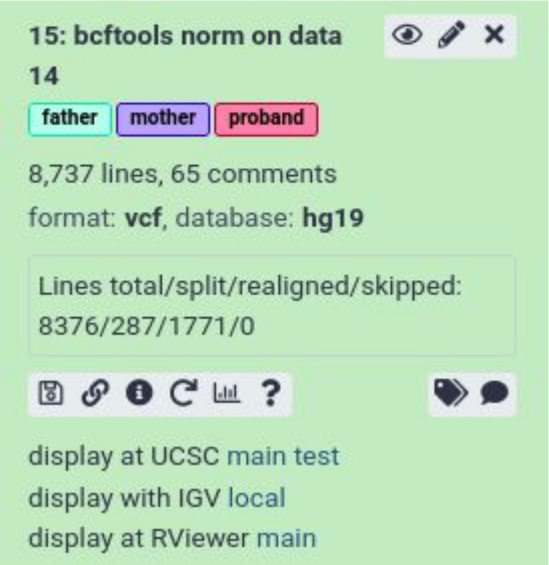
format: **vcf**, database: **hg19**

display at UCSC main test

display with IGV local

display at RViewer main

```
1.Chrom
##fileformat=VCFv4.2
##fileDate=20220324
##source=freeBayes v1.3.6
##reference=/shared/bank/data.galaxypro
##contig=<ID=chr8,length=146364022>
```



15: bcftools norm on data 14

father mother proband

8,737 lines, 65 comments

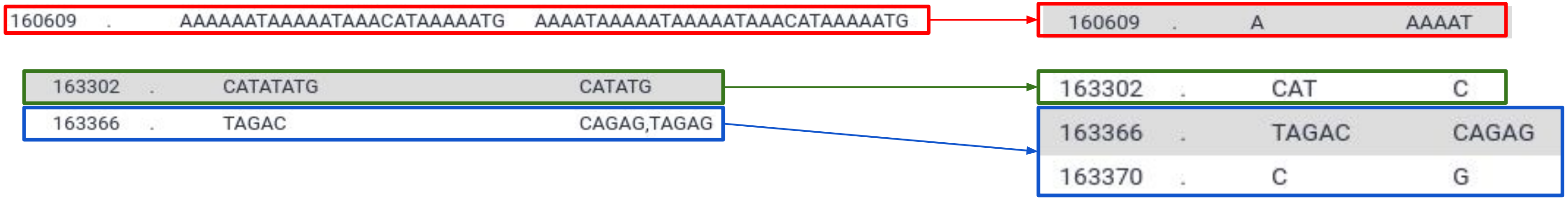
format: **vcf**, database: **hg19**

Lines total/split/realigned/skipped:
8376/287/1771/0

display at UCSC main test

display with IGV local

display at RViewer main



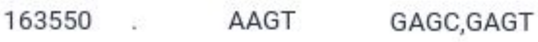
160609 . AAAAAATAAAAATAAACATAAAAATG AAAATAAAAAATAAAAATAAACATAAAAATG → 160609 . A AAAAT

163302 . CATATATG CATATG → 163302 . CAT C

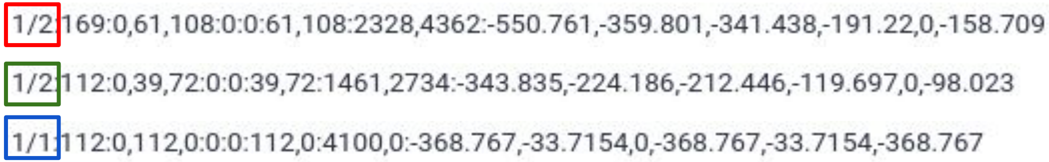
163366 . TAGAC CAGAG,TAGAG → 163366 . TAGAC CAGAG
163370 . C G

Variant normalization - Genotypes

Initial file

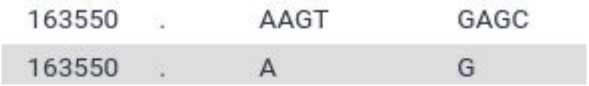


```
163550    .    AAGT    GAGC,GAGT
```

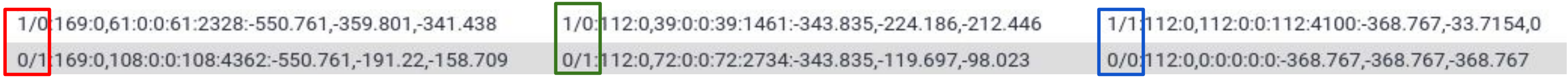


```
1/2:169:0,61,108:0:0:61,108:2328,4362:-550.761,-359.801,-341.438,-191.22,0,-158.709
1/2:112:0,39,72:0:0:39,72:1461,2734:-343.835,-224.186,-212.446,-119.697,0,-98.023
1/1:112:0,112,0:0:0:112,0:4100,0:-368.767,-33.7154,0,-368.767,-33.7154,-368.767
```

Normalized file



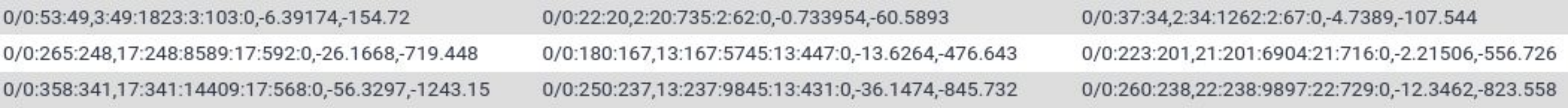
```
163550    .    AAGT    GAGC
163550    .    A       G
```



```
1/0:169:0,61:0:0:61:2328:-550.761,-359.801,-341.438     1/0:112:0,39:0:0:39:1461:-343.835,-224.186,-212.446     1/1:112:0,112:0:0:112:4100:-368.767,-33.7154,0
0/1:169:0,108:0:0:108:4362:-550.761,-191.22,-158.709    0/1:112:0,72:0:0:72:2734:-343.835,-119.697,-98.023      0/0:112:0,0:0:0:0:0:-368.767,-368.767,-368.767
```

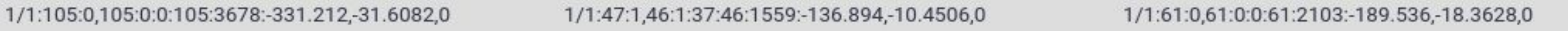
Variant filtering

Only Homozygous reference



0/0:53:49,3:49:1823:3:103:0,-6.39174,-154.72	0/0:22:20,2:20:735:2:62:0,-0.733954,-60.5893	0/0:37:34,2:34:1262:2:67:0,-4.7389,-107.544
0/0:265:248,17:248:8589:17:592:0,-26.1668,-719.448	0/0:180:167,13:167:5745:13:447:0,-13.6264,-476.643	0/0:223:201,21:201:6904:21:716:0,-2.21506,-556.726
0/0:358:341,17:341:14409:17:568:0,-56.3297,-1243.15	0/0:250:237,13:237:9845:13:431:0,-36.1474,-845.732	0/0:260:238,22:238:9897:22:729:0,-12.3462,-823.558

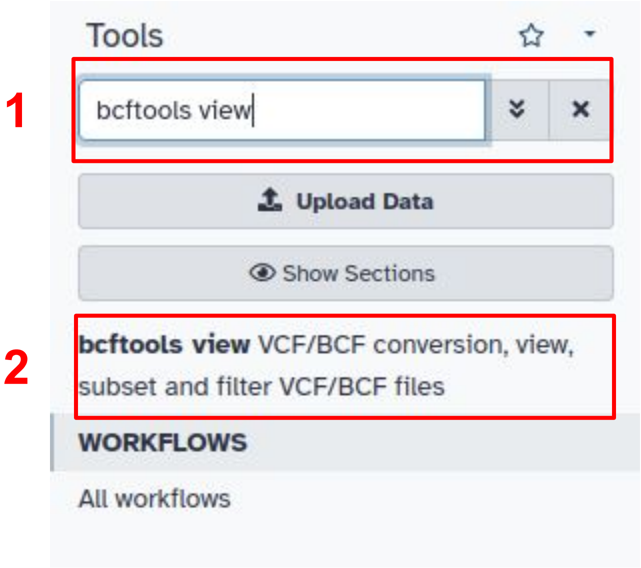
Only Homozygous alternate



1/1:105:0,105:0:0:105:3678:-331.212,-31.6082,0	1/1:47:1,46:1:37:46:1559:-136.894,-10.4506,0	1/1:61:0,61:0:0:61:2103:-189.536,-18.3628,0

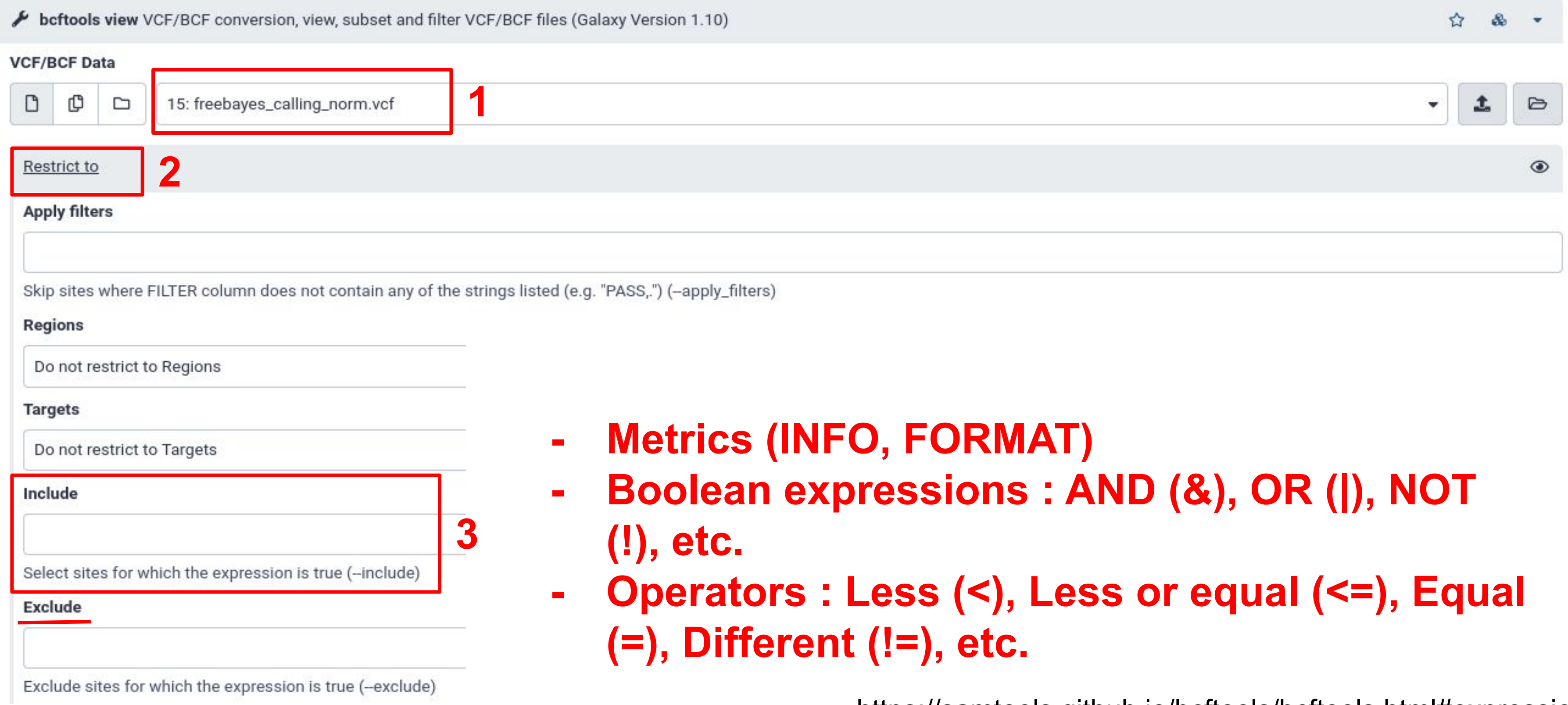
Do they bring some information in our case (proband affected) if we only consider genotypes?

Variant filtering



bcftools view VCF/BCF conversion, view, subset and filter VCF/BCF files

Variant filtering



bcftools view VCF/BCF conversion, view, subset and filter VCF/BCF files (Galaxy Version 1.10)

VCF/BCF Data

15: freebayes_calling_norm.vcf

Restrict to

Apply filters

Skip sites where FILTER column does not contain any of the strings listed (e.g. "PASS,.") (--apply_filters)

Regions

Do not restrict to Regions

Targets

Do not restrict to Targets

Include

Select sites for which the expression is true (--include)

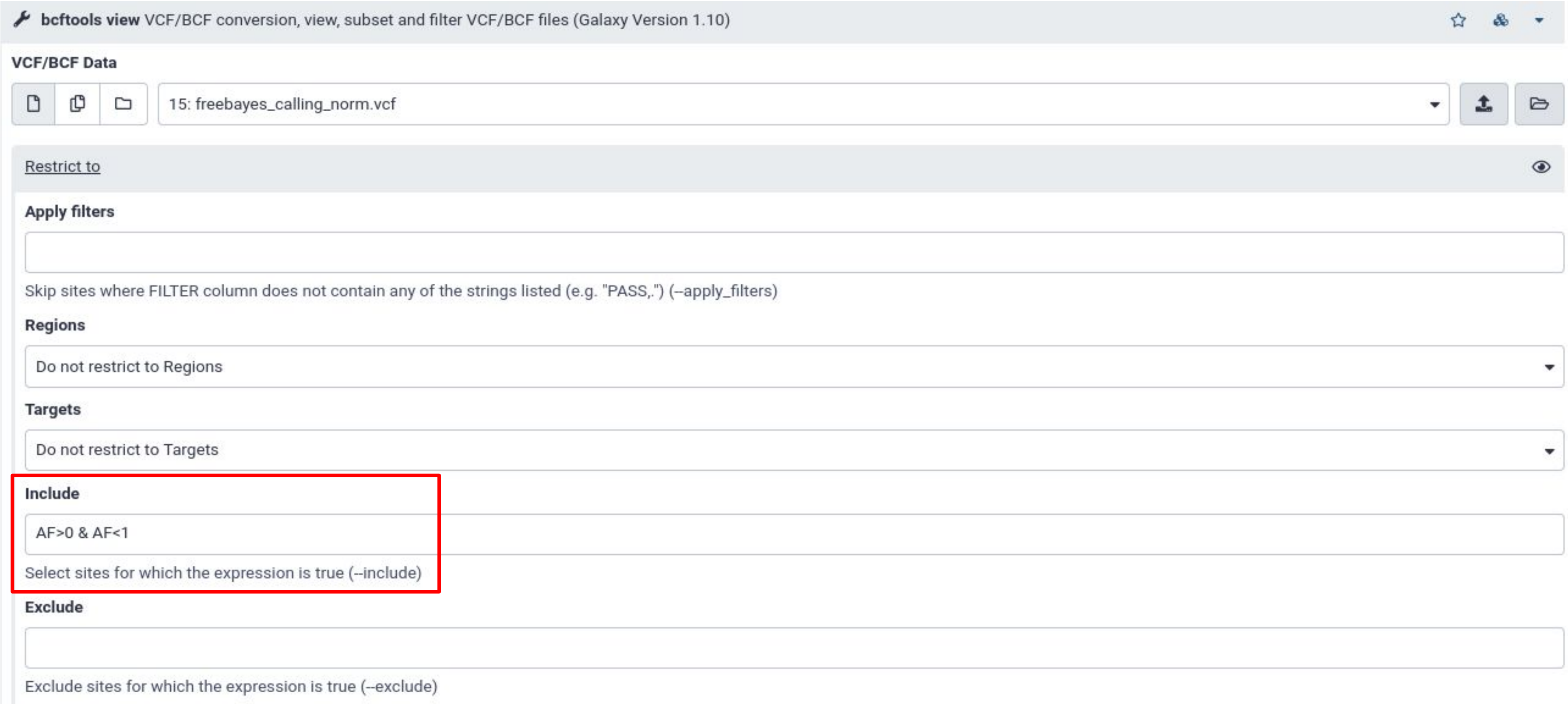
Exclude

Exclude sites for which the expression is true (--exclude)

- Metrics (INFO, FORMAT)
- Boolean expressions : AND (&), OR (|), NOT (!), etc.
- Operators : Less (<), Less or equal (<=), Equal (=), Different (!=), etc.

https://samtools.github.io/bcftools/bcftools.html#expressions

Variant filtering



bcftools view VCF/BCF conversion, view, subset and filter VCF/BCF files (Galaxy Version 1.10)

VCF/BCF Data

15: freebayes_calling_norm.vcf

Restrict to

Apply filters

Skip sites where FILTER column does not contain any of the strings listed (e.g. "PASS,.") (--apply_filters)

Regions

Do not restrict to Regions

Targets

Do not restrict to Targets

Include

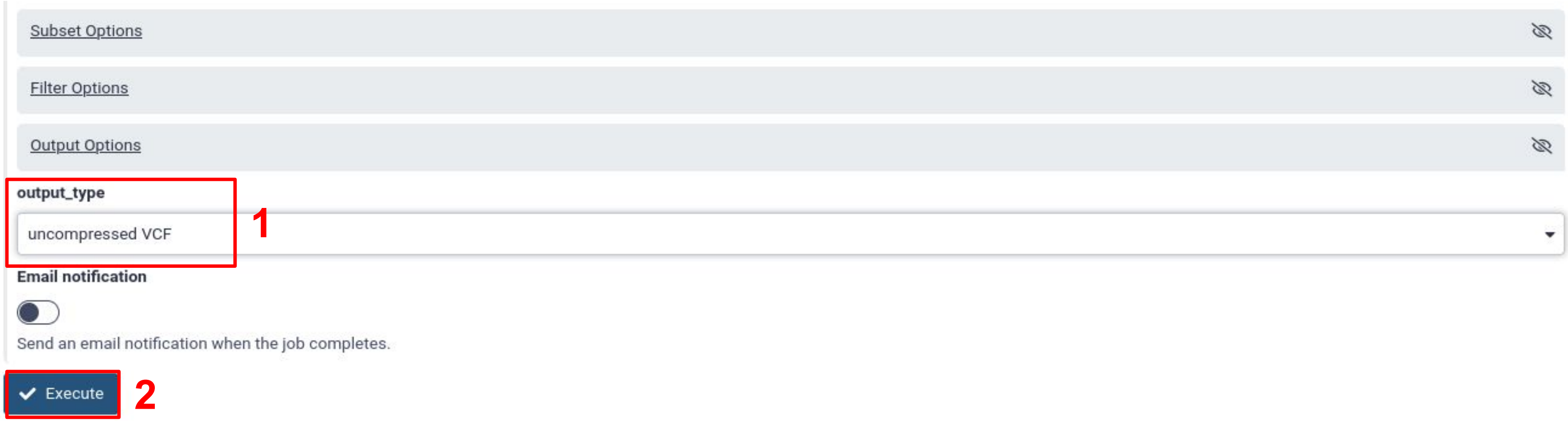
AF>0 & AF<1

Select sites for which the expression is true (--include)

Exclude

Exclude sites for which the expression is true (--exclude)

Variant filtering

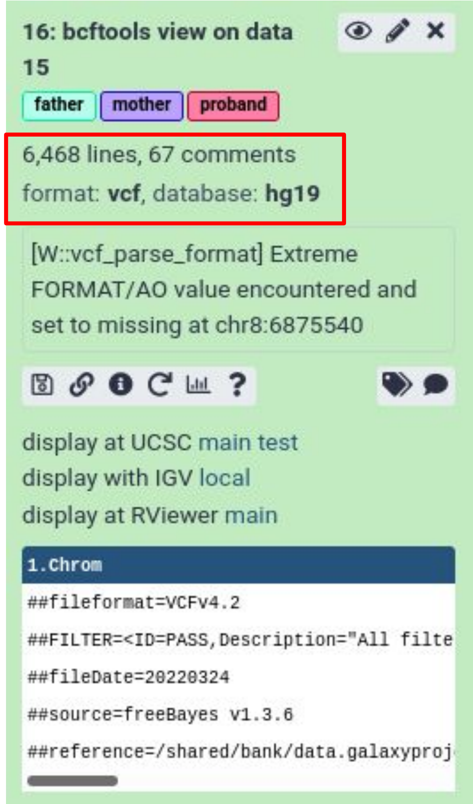


output_type: uncompressed VCF

Email notification

Send an email notification when the job completes.

Variant filtering



16: bcftools view on data 15

6,468 lines, 67 comments

format: **vcf**, database: **hg19**

[W::vcf_parse_format] Extreme FORMAT/AO value encountered and set to missing at chr8:6875540

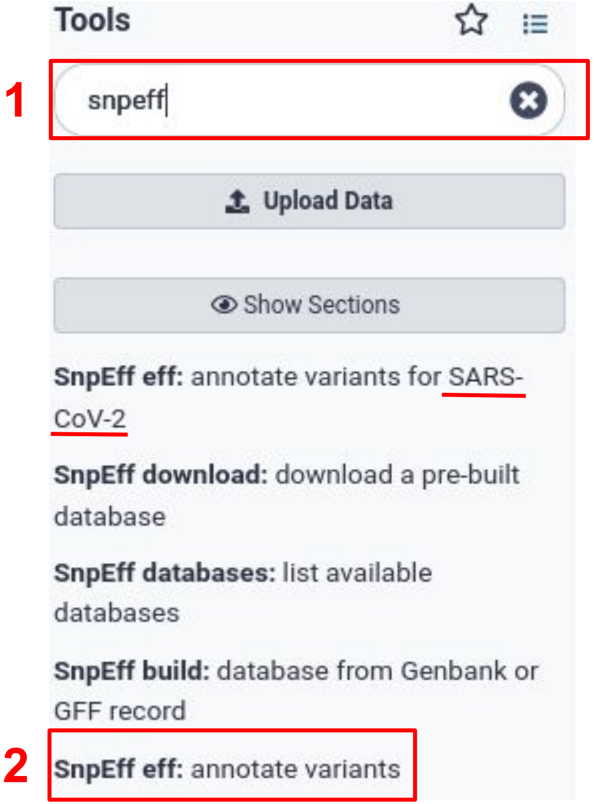
display at UCSC main test

display with IGV local

display at RViewer main

```
1.Chrom
##fileformat=VCFv4.2
##FILTER=<ID=PASS,Description="All filte
##fileDate=20220324
##source=freeBayes v1.3.6
##reference=/shared/bank/data.galaxyproj
```

Variant annotation



SnpEff eff: annotate variants for SARS-CoV-2

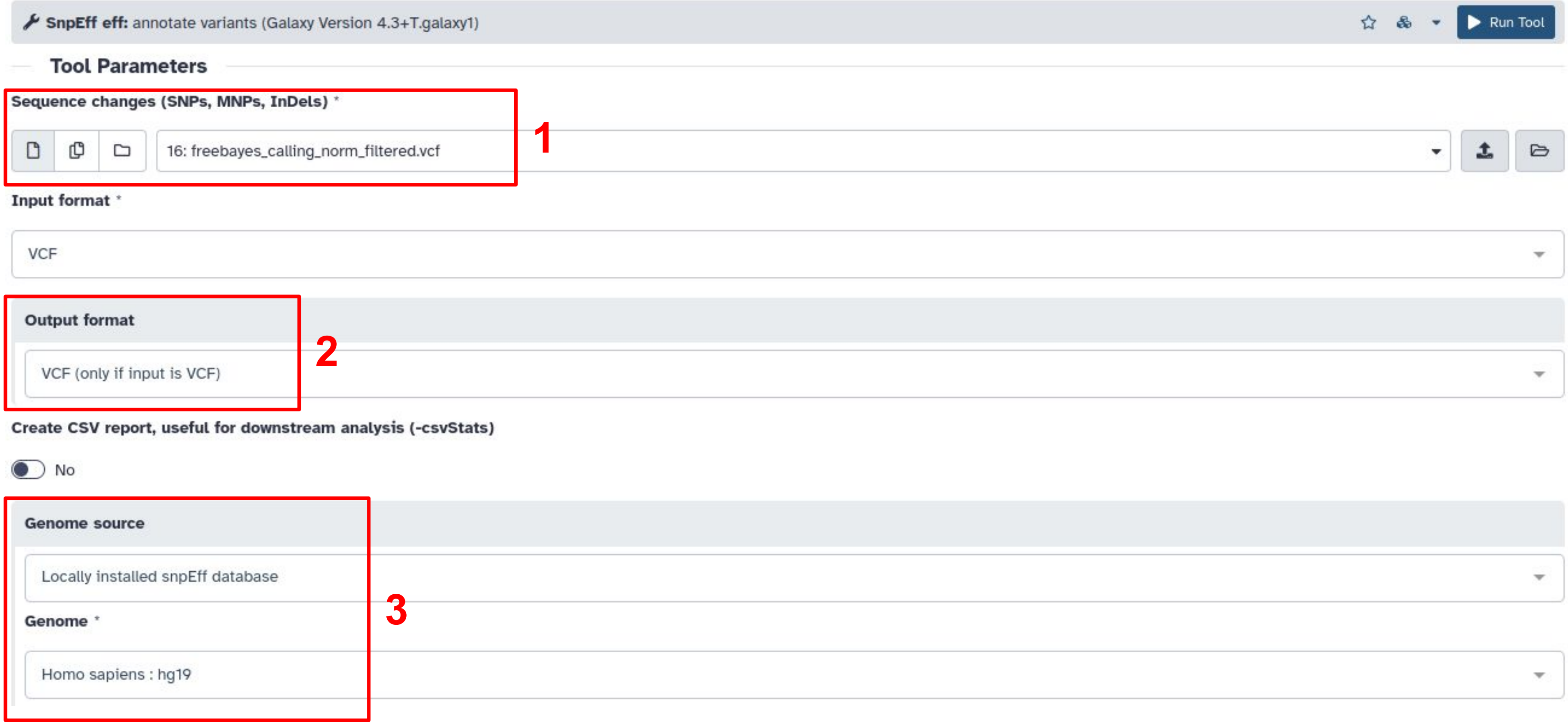
SnpEff download: download a pre-built database

SnpEff databases: list available databases

SnpEff build: database from Genbank or GFF record

SnpEff eff: annotate variants

Variant annotation



SnpEff eff: annotate variants (Galaxy Version 4.3+T.galaxy1)

Tool Parameters

Sequence changes (SNPs, MNPs, InDels) *

16: freebayes_calling_norm_filtered.vcf

Input format *

VCF

Output format

VCF (only if input is VCF)

Create CSV report, useful for downstream analysis (-csvStats)

○ No

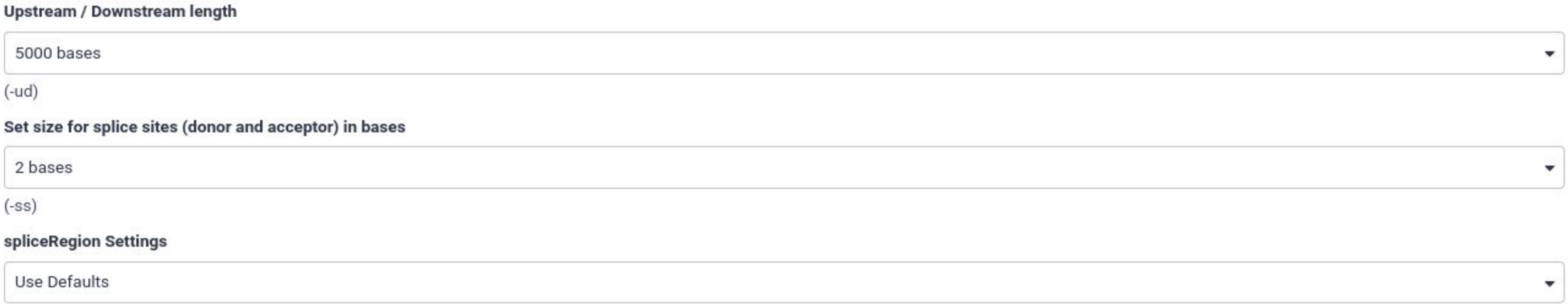
Genome source

Locally installed snpEff database

Genome *

Homo sapiens : hg19

Variant annotation



Upstream / Downstream length

5000 bases

(-ud)

Set size for splice sites (donor and acceptor) in bases

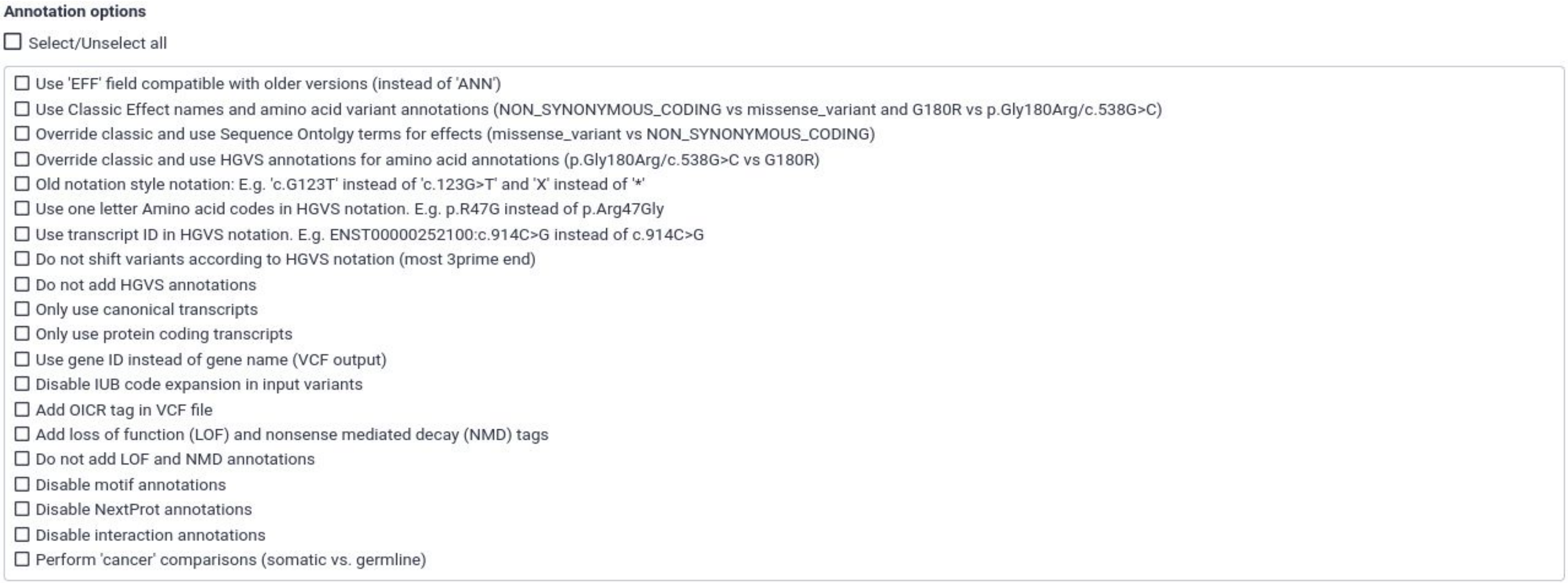
2 bases

(-ss)

spliceRegion Settings

Use Defaults

Variant annotation

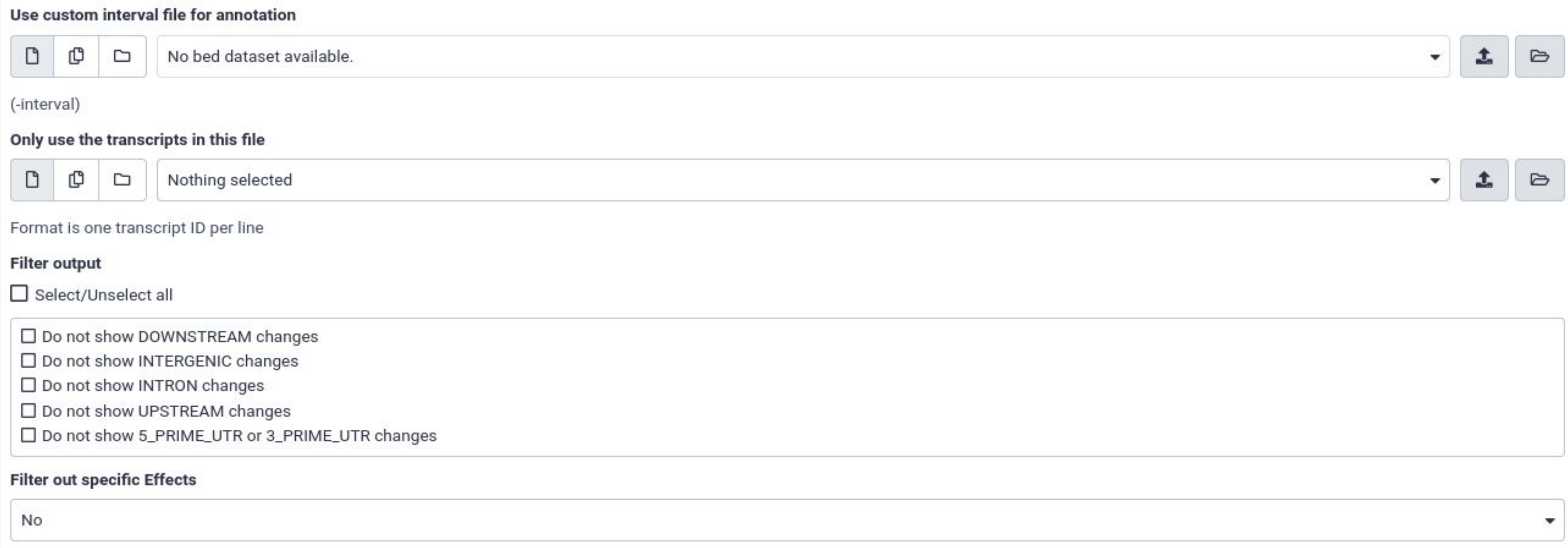


Annotation options

□ Select/Unselect all

□ Use 'EFF' field compatible with older versions (instead of 'ANN')
□ Use Classic Effect names and amino acid variant annotations (NON_SYNONYMOUS_CODING vs missense_variant and G180R vs p.Gly180Arg/c.538G>C)
□ Override classic and use Sequence Ontolgy terms for effects (missense_variant vs NON_SYNONYMOUS_CODING)
□ Override classic and use HGVS annotations for amino acid annotations (p.Gly180Arg/c.538G>C vs G180R)
□ Old notation style notation: E.g. 'c.G123T' instead of 'c.123G>T' and 'X' instead of '*'
□ Use one letter Amino acid codes in HGVS notation. E.g. p.R47G instead of p.Arg47Gly
□ Use transcript ID in HGVS notation. E.g. ENST00000252100:c.914C>G instead of c.914C>G
□ Do not shift variants according to HGVS notation (most 3prime end)
□ Do not add HGVS annotations
□ Only use canonical transcripts
□ Only use protein coding transcripts
□ Use gene ID instead of gene name (VCF output)
□ Disable IUB code expansion in input variants
□ Add OICR tag in VCF file
□ Add loss of function (LOF) and nonsense mediated decay (NMD) tags
□ Do not add LOF and NMD annotations
□ Disable motif annotations
□ Disable NextProt annotations
□ Disable interaction annotations
□ Perform 'cancer' comparisons (somatic vs. germline)

Variant annotation



Use custom interval file for annotation

No bed dataset available.

(-interval)

Only use the transcripts in this file

Nothing selected

Format is one transcript ID per line

Filter output

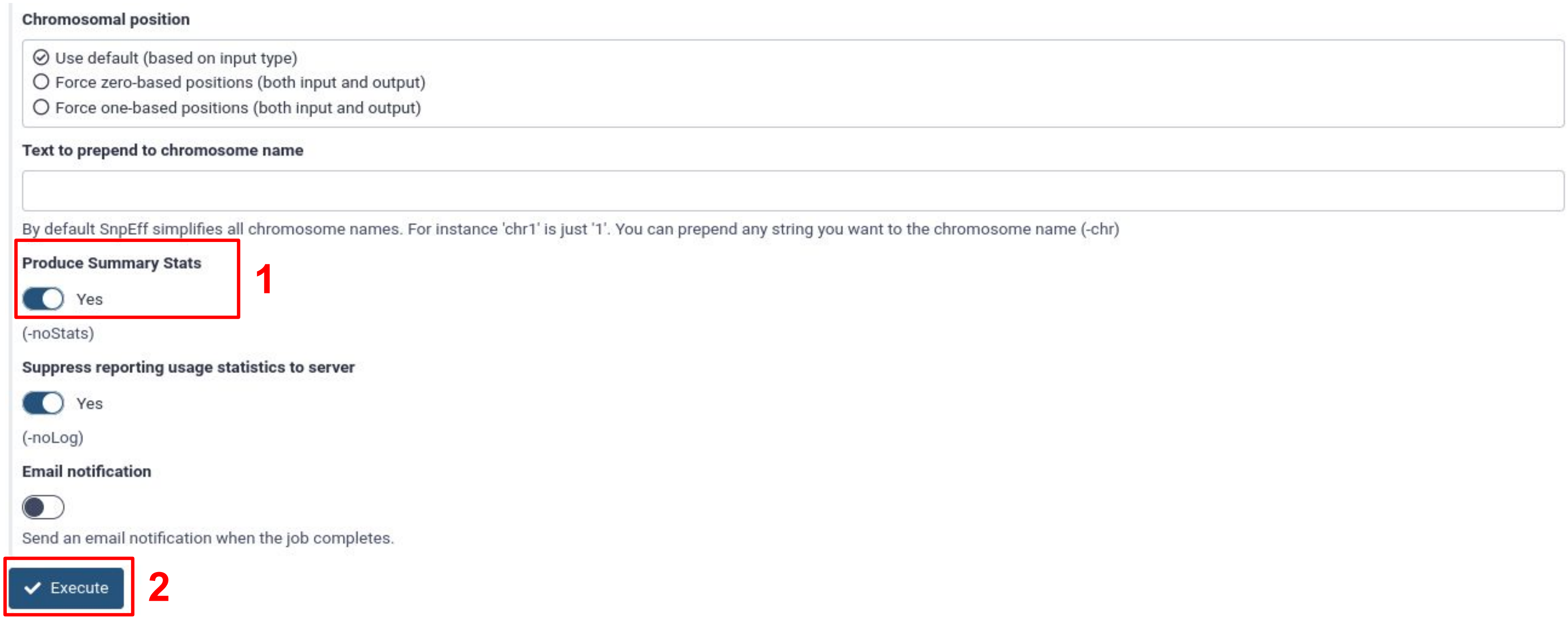
□ Select/Unselect all

□ Do not show DOWNSTREAM changes
□ Do not show INTERGENIC changes
□ Do not show INTRON changes
□ Do not show UPSTREAM changes
□ Do not show 5_PRIME_UTR or 3_PRIME_UTR changes

Filter out specific Effects

No

Variant annotation



Chromosomal position

◉ Use default (based on input type)
○ Force zero-based positions (both input and output)
○ Force one-based positions (both input and output)

Text to prepend to chromosome name

By default SnpEff simplifies all chromosome names. For instance 'chr1' is just '1'. You can prepend any string you want to the chromosome name (-chr)

Produce Summary Stats

Yes

(-noStats)

Suppress reporting usage statistics to server

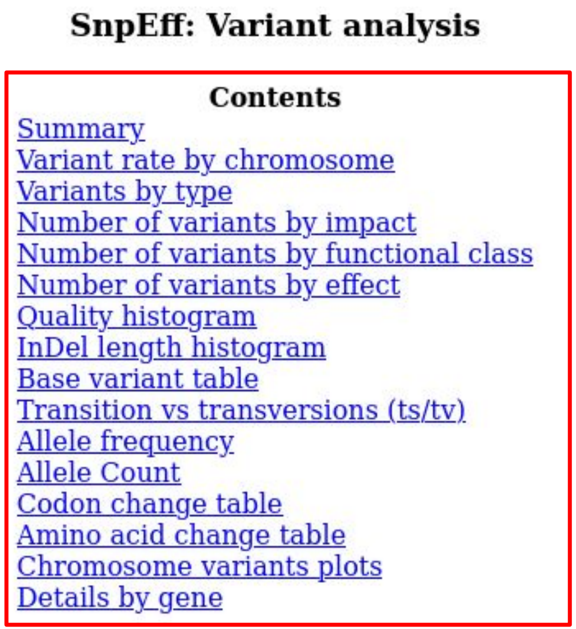
Yes

(-noLog)

Email notification

Send an email notification when the job completes.

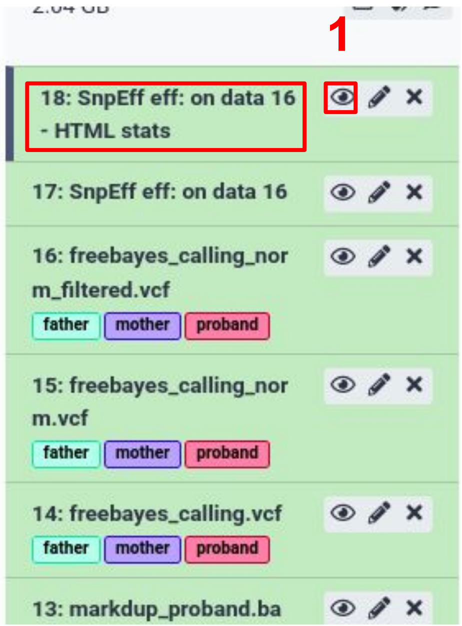
Variant annotation - Content



SnpEff: Variant analysis

Contents

- Summary
- Variant rate by chromosome
- Variants by type
- Number of variants by impact
- Number of variants by functional class
- Number of variants by effect
- Quality histogram
- InDel length histogram
- Base variant table
- Transition vs transversions (ts/tv)
- Allele frequency
- Allele Count
- Codon change table
- Amino acid change table
- Chromosome variants plots
- Details by gene



2.04 GB

1

18: SnpEff eff: on data 16 - HTML stats

17: SnpEff eff: on data 16

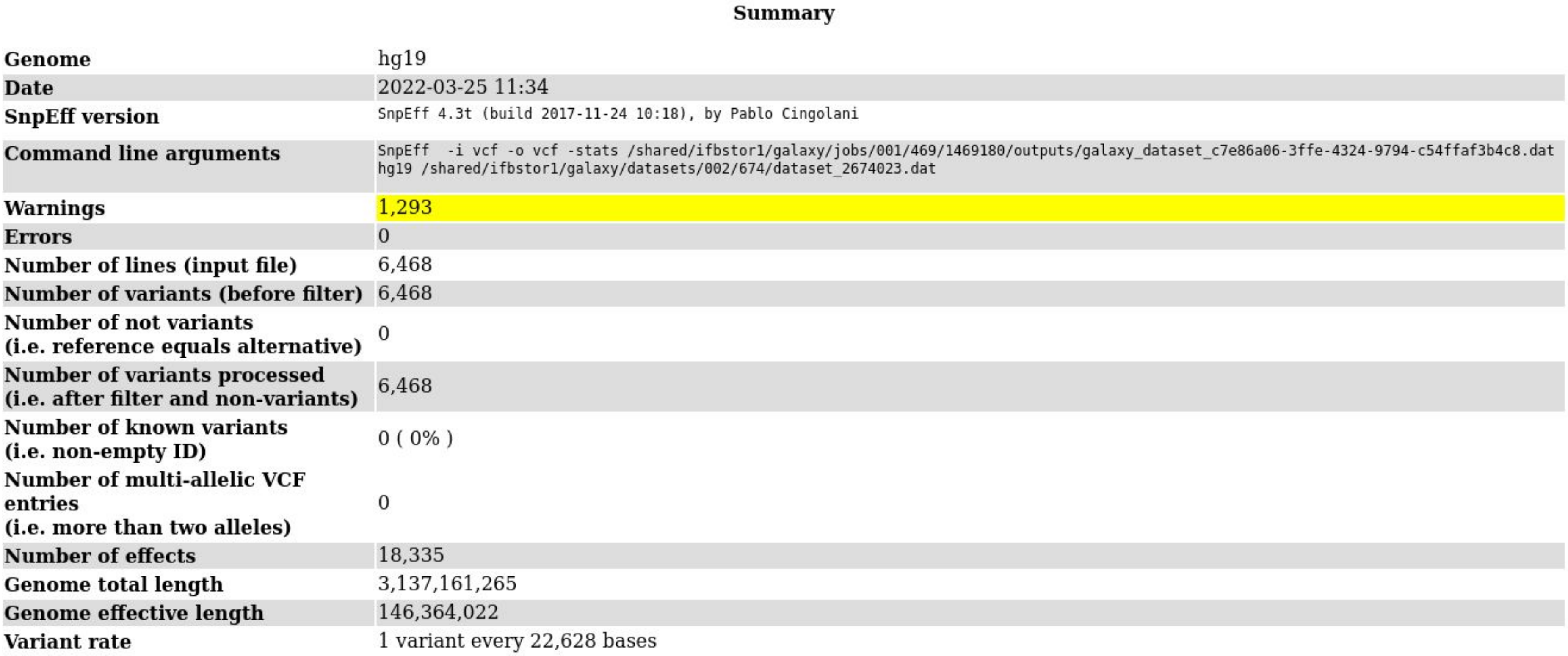
16: freebayes_calling_norm_filtered.vcf
father mother proband

15: freebayes_calling_norm.vcf
father mother proband

14: freebayes_calling.vcf
father mother proband

13: markdup_proband.ba

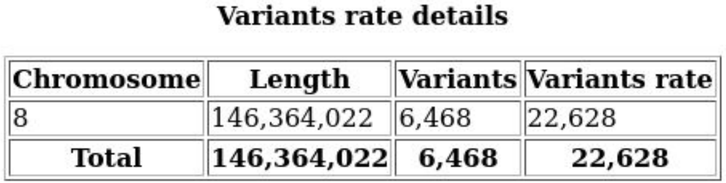
Variant annotation - Summary



Summary

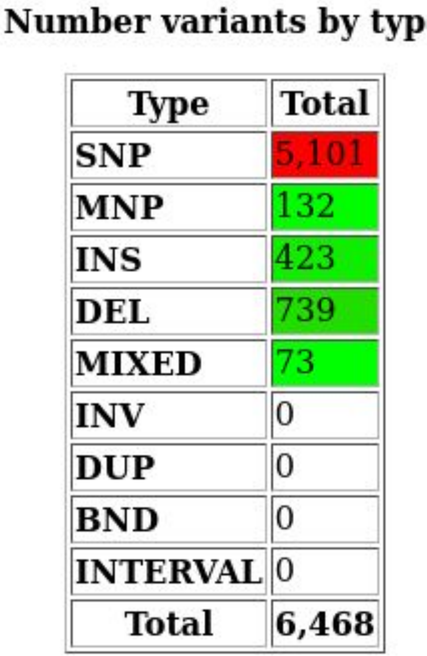
Genome	hg19
Date	2022-03-25 11:34
SnpEff version	SnpEff 4.3t (build 2017-11-24 10:18), by Pablo Cingolani
Command line arguments	SnpEff -i vcf -o vcf -stats /shared/ifbstor1/galaxy/jobs/001/469/1469180/outputs/galaxy_dataset_c7e86a06-3ffe-4324-9794-c54ffaf3b4c8.dat hg19 /shared/ifbstor1/galaxy/datasets/002/674/dataset_2674023.dat
Warnings	1,293
Errors	0
Number of lines (input file)	6,468
Number of variants (before filter)	6,468
Number of not variants (i.e. reference equals alternative)	0
Number of variants processed (i.e. after filter and non-variants)	6,468
Number of known variants (i.e. non-empty ID)	0 (0%)
Number of multi-allelic VCF entries (i.e. more than two alleles)	0
Number of effects	18,335
Genome total length	3,137,161,265
Genome effective length	146,364,022
Variant rate	1 variant every 22,628 bases

Variant annotation - Variants details



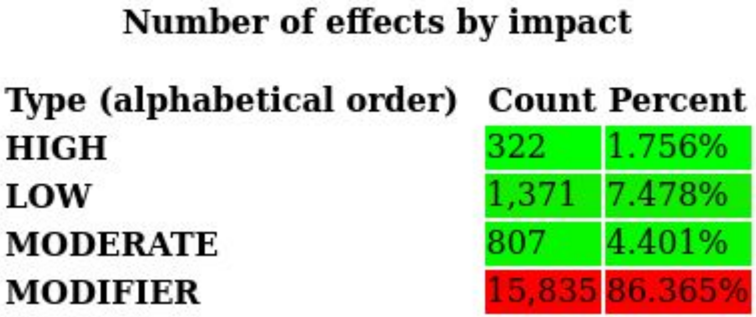
Variants rate details

Chromosome	Length	Variants	Variants rate
8	146,364,022	6,468	22,628
Total	**146,364,022**	**6,468**	**22,628**



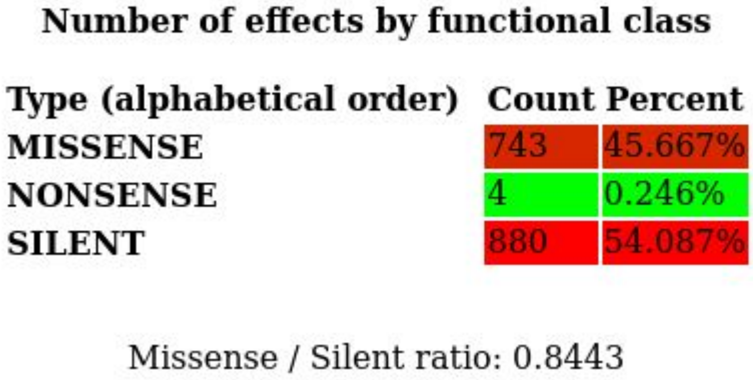
Number variants by type

Type	Total
SNP	5,101
MNP	132
INS	423
DEL	739
MIXED	73
INV	0
DUP	0
BND	0
INTERVAL	0
Total	**6,468**



Number of effects by impact

Type (alphabetical order)	Count	Percent
HIGH	322	1.756%
LOW	1,371	7.478%
MODERATE	807	4.401%
MODIFIER	15,835	86.365%

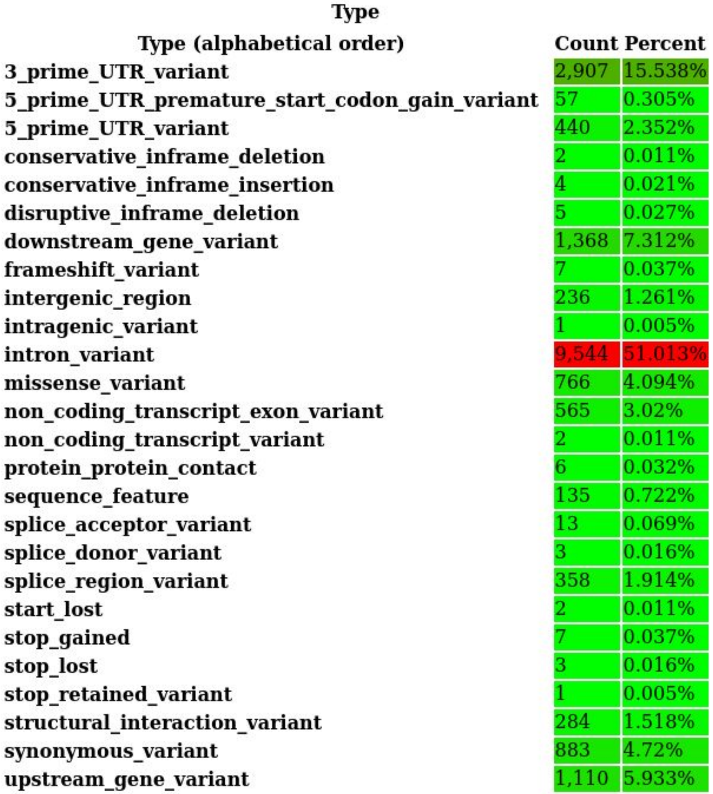


Number of effects by functional class

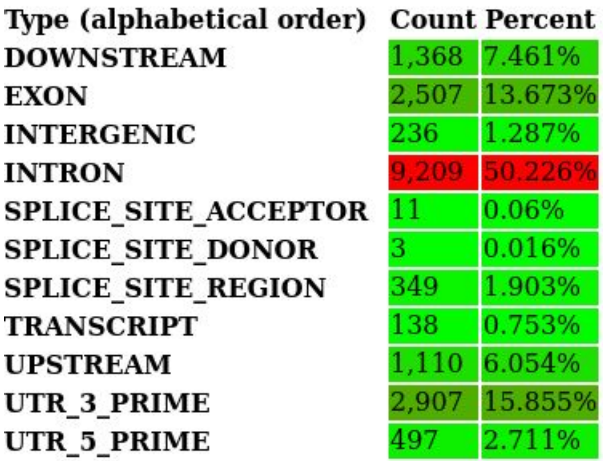
Type (alphabetical order)	Count	Percent
MISSENSE	743	45.667%
NONSENSE	4	0.246%
SILENT	880	54.087%

Missense / Silent ratio: 0.8443

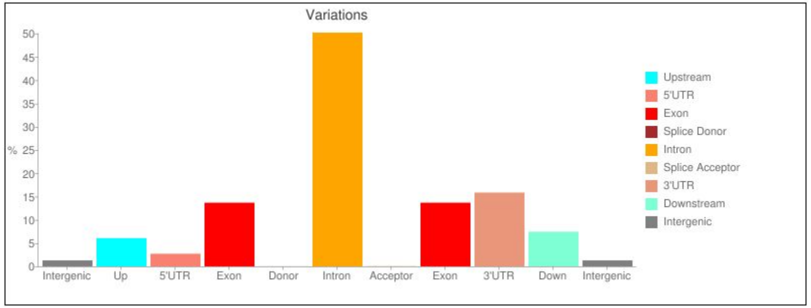
Variant annotation - Variants details



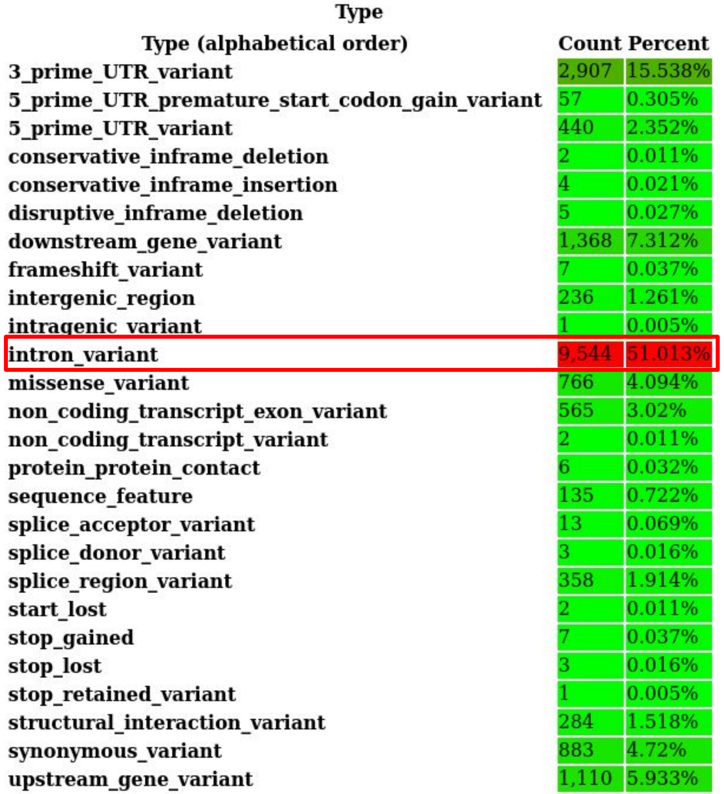
Type Type (alphabetical order)	Count	Percent
3_prime_UTR_variant	2,907	15.538%
5_prime_UTR_premature_start_codon_gain_variant	57	0.305%
5_prime_UTR_variant	440	2.352%
conservative_inframe_deletion	2	0.011%
conservative_inframe_insertion	4	0.021%
disruptive_inframe_deletion	5	0.027%
downstream_gene_variant	1,368	7.312%
frameshift_variant	7	0.037%
intergenic_region	236	1.261%
intragenic_variant	1	0.005%
intron_variant	9,544	51.013%
missense_variant	766	4.094%
non_coding_transcript_exon_variant	565	3.02%
non_coding_transcript_variant	2	0.011%
protein_protein_contact	6	0.032%
sequence_feature	135	0.722%
splice_acceptor_variant	13	0.069%
splice_donor_variant	3	0.016%
splice_region_variant	358	1.914%
start_lost	2	0.011%
stop_gained	7	0.037%
stop_lost	3	0.016%
stop_retained_variant	1	0.005%
structural_interaction_variant	284	1.518%
synonymous_variant	883	4.72%
upstream_gene_variant	1,110	5.933%



Type (alphabetical order)	Count	Percent
DOWNSTREAM	1,368	7.461%
EXON	2,507	13.673%
INTERGENIC	236	1.287%
INTRON	9,209	50.226%
SPLICE_SITE_ACCEPTOR	11	0.06%
SPLICE_SITE_DONOR	3	0.016%
SPLICE_SITE_REGION	349	1.903%
TRANSCRIPT	138	0.753%
UPSTREAM	1,110	6.054%
UTR_3_PRIME	2,907	15.855%
UTR_5_PRIME	497	2.711%



Variant annotation - Variants details



Type Type (alphabetical order)	Count	Percent
3_prime_UTR_variant	2,907	15.538%
5_prime_UTR_premature_start_codon_gain_variant	57	0.305%
5_prime_UTR_variant	440	2.352%
conservative_inframe_deletion	2	0.011%
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downstream_gene_variant	1,368	7.312%
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intergenic_region	236	1.261%
intragenic_variant	1	0.005%
intron_variant	9,544	51.013%
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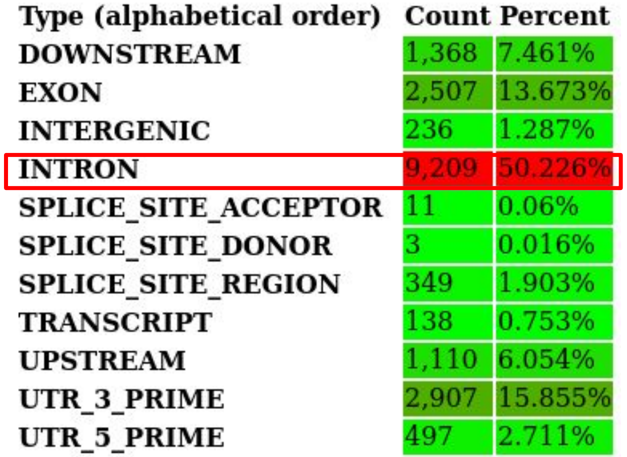
synonymous_variant	883	4.72%
upstream_gene_variant	1,110	5.933%



Type (alphabetical order)	Count	Percent
DOWNSTREAM	1,368	7.461%
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INTERGENIC	236	1.287%
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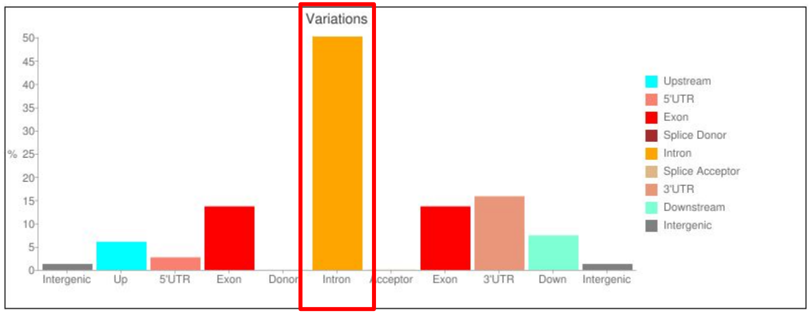
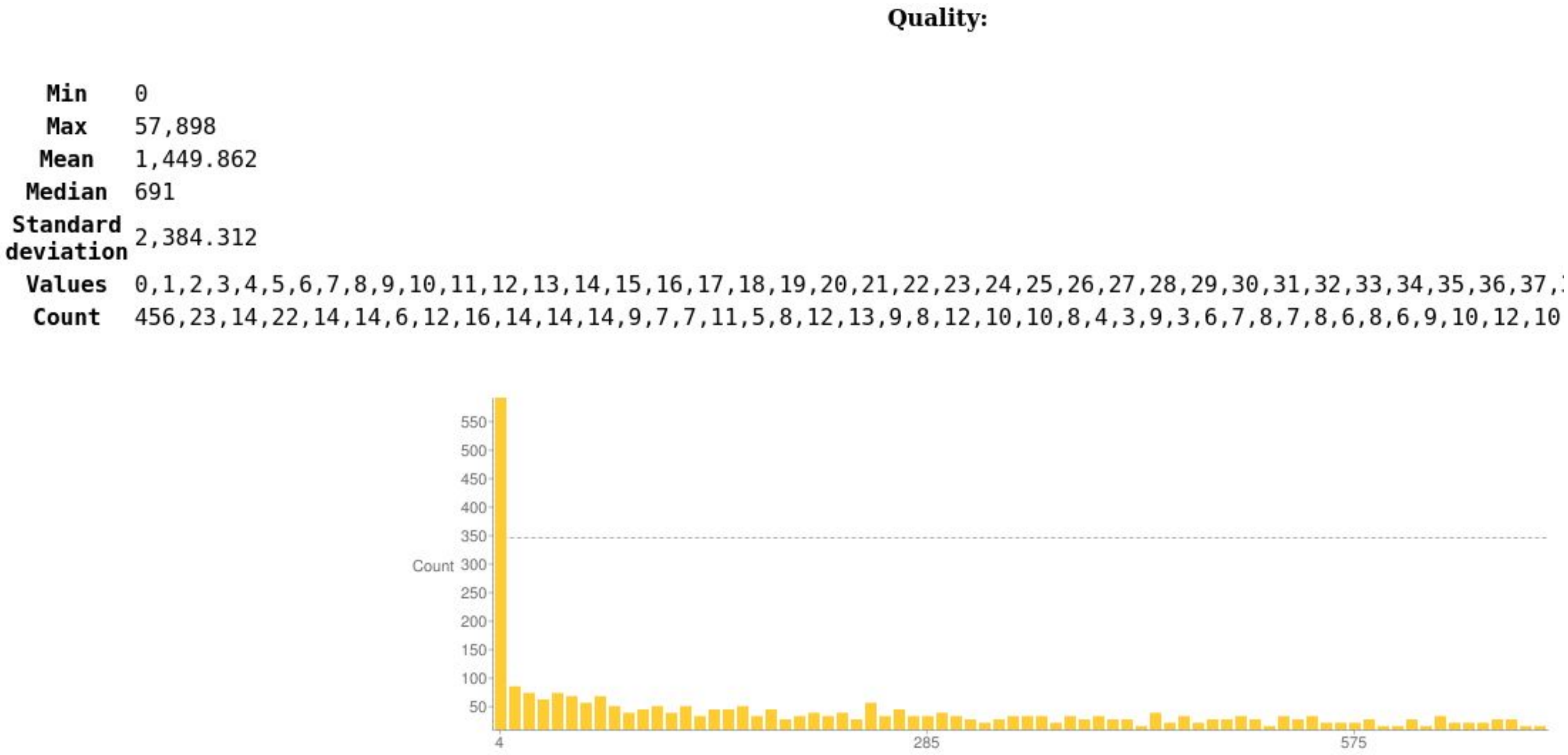
SPLICE_SITE_REGION	349	1.903%
TRANSCRIPT	138	0.753%
UPSTREAM	1,110	6.054%
UTR_3_PRIME	2,907	15.855%
UTR_5_PRIME	497	2.711%



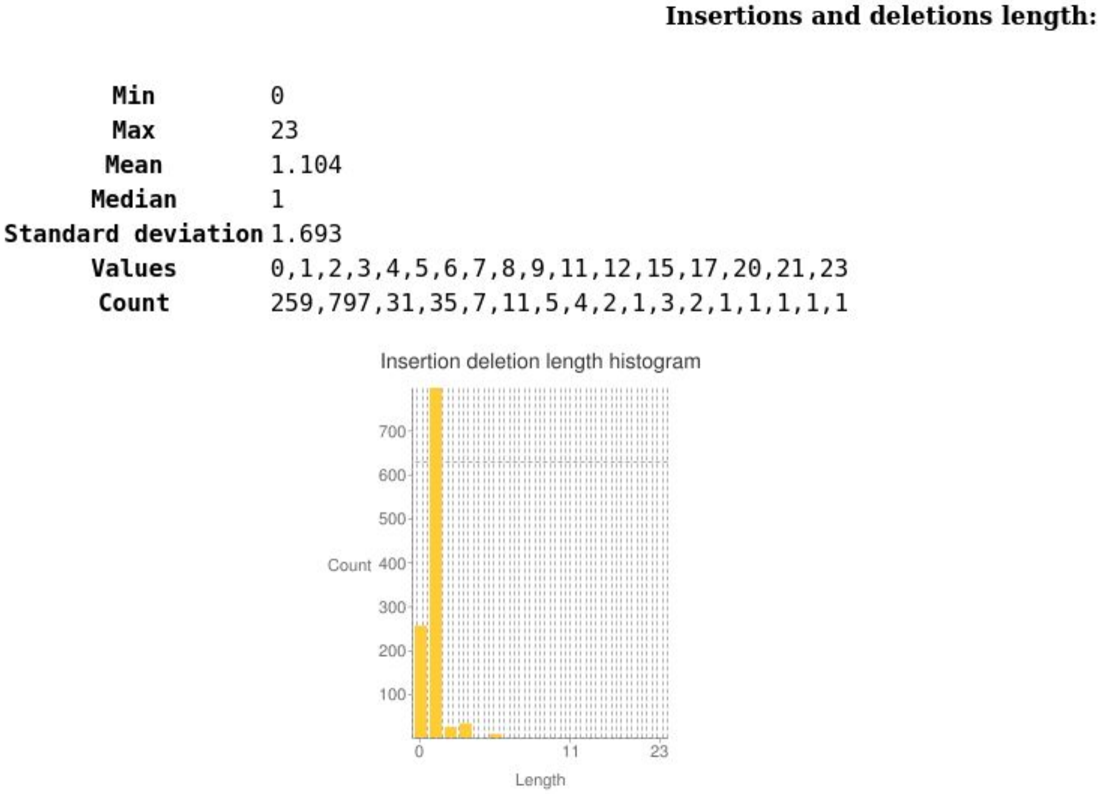
Variant annotation - Variants quality



Quality:

Min	0
Max	57,898
Mean	1,449.862
Median	691
Standard deviation	2,384.312
Values	0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,
Count	456,23,14,22,14,14,6,12,16,14,14,14,9,7,7,11,5,8,12,13,9,8,12,10,10,8,4,3,9,3,6,7,8,7,8,6,8,6,9,10,12,10

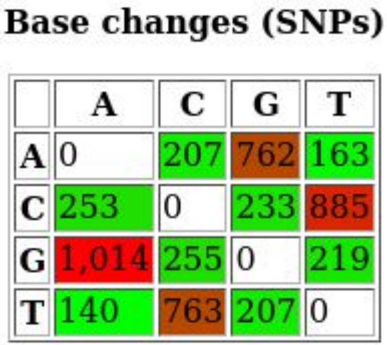
Variant annotation - Insertions/Deletions



Insertions and deletions length:

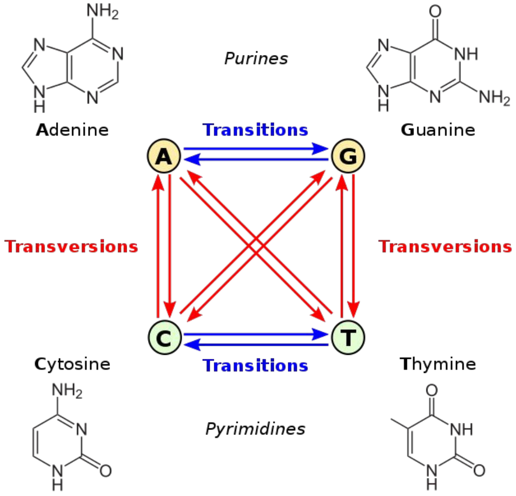
Min	0
Max	23
Mean	1.104
Median	1
Standard deviation	1.693
Values	0,1,2,3,4,5,6,7,8,9,11,12,15,17,20,21,23
Count	259,797,31,35,7,11,5,4,2,1,3,2,1,1,1,1,1

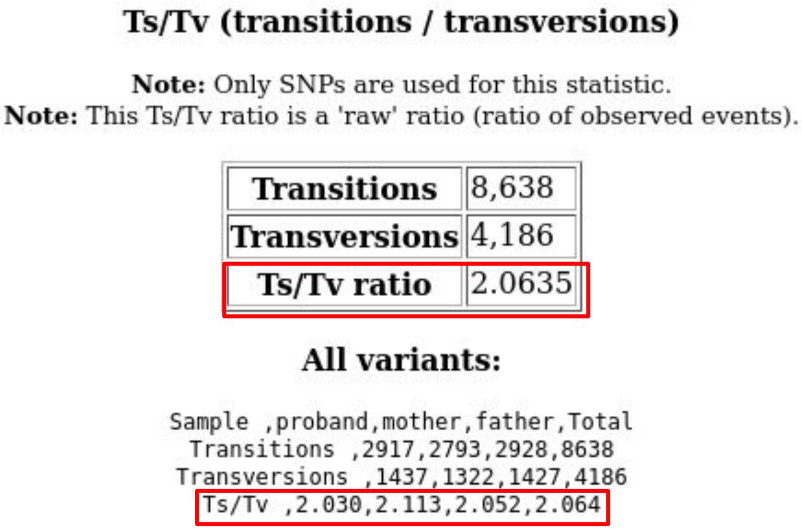
Variant annotation - Transitions/Transversions



Base changes (SNPs)

	A	C	G	T
A	0	207	762	163
C	253	0	233	885
G	1,014	255	0	219
T	140	763	207	0





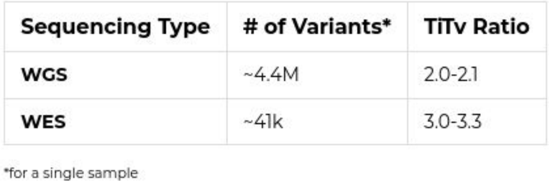
Ts/Tv (transitions / transversions)

Note: Only SNPs are used for this statistic.
Note: This Ts/Tv ratio is a 'raw' ratio (ratio of observed events).

Transitions	8,638
Transversions	4,186
Ts/Tv ratio	2.0635

All variants:

```
Sample ,proband,mother,father,Total
Transitions ,2917,2793,2928,8638
Transversions ,1437,1322,1427,4186
Ts/Tv ,2.030,2.113,2.052,2.064
```



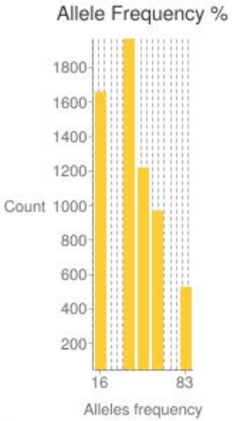
Sequencing Type	# of Variants*	TiTv Ratio
WGS	~4.4M	2.0-2.1
WES	~41k	3.0-3.3

*for a single sample

https://en.wikipedia.org/wiki/Transversion
https://gatk.broadinstitute.org/hc/en-us/articles/360035531572-Evaluating-the-quality-of-a-germline-short-variant-callset

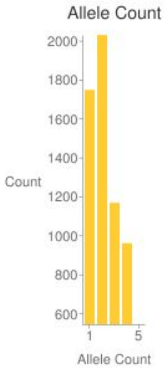
Variant annotation - Allele details

Allele frequency



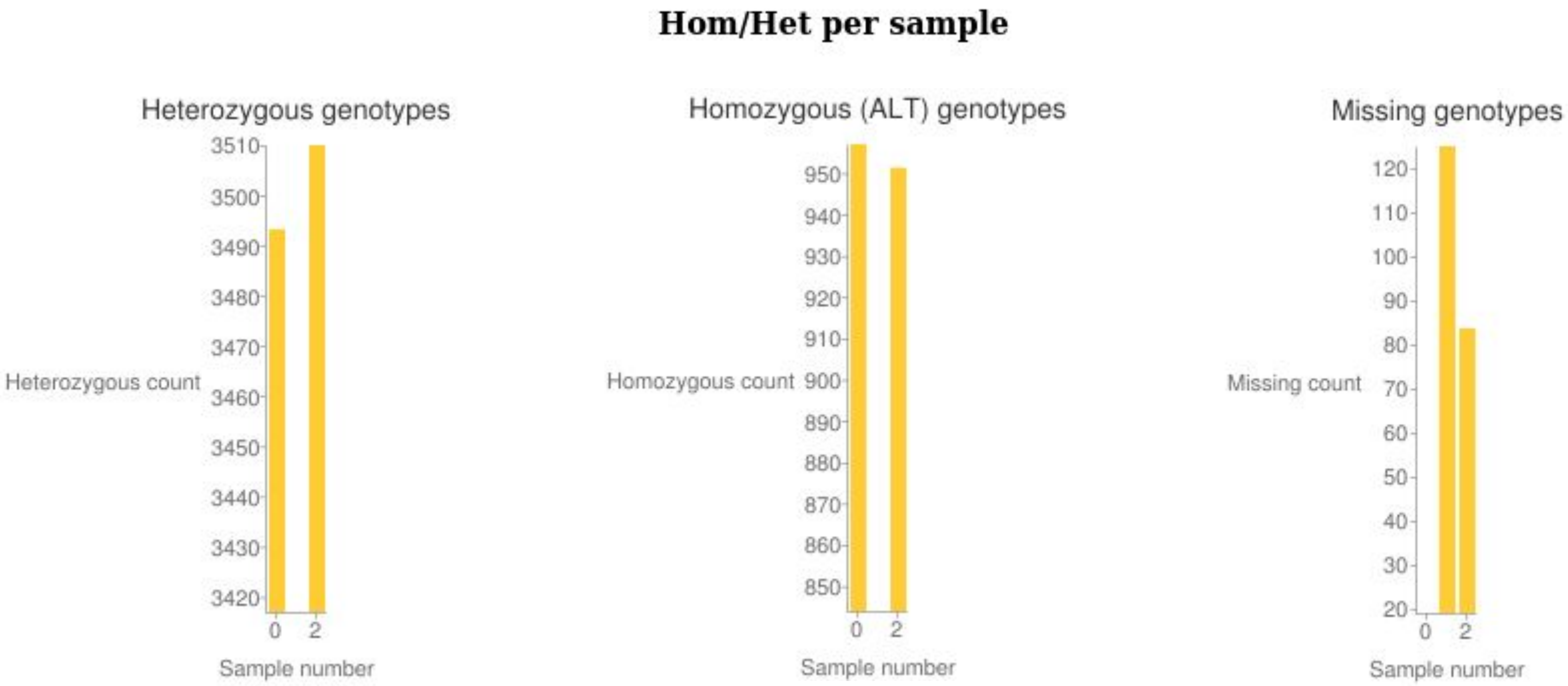
Min	16
Max	83
Mean	41.217
Median	33
Standard deviation	21.155
Values	16,25,33,50,66,75,83
Count	1665,53,1965,1229,968,45,543

Allele Count



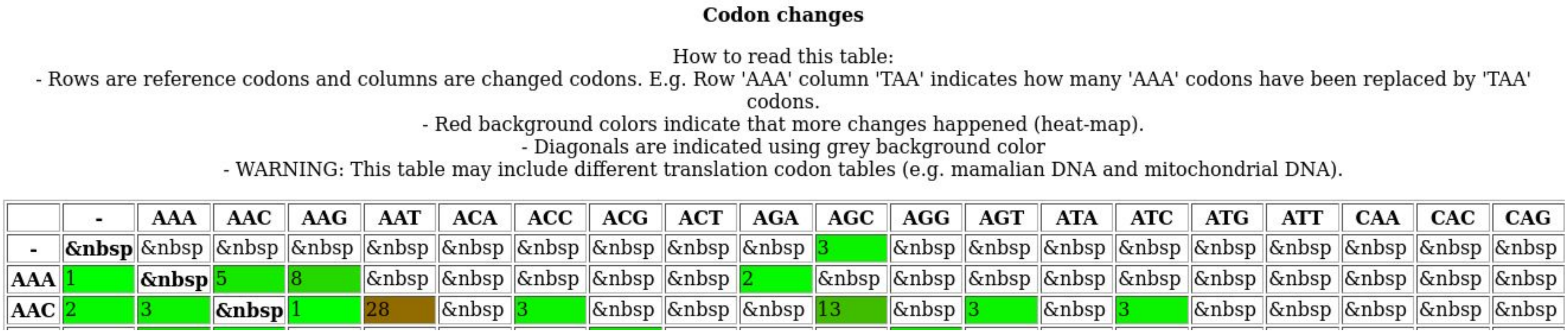
Min	1
Max	5
Mean	2.462
Median	2
Standard deviation	1.262
Values	1,2,3,4,5
Count	1751,2029,1177,968,543

Variant annotation - Genotypes details



```
Sample_names , proband, mother, father
Reference , 1998, 2082, 1922
Het , 3494, 3417, 3510
Hom , 957, 844, 952
Missing , 19, 125, 84
```

Variant annotation - Codon changes

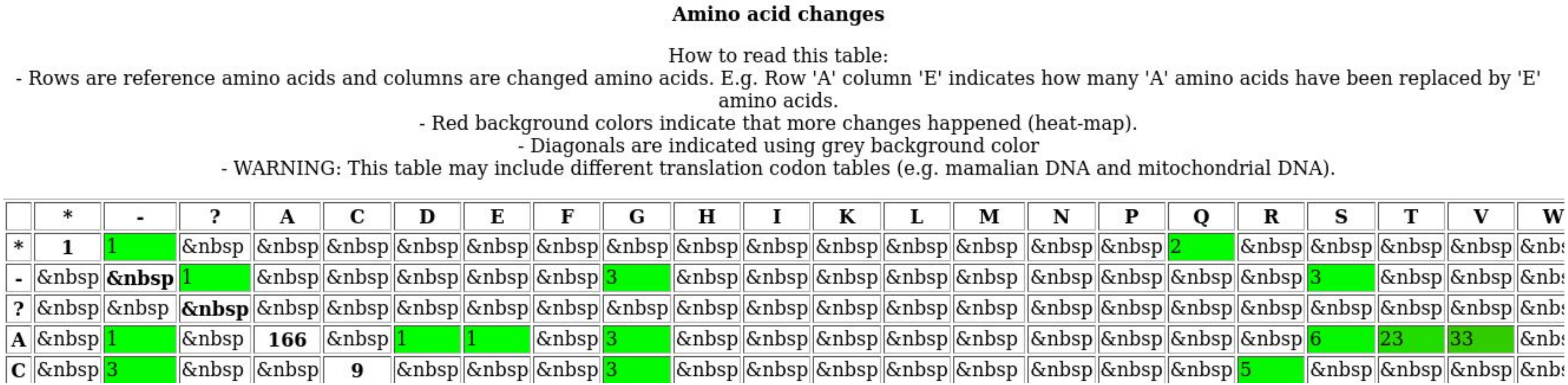


Codon changes

How to read this table:
- Rows are reference codons and columns are changed codons. E.g. Row 'AAA' column 'TAA' indicates how many 'AAA' codons have been replaced by 'TAA' codons.
- Red background colors indicate that more changes happened (heat-map).
- Diagonals are indicated using grey background color
- WARNING: This table may include different translation codon tables (e.g. mamalian DNA and mitochondrial DNA).

	-	AAA	AAC	AAG	AAT	ACA	ACC	ACG	ACT	AGA	AGC	AGG	AGT	ATA	ATC	ATG	ATT	CAA	CAC	CAG
-	**&nbsp**	 	 	 	 	 	 	 	 	 	3	 	 	 	 	 	 	 	 	
AAA	1	**&nbsp**	5	8	 	 	 	 	 	2	 	 	 	 	 	 	 	 	 	
AAC	2	3	**&nbsp**	1	28	 	3	 	 	 	13	 	3	 	3	 	 	 	 	

Variant annotation - Amino acid changes

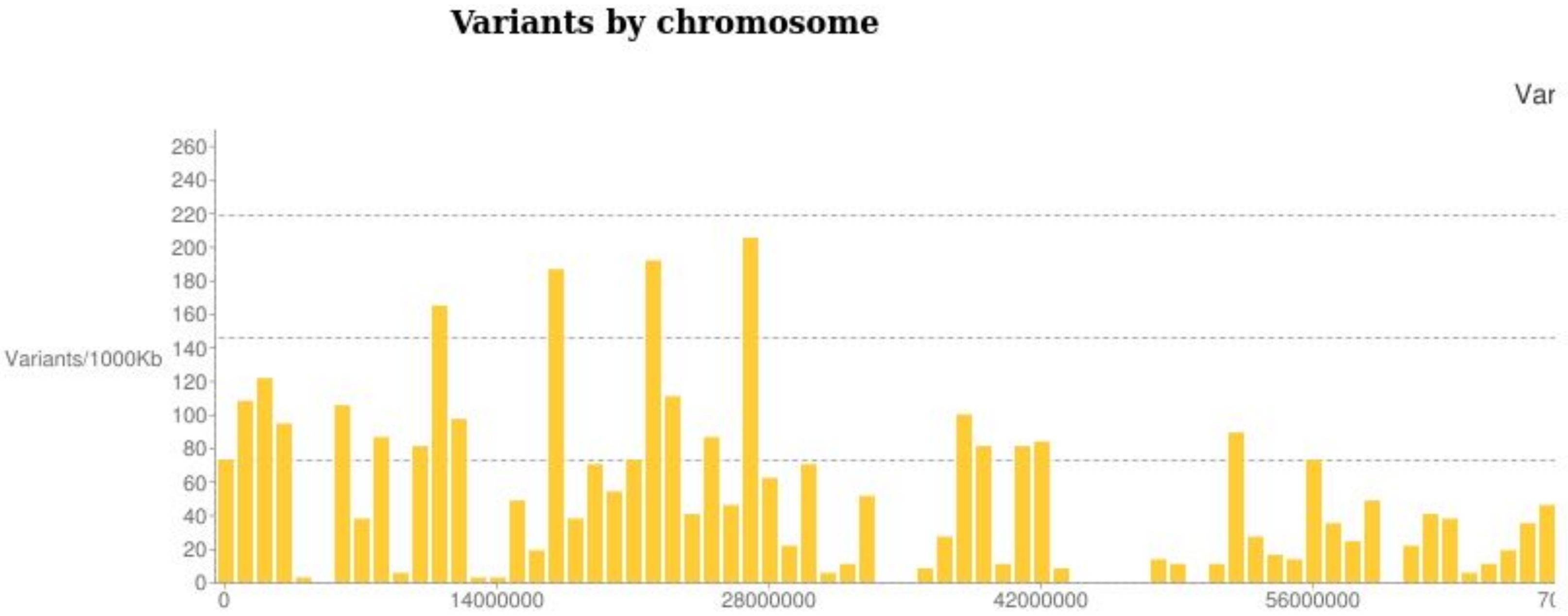


Amino acid changes

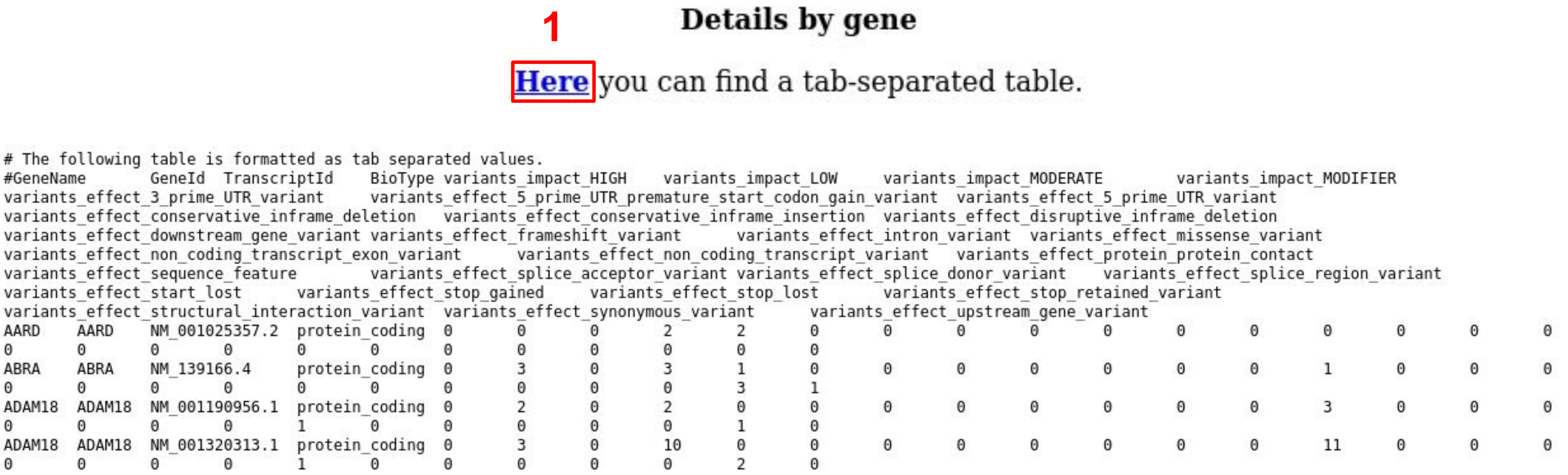
How to read this table:
- Rows are reference amino acids and columns are changed amino acids. E.g. Row 'A' column 'E' indicates how many 'A' amino acids have been replaced by 'E' amino acids.
- Red background colors indicate that more changes happened (heat-map).
- Diagonals are indicated using grey background color
- WARNING: This table may include different translation codon tables (e.g. mamalian DNA and mitochondrial DNA).

	*	-	?	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W
*	**1**	1	 	 	 	 	 	 	 	 	 	 	 	 	 	 	2	 	 	 	 	&nbs
-	 	**&nbsp**	1	 	 	 	 	 	3	 	 	 	 	 	 	 	 	 	3	 	 	&nbs
?	 	 	**&nbsp**	 	 	 	 	 	 	 	 	 	 	 	 	 	 	 	 	 	 	&nbs
A	 	1	 	**166**	 	1	1	 	3	 	 	 	 	 	 	 	 	 	6	23	33	&nbs
C	 	3	 	 	**9**	 	 	 	3	 	 	 	 	 	 	 	 	5	 	 	 	&nbs

Variant annotation - Chromosomes details



Variant annotation - Genes information



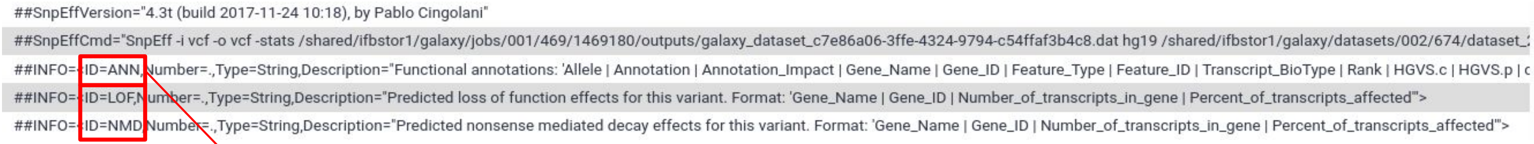
1

Details by gene

Here you can find a tab-separated table.

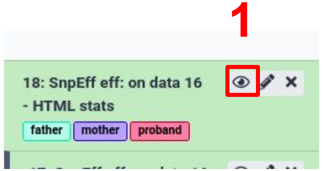
```
# The following table is formatted as tab separated values.
#GeneName       GeneId  TranscriptId    BioType variants_impact_HIGH    variants_impact_LOW     variants_impact_MODERATE        variants_impact_MODIFIER
variants_effect_3_prime_UTR_variant     variants_effect_5_prime_UTR_premature_start_codon_gain_variant  variants_effect_5_prime_UTR_variant
variants_effect_conservative_inframe_deletion   variants_effect_conservative_inframe_insertion  variants_effect_disruptive_inframe_deletion
variants_effect_downstream_gene_variant variants_effect_frameshift_variant      variants_effect_intron_variant  variants_effect_missense_variant
variants_effect_non_coding_transcript_exon_variant      variants_effect_non_coding_transcript_variant   variants_effect_protein_protein_contact
variants_effect_sequence_feature        variants_effect_splice_acceptor_variant variants_effect_splice_donor_variant    variants_effect_splice_region_variant
variants_effect_start_lost      variants_effect_stop_gained     variants_effect_stop_lost       variants_effect_stop_retained_variant
variants_effect_structural_interaction_variant  variants_effect_synonymous_variant      variants_effect_upstream_gene_variant
AARD    AARD    NM_001025357.2  protein_coding  0       0       0       2       2       0       0       0       0       0       0       0       0       0       0       0
0       0       0       0       0       0       0       0       0       0       0       0
ABRA    ABRA    NM_139166.4     protein_coding  0       3       0       3       1       0       0       0       0       0       0       0       1       0       0       0
0       0       0       0       0       0       0       0       0       0       3       1
ADAM18  ADAM18  NM_001190956.1  protein_coding  0       2       0       2       0       0       0       0       0       0       0       0       3       0       0       0
0       0       0       0       1       0       0       0       0       0       1       0
ADAM18  ADAM18  NM_001320313.1  protein_coding  0       3       0       10      0       0       0       0       0       0       0       0       11      0       0       0
0       0       0       0       1       0       0       0       0       0       2       0
```

Variant annotation - ANN field



```
##SnpEffVersion="4.3t (build 2017-11-24 10:18), by Pablo Cingolani"
##SnpEffCmd="SnpEff -i vcf -o vcf -stats /shared/ifbstor1/galaxy/jobs/001/469/1469180/outputs/galaxy_dataset_c7e86a06-3ffe-4324-9794-c54ffaf3b4c8.dat hg19 /shared/ifbstor1/galaxy/datasets/002/674/dataset_2
##INFO=<ID=ANN,Number=.,Type=String,Description="Functional annotations: 'Allele | Annotation | Annotation_Impact | Gene_Name | Gene_ID | Feature_Type | Feature_ID | Transcript_BioType | Rank | HGVS.c | HGVS.p | c
##INFO=<ID=LOF,Number=.,Type=String,Description="Predicted loss of function effects for this variant. Format: 'Gene_Name | Gene_ID | Number_of_transcripts_in_gene | Percent_of_transcripts_affected'">
##INFO=<ID=NMD,Number=.,Type=String,Description="Predicted nonsense mediated decay effects for this variant. Format: 'Gene_Name | Gene_ID | Number_of_transcripts_in_gene | Percent_of_transcripts_affected'">
```

1

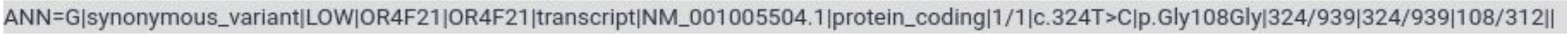


18: SnpEff eff: on data 16 - HTML stats

'Allele | Annotation | Annotation_Impact | Gene_Name | Gene_ID | Feature_Type | Feature_ID | Transcript_BioType | Rank | HGVS.c | HGVS.p | cDNA.pos / cDNA.length | CDS.pos / CDS.length | AA.pos / AA.length | Distance | ERRORS / WARNINGS / INFO' ">

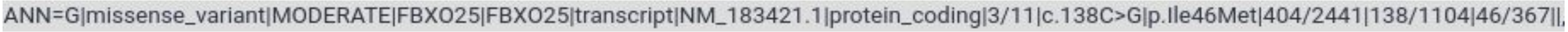
Variant annotation - Examples

Synonymous



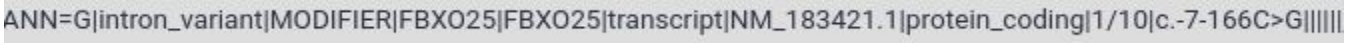
```
ANN=G|synonymous_variant|LOW|OR4F21|OR4F21|transcript|NM_001005504.1|protein_coding|1/1|c.324T>C|p.Gly108Gly|324/939|324/939|108/312||
```

Missense

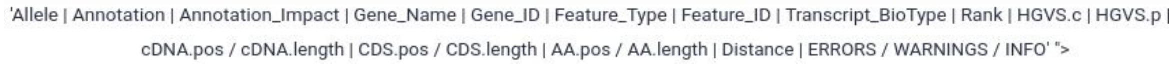


```
ANN=G|missense_variant|MODERATE|FBXO25|FBXO25|transcript|NM_183421.1|protein_coding|3/11|c.138C>G|p.Ile46Met|404/2441|138/1104|46/367||,
```

Intronic



```
ANN=G|intron_variant|MODIFIER|FBXO25|FBXO25|transcript|NM_183421.1|protein_coding|1/10|c.-7-166C>G||||||
```



```
'Allele | Annotation | Annotation_Impact | Gene_Name | Gene_ID | Feature_Type | Feature_ID | Transcript_BioType | Rank | HGVS.c | HGVS.p |
cDNA.pos / cDNA.length | CDS.pos / CDS.length | AA.pos / AA.length | Distance | ERRORS / WARNINGS / INFO' ">
```

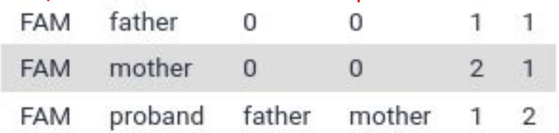
Variant reporting - Pedigree

Individual

Father ID

Family ID

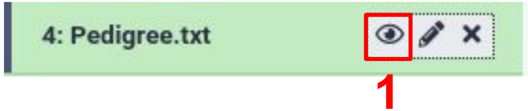
Mother ID



FAM	father	0	0	1	1
FAM	mother	0	0	2	1
FAM	proband	father	mother	1	2

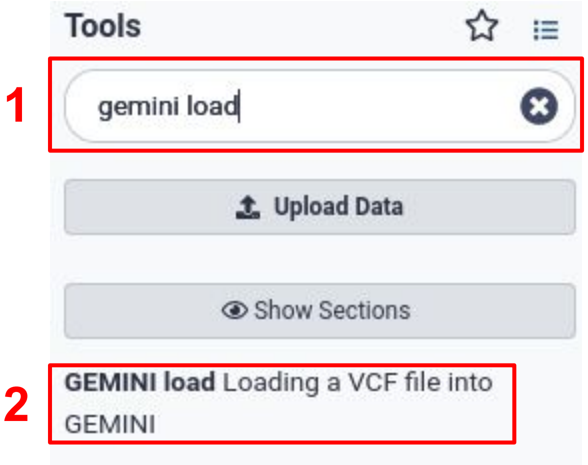
Sex (1: male; 2: female)

Status (1: control; 2: case)



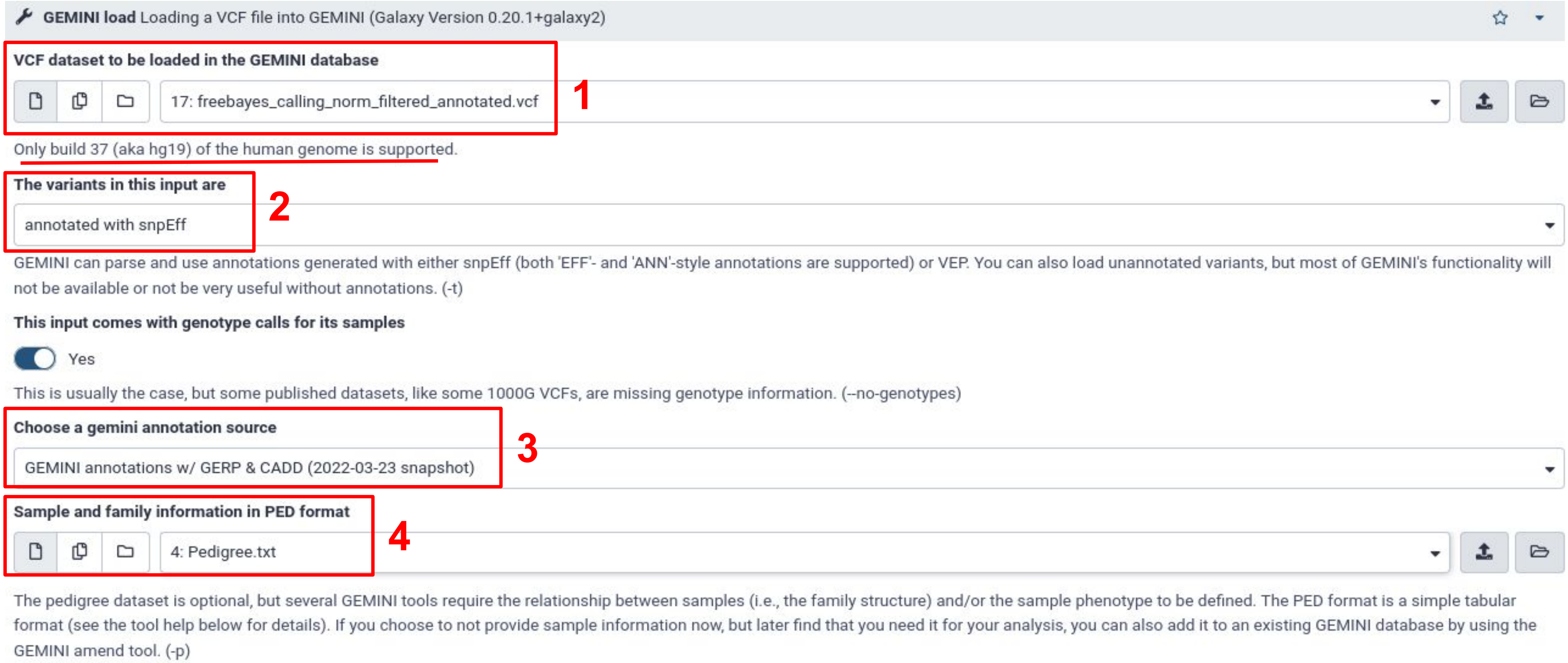
1

Variant reporting - Database creation



GEMINI load Loading a VCF file into GEMINI

Variant reporting - Database creation



GEMINI load Loading a VCF file into GEMINI (Galaxy Version 0.20.1+galaxy2)

VCF dataset to be loaded in the GEMINI database

17: freebayes_calling_norm_filtered_annotated.vcf

Only build 37 (aka hg19) of the human genome is supported.

The variants in this input are

annotated with snpEff

GEMINI can parse and use annotations generated with either snpEff (both 'EFF'- and 'ANN'-style annotations are supported) or VEP. You can also load unannotated variants, but most of GEMINI's functionality will not be available or not be very useful without annotations. (-t)

This input comes with genotype calls for its samples

Yes

This is usually the case, but some published datasets, like some 1000G VCFs, are missing genotype information. (--no-genotypes)

Choose a gemini annotation source

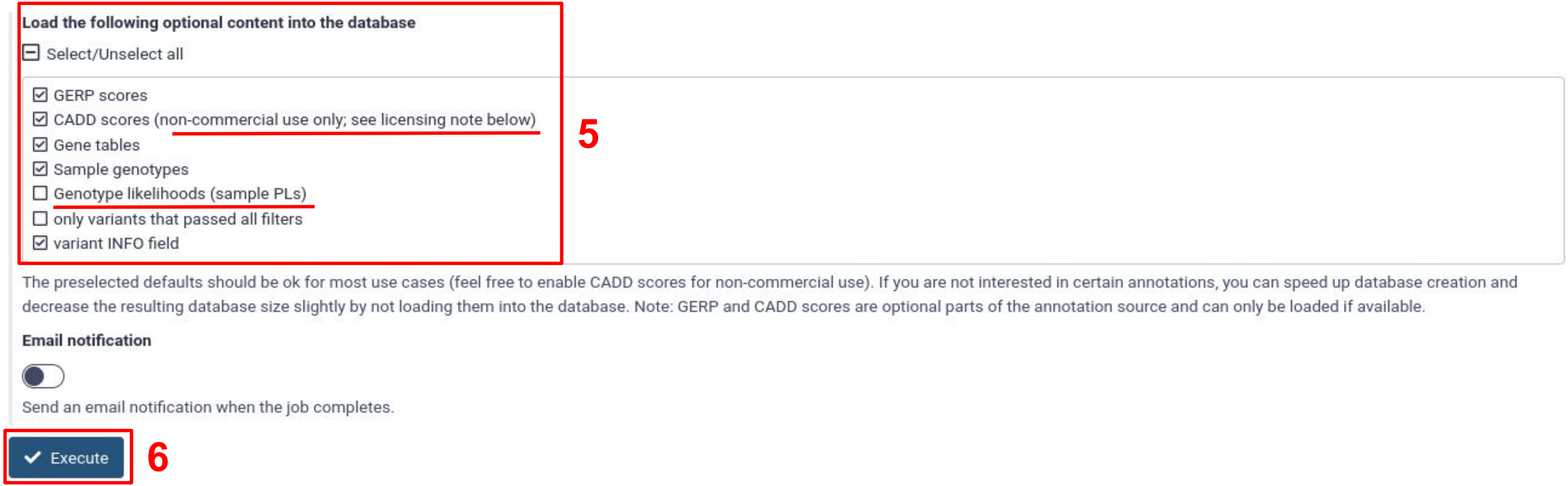
GEMINI annotations w/ GERP & CADD (2022-03-23 snapshot)

Sample and family information in PED format

4: Pedigree.txt

The pedigree dataset is optional, but several GEMINI tools require the relationship between samples (i.e., the family structure) and/or the sample phenotype to be defined. The PED format is a simple tabular format (see the tool help below for details). If you choose to not provide sample information now, but later find that you need it for your analysis, you can also add it to an existing GEMINI database by using the GEMINI amend tool. (-p)

Variant reporting - Database creation



Load the following optional content into the database

⊟ Select/Unselect all

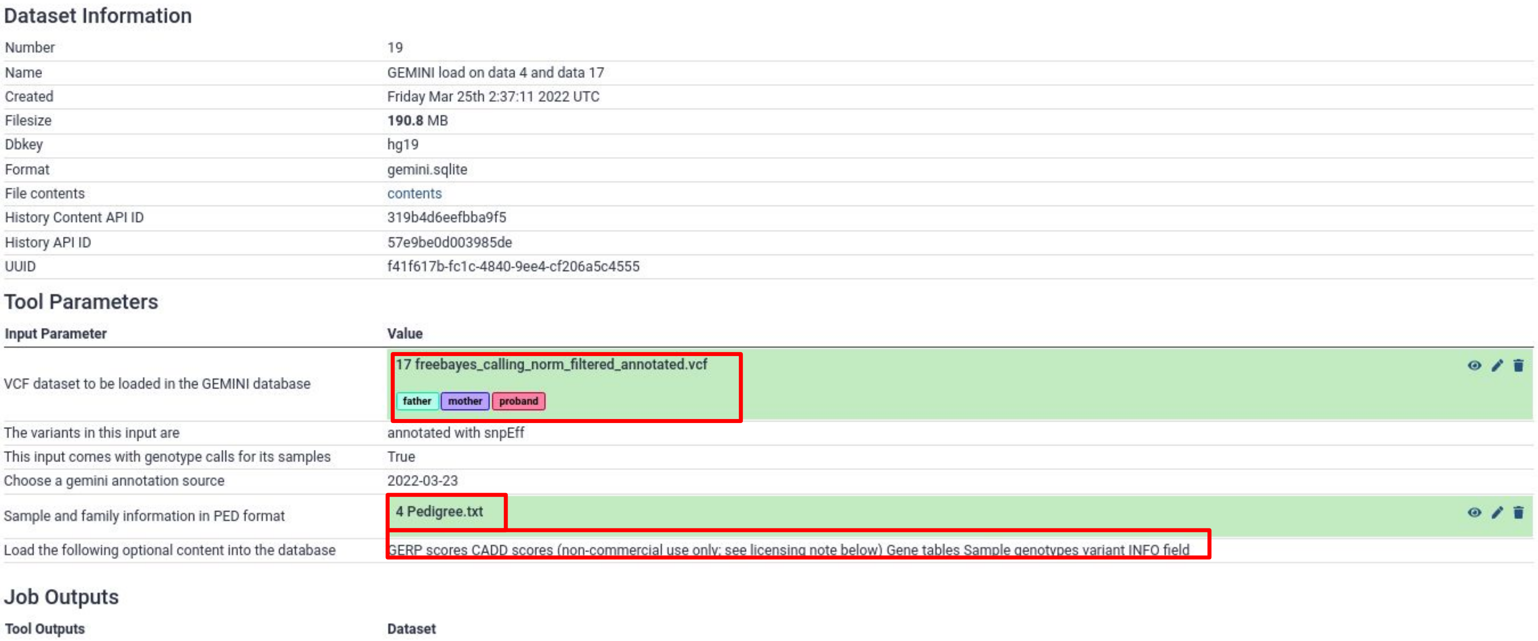
- ☑ GERP scores
- ☑ CADD scores (non-commercial use only; see licensing note below)
- ☑ Gene tables
- ☑ Sample genotypes
- ☐ Genotype likelihoods (sample PLs)
- ☐ only variants that passed all filters
- ☑ variant INFO field

The preselected defaults should be ok for most use cases (feel free to enable CADD scores for non-commercial use). If you are not interested in certain annotations, you can speed up database creation and decrease the resulting database size slightly by not loading them into the database. Note: GERP and CADD scores are optional parts of the annotation source and can only be loaded if available.

Email notification

Send an email notification when the job completes.

Variant reporting - Database creation



Dataset Information

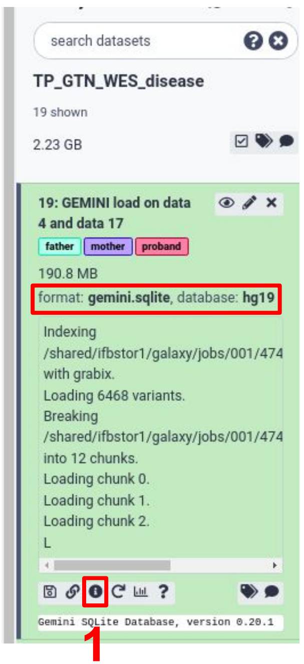
Number	19
Name	GEMINI load on data 4 and data 17
Created	Friday Mar 25th 2:37:11 2022 UTC
Filesize	**190.8** MB
Dbkey	hg19
Format	gemini.sqlite
File contents	contents
History Content API ID	319b4d6eefbba9f5
History API ID	57e9be0d003985de
UUID	f41f617b-fc1c-4840-9ee4-cf206a5c4555

Tool Parameters

Input Parameter	Value
VCF dataset to be loaded in the GEMINI database	17 freebayes_calling_norm_filtered_annotated.vcf father mother proband
The variants in this input are	annotated with snpEff
This input comes with genotype calls for its samples	True
Choose a gemini annotation source	2022-03-23
Sample and family information in PED format	4 Pedigree.txt
Load the following optional content into the database	GERP scores CADD scores (non-commercial use only; see licensing note below) Gene tables Sample genotypes variant INFO field

Job Outputs

Tool Outputs	Dataset



TP_GTN_WES_disease

19 shown

2.23 GB

19: GEMINI load on data 4 and data 17

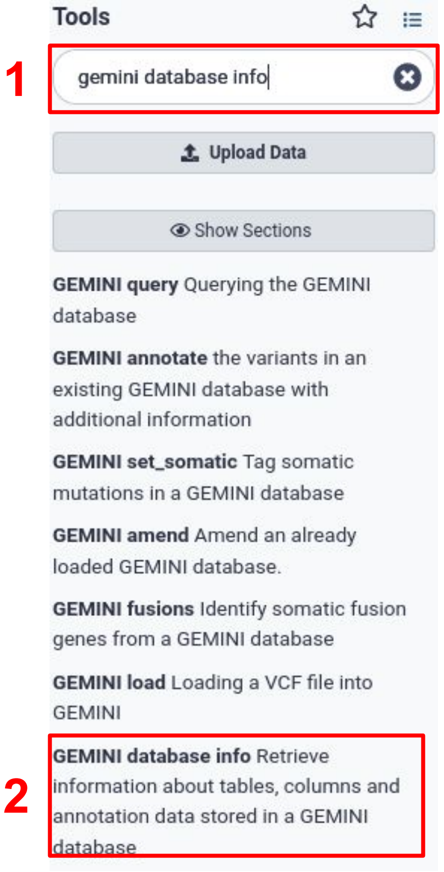
190.8 MB

format: **gemini.sqlite**, database: **hg19**

```
Indexing
/shared/ifbstor1/galaxy/jobs/001/474
with grabix.
Loading 6468 variants.
Breaking
/shared/ifbstor1/galaxy/jobs/001/474
into 12 chunks.
Loading chunk 0.
Loading chunk 1.
Loading chunk 2.
L
```

```
Gemini SQLite Database, version 0.20.1
```

Variant reporting - Database content



GEMINI query Querying the GEMINI database

GEMINI annotate the variants in an existing GEMINI database with additional information

GEMINI set_somatic Tag somatic mutations in a GEMINI database

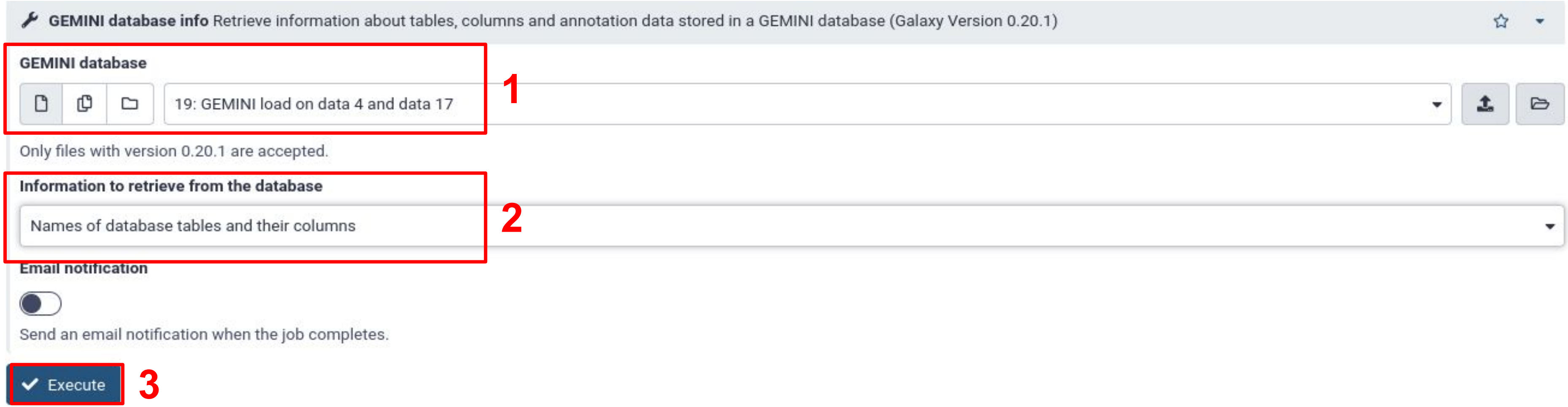
GEMINI amend Amend an already loaded GEMINI database.

GEMINI fusions Identify somatic fusion genes from a GEMINI database

GEMINI load Loading a VCF file into GEMINI

GEMINI database info Retrieve information about tables, columns and annotation data stored in a GEMINI database

Variant reporting - Database content



GEMINI database info Retrieve information about tables, columns and annotation data stored in a GEMINI database (Galaxy Version 0.20.1)

GEMINI database

19: GEMINI load on data 4 and data 17

Only files with version 0.20.1 are accepted.

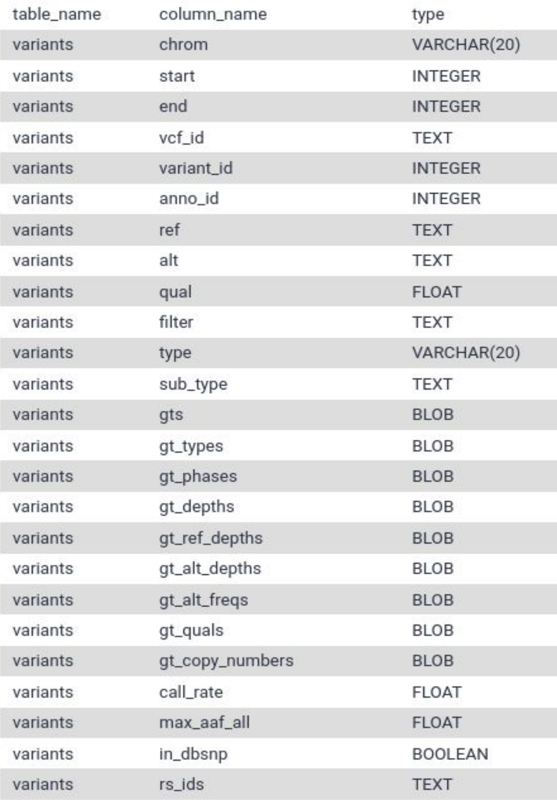
Information to retrieve from the database

Names of database tables and their columns

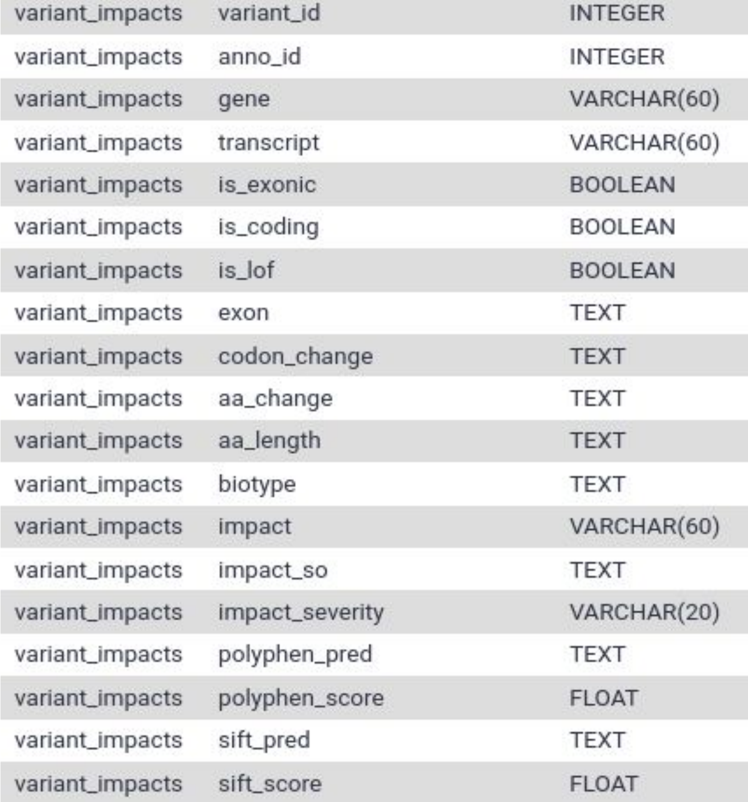
Email notification

Send an email notification when the job completes.

Variant reporting - Database content

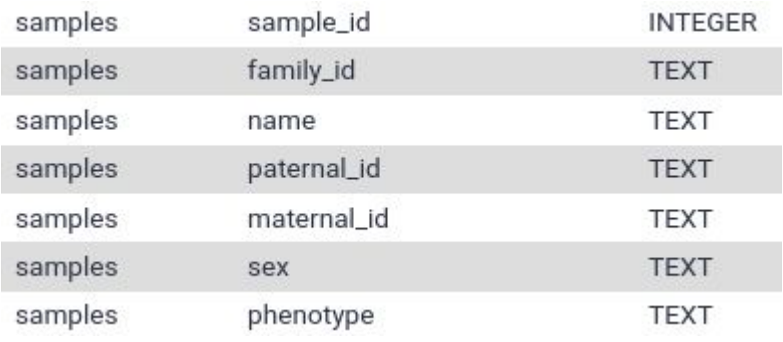


table_name	column_name	type
variants	chrom	VARCHAR(20)
variants	start	INTEGER
variants	end	INTEGER
variants	vcf_id	TEXT
variants	variant_id	INTEGER
variants	anno_id	INTEGER
variants	ref	TEXT
variants	alt	TEXT
variants	qual	FLOAT
variants	filter	TEXT
variants	type	VARCHAR(20)
variants	sub_type	TEXT
variants	gts	BLOB
variants	gt_types	BLOB
variants	gt_phases	BLOB
variants	gt_depths	BLOB
variants	gt_ref_depths	BLOB
variants	gt_alt_depths	BLOB
variants	gt_alt_freqs	BLOB
variants	gt_quals	BLOB
variants	gt_copy_numbers	BLOB
variants	call_rate	FLOAT
variants	max_aaf_all	FLOAT
variants	in_dbsnp	BOOLEAN
variants	rs_ids	TEXT

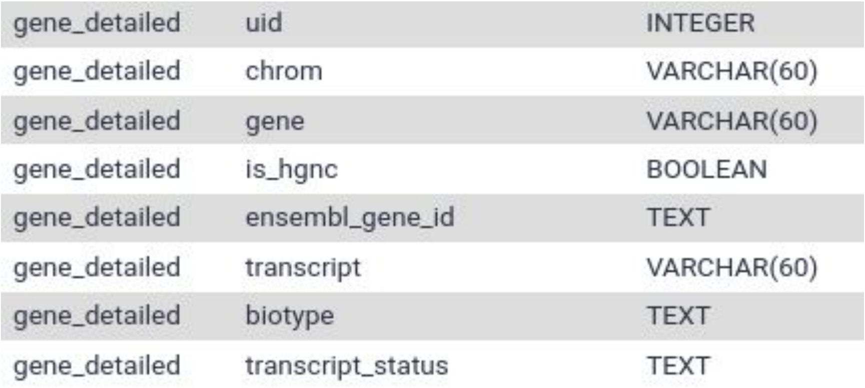


variant_impacts	variant_id	INTEGER
variant_impacts	anno_id	INTEGER
variant_impacts	gene	VARCHAR(60)
variant_impacts	transcript	VARCHAR(60)
variant_impacts	is_exonic	BOOLEAN
variant_impacts	is_coding	BOOLEAN
variant_impacts	is_lof	BOOLEAN
variant_impacts	exon	TEXT
variant_impacts	codon_change	TEXT
variant_impacts	aa_change	TEXT
variant_impacts	aa_length	TEXT
variant_impacts	biotype	TEXT
variant_impacts	impact	VARCHAR(60)
variant_impacts	impact_so	TEXT
variant_impacts	impact_severity	VARCHAR(20)
variant_impacts	polyphen_pred	TEXT
variant_impacts	polyphen_score	FLOAT
variant_impacts	sift_pred	TEXT
variant_impacts	sift_score	FLOAT

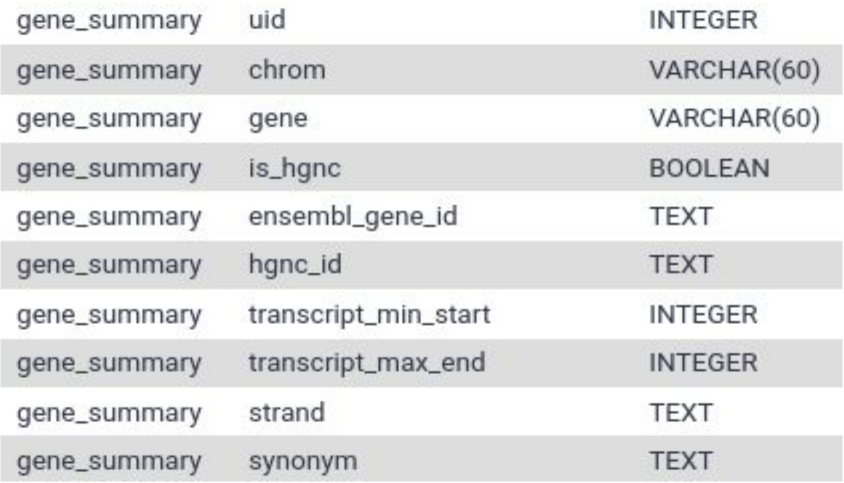
Variant reporting - Database content



samples	sample_id	INTEGER
samples	family_id	TEXT
samples	name	TEXT
samples	paternal_id	TEXT
samples	maternal_id	TEXT
samples	sex	TEXT
samples	phenotype	TEXT

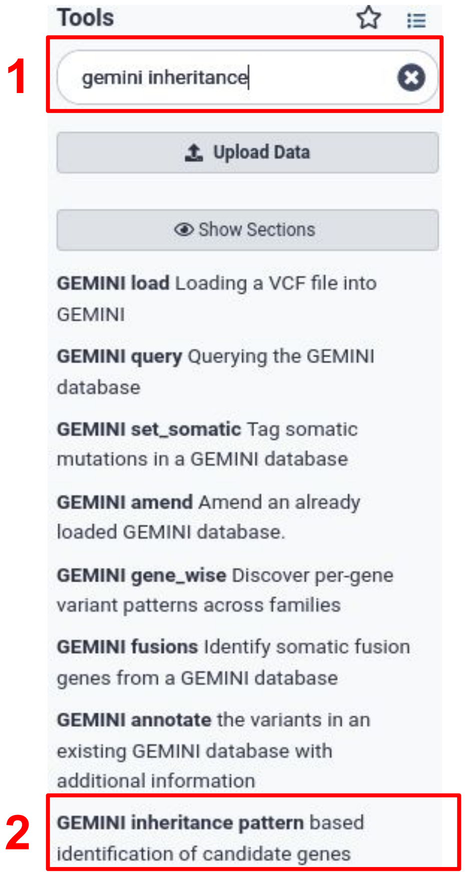


gene_detailed	uid	INTEGER
gene_detailed	chrom	VARCHAR(60)
gene_detailed	gene	VARCHAR(60)
gene_detailed	is_hgnc	BOOLEAN
gene_detailed	ensembl_gene_id	TEXT
gene_detailed	transcript	VARCHAR(60)
gene_detailed	biotype	TEXT
gene_detailed	transcript_status	TEXT



gene_summary	uid	INTEGER
gene_summary	chrom	VARCHAR(60)
gene_summary	gene	VARCHAR(60)
gene_summary	is_hgnc	BOOLEAN
gene_summary	ensembl_gene_id	TEXT
gene_summary	hgnc_id	TEXT
gene_summary	transcript_min_start	INTEGER
gene_summary	transcript_max_end	INTEGER
gene_summary	strand	TEXT
gene_summary	synonym	TEXT

Variant reporting - Querying



gemini inheritance

GEMINI load Loading a VCF file into GEMINI

GEMINI query Querying the GEMINI database

GEMINI set_somatic Tag somatic mutations in a GEMINI database

GEMINI amend Amend an already loaded GEMINI database.

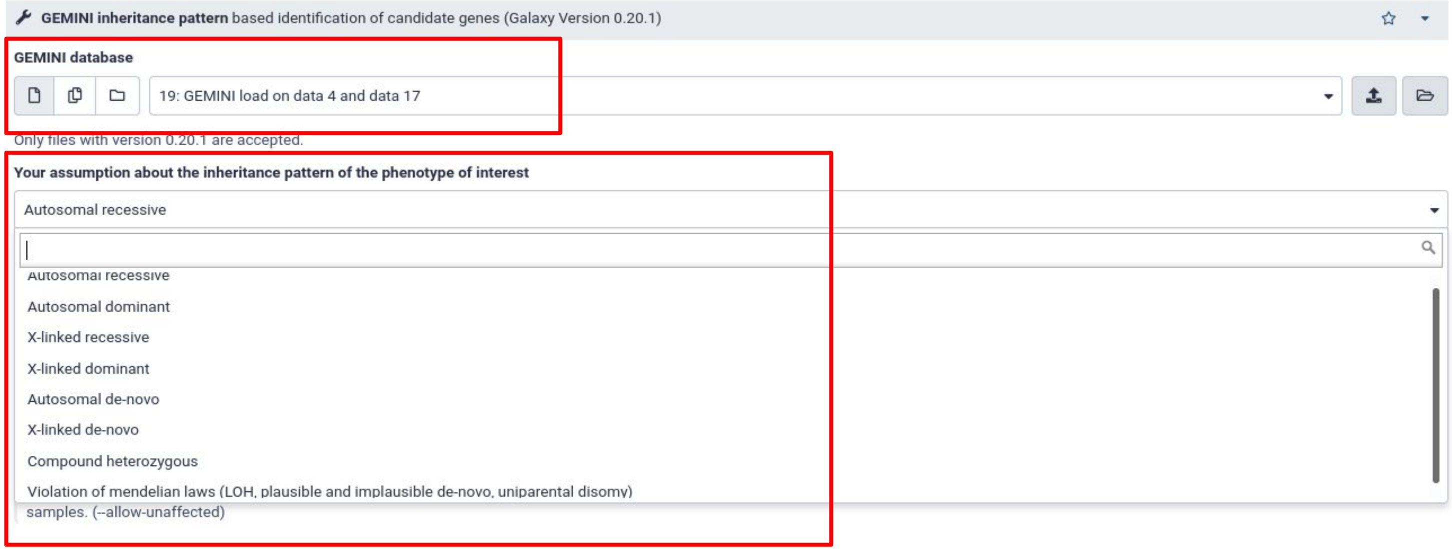
GEMINI gene_wise Discover per-gene variant patterns across families

GEMINI fusions Identify somatic fusion genes from a GEMINI database

GEMINI annotate the variants in an existing GEMINI database with additional information

GEMINI inheritance pattern based identification of candidate genes

Variant reporting - Querying



GEMINI inheritance pattern based identification of candidate genes (Galaxy Version 0.20.1)

GEMINI database

19: GEMINI load on data 4 and data 17

Only files with version 0.20.1 are accepted.

Your assumption about the inheritance pattern of the phenotype of interest

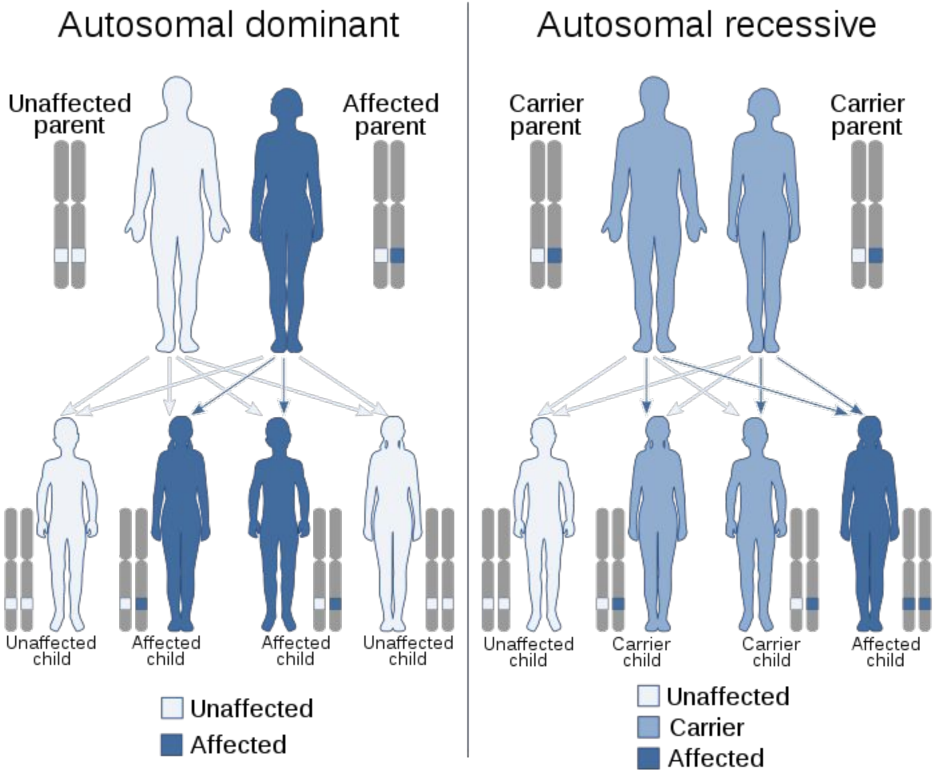
Autosomal recessive

- Autosomal recessive
- Autosomal dominant
- X-linked recessive
- X-linked dominant
- Autosomal de-novo
- X-linked de-novo
- Compound heterozygous
- Violation of mendelian laws (LOH, plausible and implausible de-novo, uniparental disomy)

samples. (--allow-unaffected)

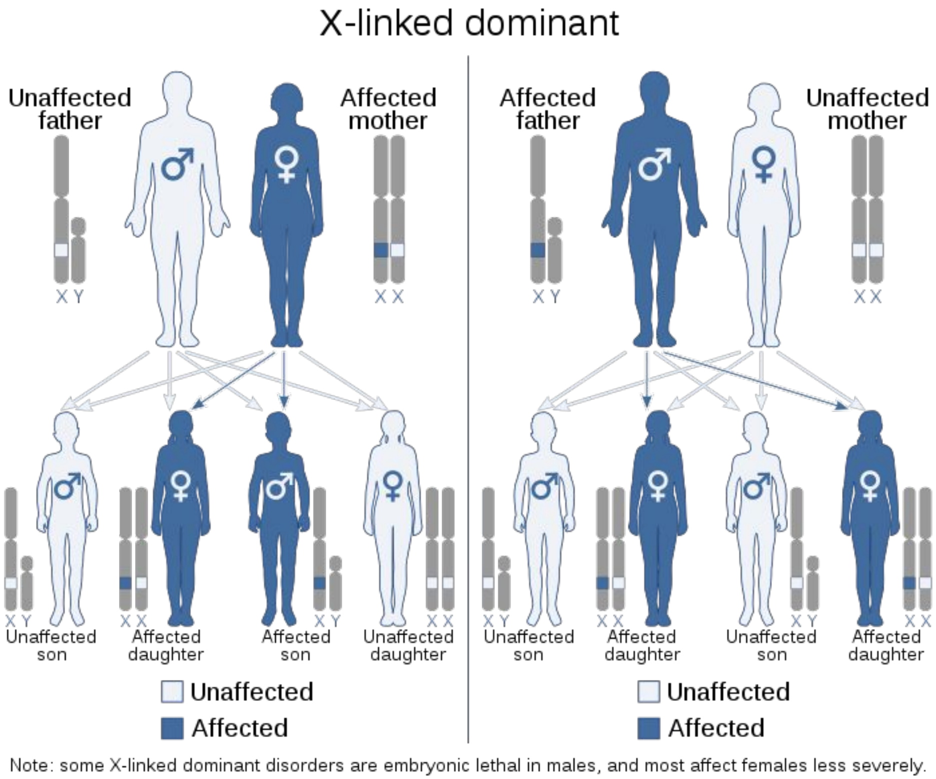
Which inheritance pattern to select ?

Variant reporting - Inheritance pattern



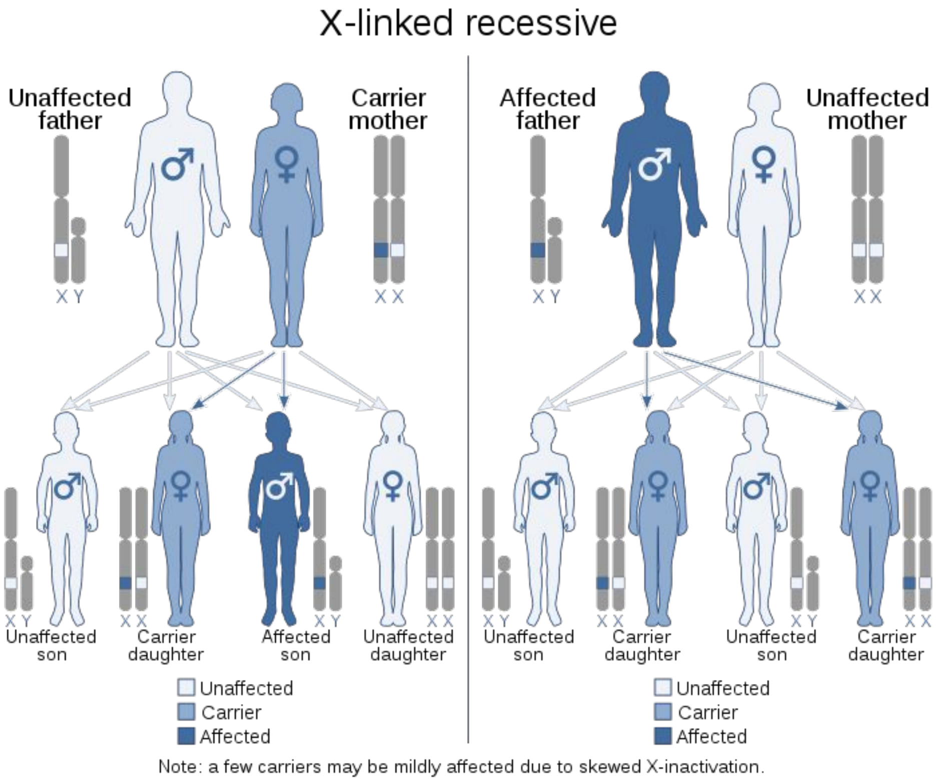
https://en.wikipedia.org

Variant reporting - Inheritance pattern



https://en.wikipedia.org

Variant reporting - Inheritance pattern



https://en.wikipedia.org

Variant reporting - Inheritance pattern

- Autosomal de-novo : mutation on autosomes (chr1-22), mutation not present in parents
- X-linked de-novo : mutation on the sex chromosome X, mutation not present in parents
- Compound heterozygous : 2 or more recessive alleles at a particular locus
- Violation of mendelian laws :
  - LOH : Loss of Heterozygosity, cross chromosomal event resulting in in loss of an entire gene and the surrounding chromosomal region
  - Plausible de-novo : parents are homozygous reference, offspring is heterozygous
  - Implausible de-novo : parents are homozygous reference, offspring is homozygous alternate
  - Uniparental disomy : one parent and the offspring are homozygous reference, the other parent is homozygous alternate OR one parent and the offspring are homozygous alternate and the other parent is homozygous reference

Variant reporting - Inheritance pattern

- Autosomal recessive
- Autosomal dominant
- X-linked recessive
- X-linked dominant
- Autosomal de-novo
- X-linked de-novo
- Compound heterozygous
- Violation of mendelian laws

Variant reporting - Inheritance pattern

- Autosomal recessive
- Autosomal dominant
- X-linked recessive
- X-linked dominant
- Autosomal de-novo
- X-linked de-novo
- Compound heterozygous
- Violation of mendelian laws

Parents are unaffected

Variant reporting - Inheritance pattern

- Autosomal recessive
- Autosomal dominant
- X-linked recessive
- X-linked dominant
- Autosomal de-novo
- X-linked de-novo
- Compound heterozygous
- Violation of mendelian laws

Parents are unaffected

Parents are consiguineous

Variant reporting - Inheritance pattern

- Autosomal recessive
- Autosomal dominant
- X-linked recessive
- X-linked dominant
- Autosomal de-novo
- X-linked de-novo
- Compound heterozygous
- Violation of mendelian laws

Parents are unaffected

Parents are consiguineous

Chromosome 8

Variant reporting - Inheritance pattern

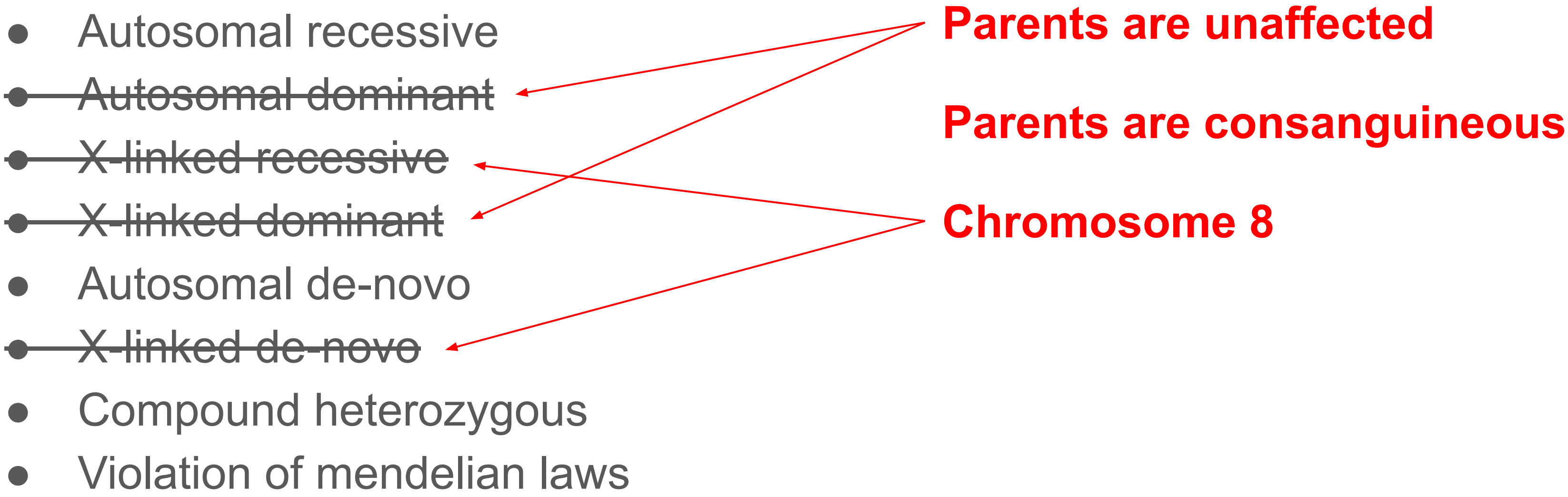
- Autosomal recessive
- ~~Autosomal dominant~~
- X-linked recessive
- ~~X-linked dominant~~
- Autosomal de-novo
- X-linked de-novo
- Compound heterozygous
- Violation of mendelian laws

Parents are unaffected

Parents are consiguineous

Chromosome 8

Variant reporting - Inheritance pattern



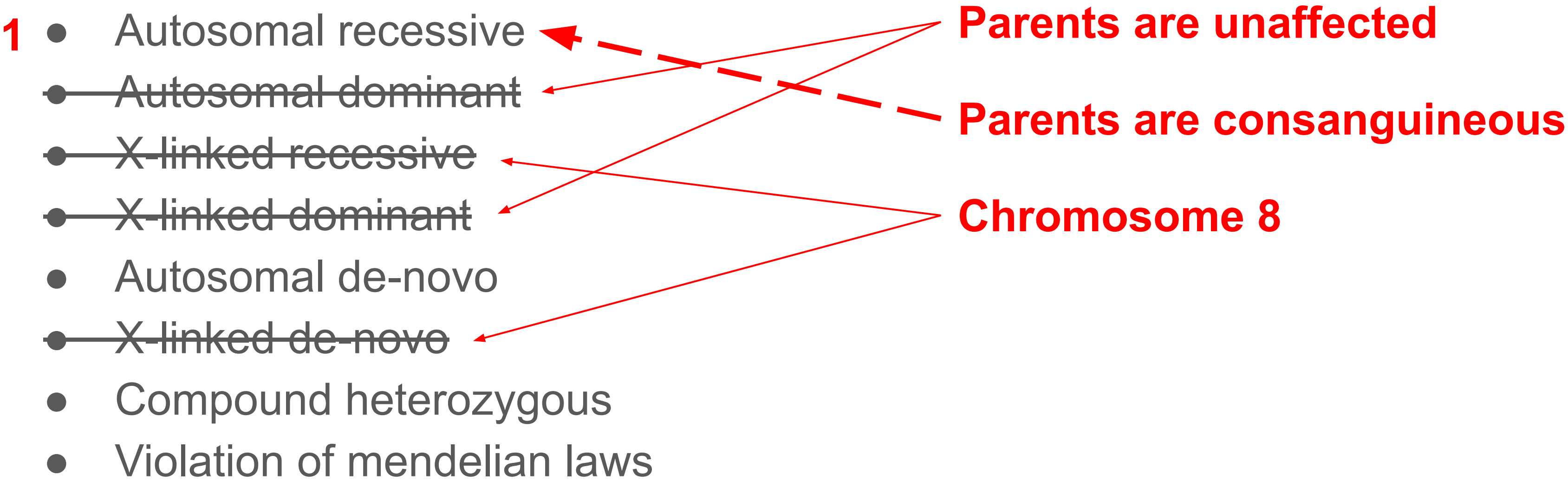
- Autosomal recessive
- ~~Autosomal dominant~~
- ~~X-linked recessive~~
- ~~X-linked dominant~~
- Autosomal de-novo
- ~~X-linked de-novo~~
- Compound heterozygous
- Violation of mendelian laws

Parents are unaffected

Parents are consanguineous

Chromosome 8

Variant reporting - Inheritance pattern



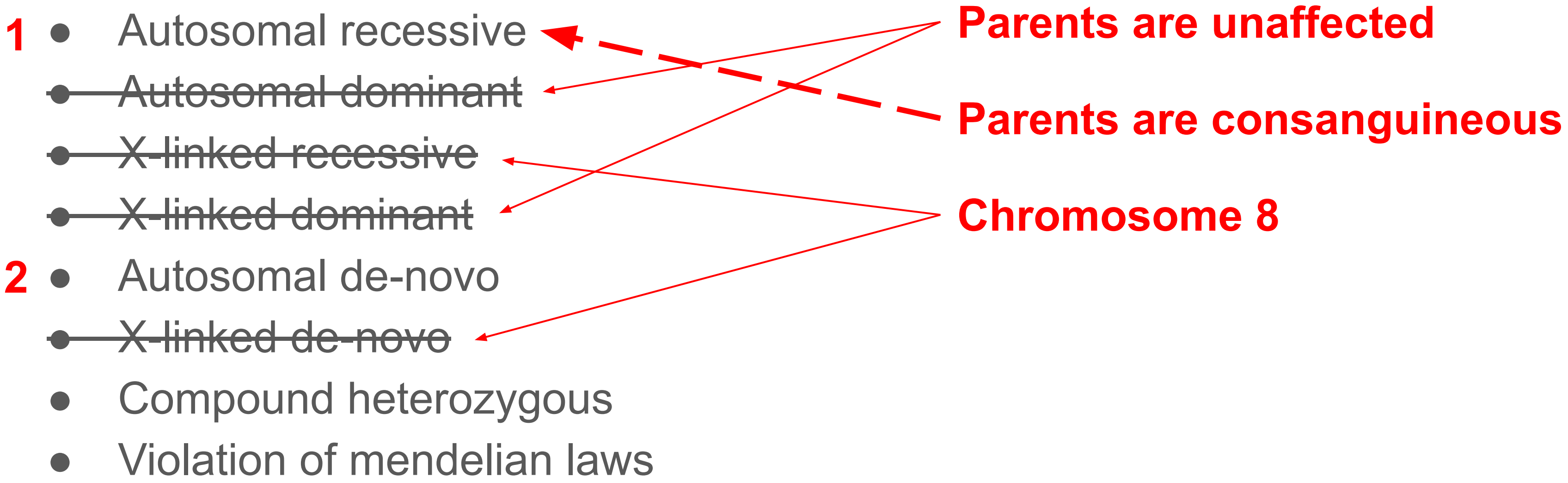
- 1 Autosomal recessive
- ~~Autosomal dominant~~
- ~~X-linked recessive~~
- ~~X-linked dominant~~
- Autosomal de-novo
- ~~X-linked de-novo~~
- Compound heterozygous
- Violation of mendelian laws

Parents are unaffected

Parents are consanguineous

Chromosome 8

Variant reporting - Inheritance pattern



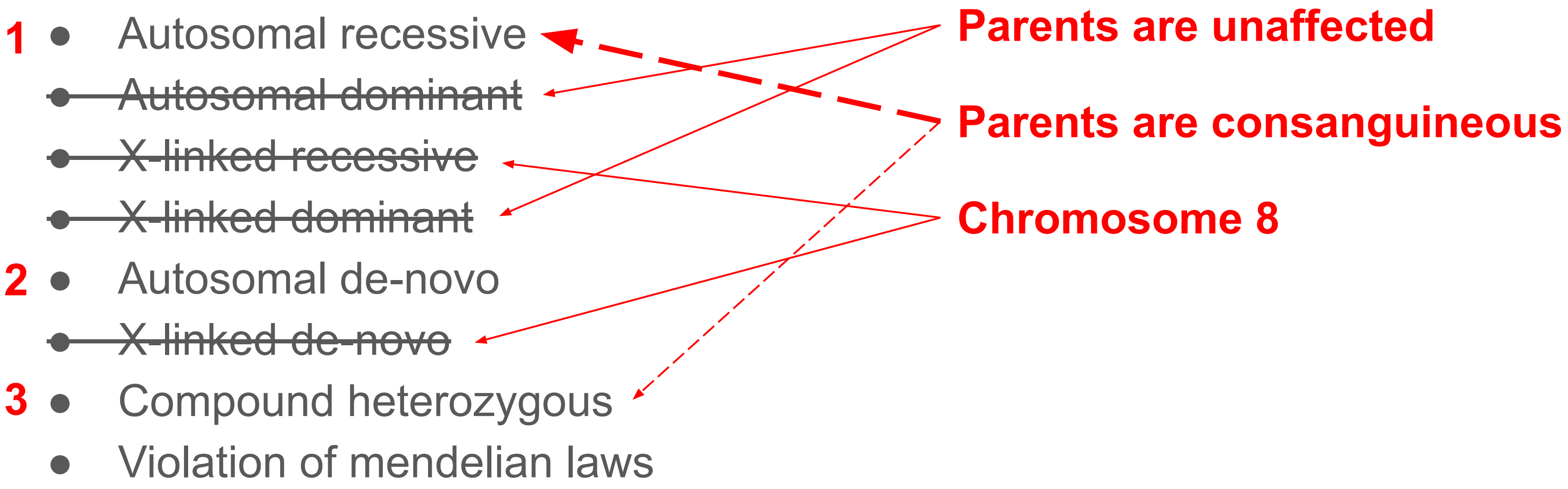
- 1 Autosomal recessive
- ~~Autosomal dominant~~
- ~~X-linked recessive~~
- ~~X-linked dominant~~
- 2 Autosomal de-novo
- ~~X-linked de-novo~~
- Compound heterozygous
- Violation of mendelian laws

Parents are unaffected

Parents are consanguineous

Chromosome 8

Variant reporting - Inheritance pattern



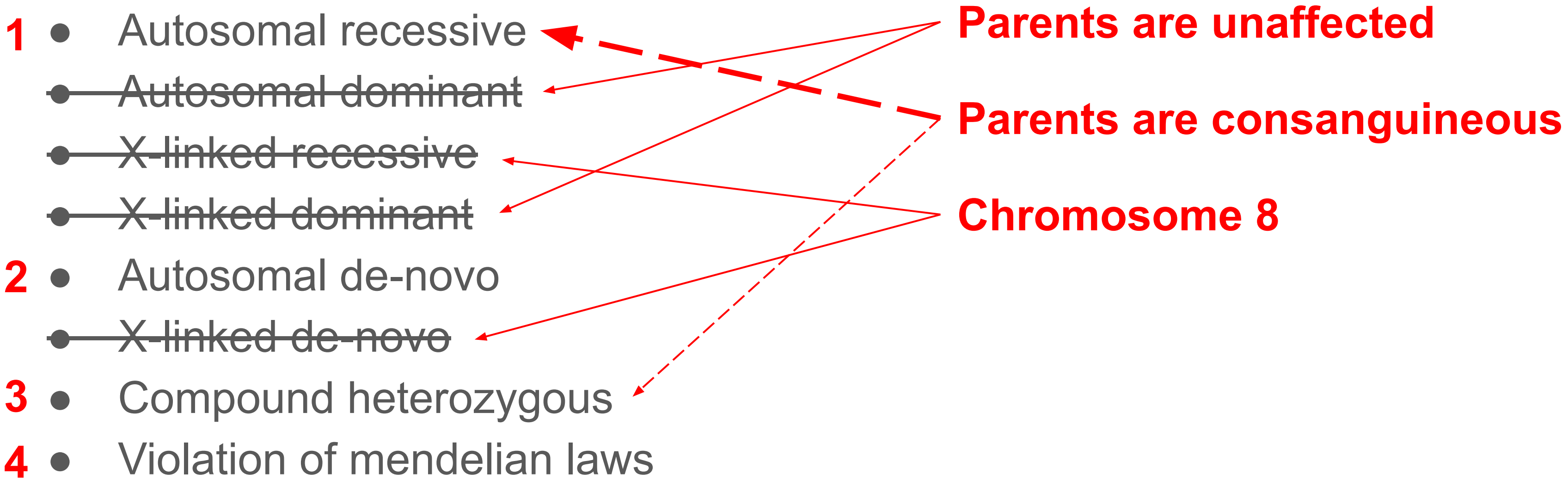
- 1 Autosomal recessive
- ~~Autosomal dominant~~
- ~~X-linked recessive~~
- ~~X-linked dominant~~
- 2 Autosomal de-novo
- ~~X-linked de-novo~~
- 3 Compound heterozygous
- Violation of mendelian laws

Parents are unaffected

Parents are consanguineous

Chromosome 8

Variant reporting - Inheritance pattern



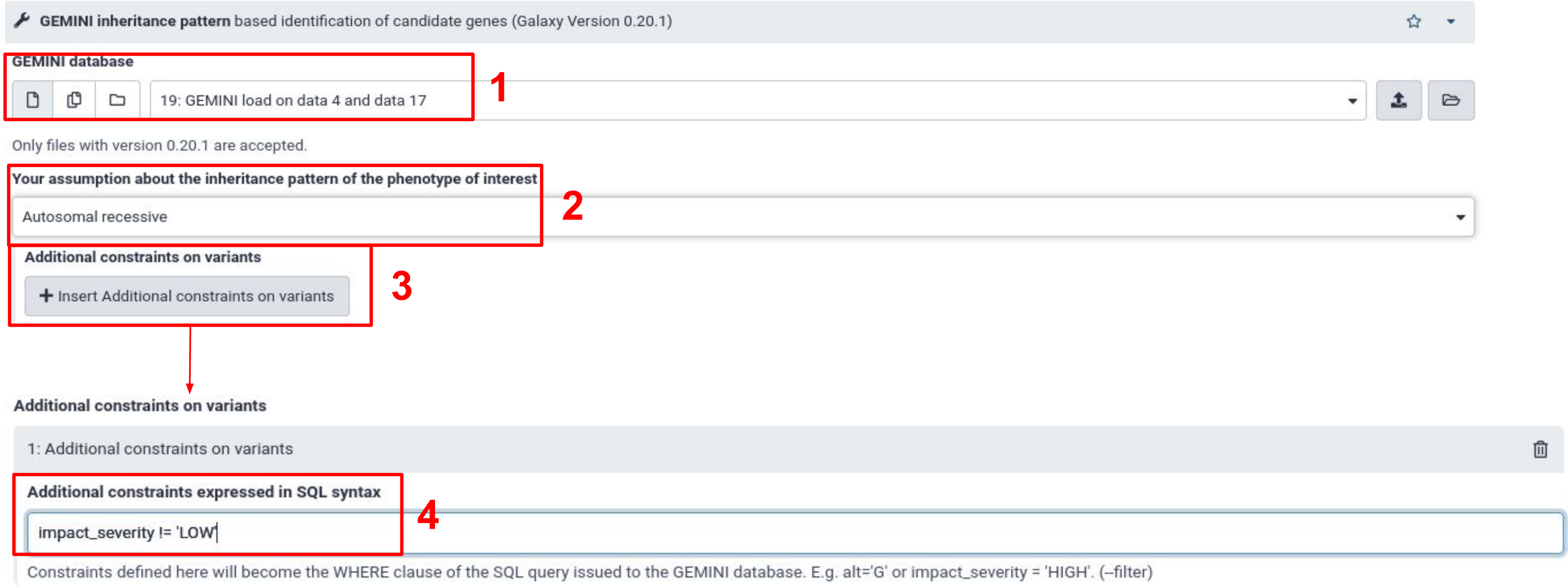
- 1 Autosomal recessive
- ~~Autosomal dominant~~
- ~~X-linked recessive~~
- ~~X-linked dominant~~
- 2 Autosomal de-novo
- ~~X-linked de-novo~~
- 3 Compound heterozygous
- 4 Violation of mendelian laws

Parents are unaffected

Parents are consanguineous

Chromosome 8

Variant reporting - Querying



GEMINI inheritance pattern based identification of candidate genes (Galaxy Version 0.20.1)

GEMINI database

19: GEMINI load on data 4 and data 17

Only files with version 0.20.1 are accepted.

Your assumption about the inheritance pattern of the phenotype of interest

Autosomal recessive

Additional constraints on variants

+ Insert Additional constraints on variants

Additional constraints on variants

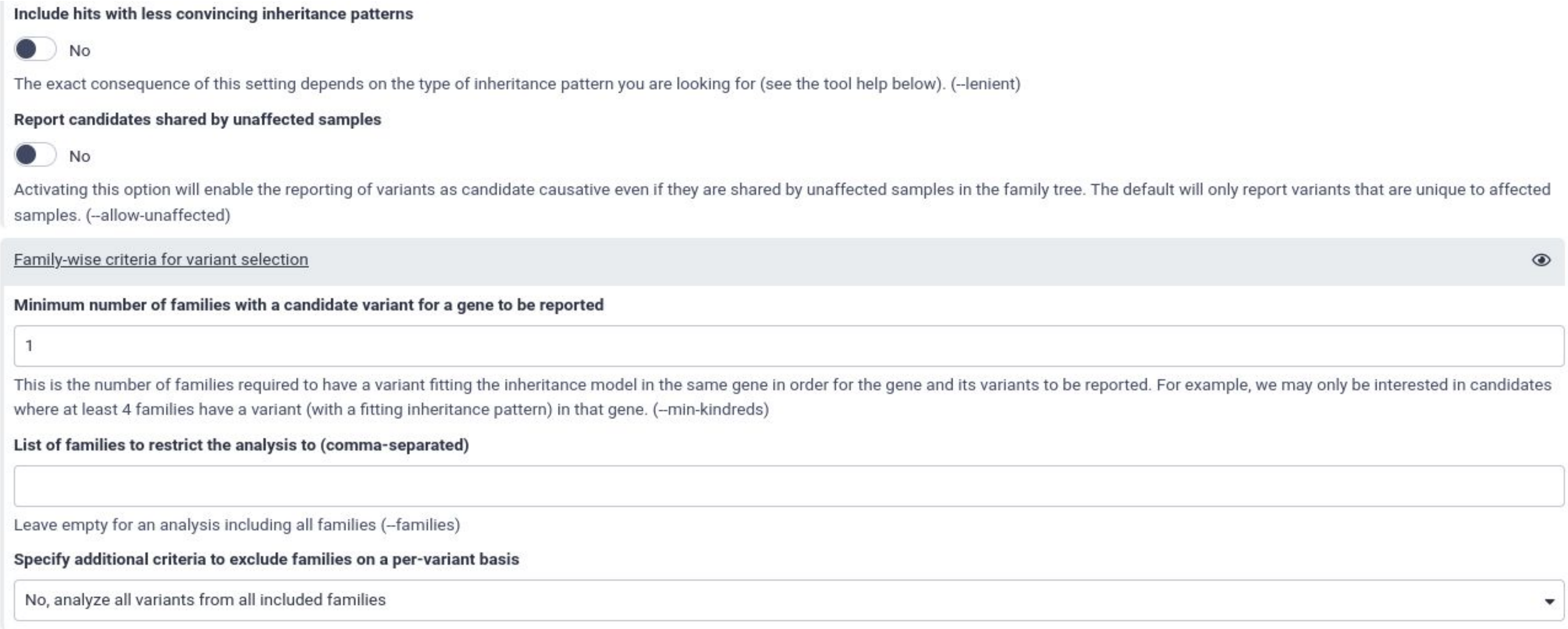
1: Additional constraints on variants

Additional constraints expressed in SQL syntax

impact_severity != 'LOW'

Constraints defined here will become the WHERE clause of the SQL query issued to the GEMINI database. E.g. alt='G' or impact_severity = 'HIGH'. (--filter)

Variant reporting - Querying



Include hits with less convincing inheritance patterns

○ No

The exact consequence of this setting depends on the type of inheritance pattern you are looking for (see the tool help below). (--lenient)

Report candidates shared by unaffected samples

○ No

Activating this option will enable the reporting of variants as candidate causative even if they are shared by unaffected samples in the family tree. The default will only report variants that are unique to affected samples. (--allow-unaffected)

Family-wise criteria for variant selection

Minimum number of families with a candidate variant for a gene to be reported

1

This is the number of families required to have a variant fitting the inheritance model in the same gene in order for the gene and its variants to be reported. For example, we may only be interested in candidates where at least 4 families have a variant (with a fitting inheritance pattern) in that gene. (--min-kindreds)

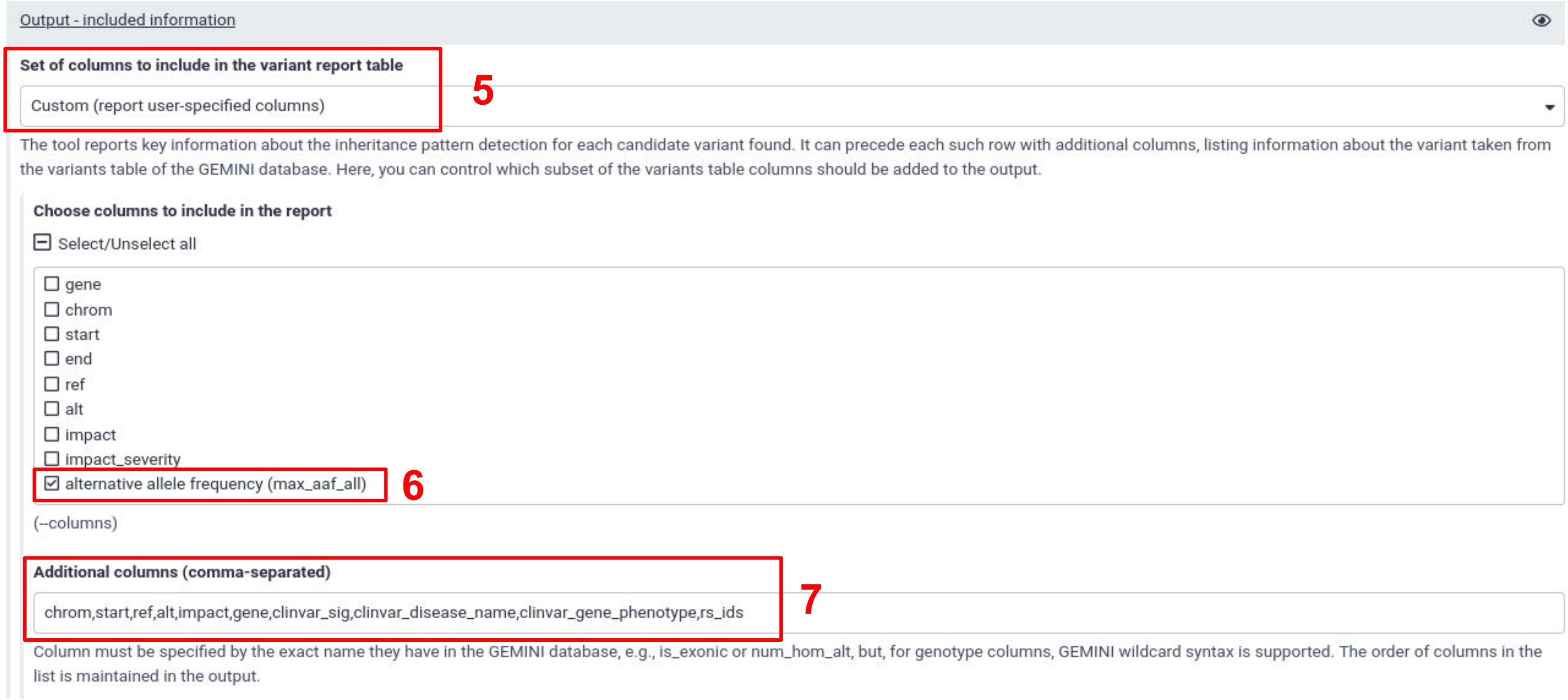
List of families to restrict the analysis to (comma-separated)

Leave empty for an analysis including all families (--families)

Specify additional criteria to exclude families on a per-variant basis

No, analyze all variants from all included families

Variant reporting - Querying



Output - included information

Set of columns to include in the variant report table

Custom (report user-specified columns)

5

The tool reports key information about the inheritance pattern detection for each candidate variant found. It can precede each such row with additional columns, listing information about the variant taken from the variants table of the GEMINI database. Here, you can control which subset of the variants table columns should be added to the output.

Choose columns to include in the report

⊟ Select/Unselect all

☐ gene
☐ chrom
☐ start
☐ end
☐ ref
☐ alt
☐ impact
☐ impact_severity
☑ alternative allele frequency (max_aaf_all)

6

(--columns)

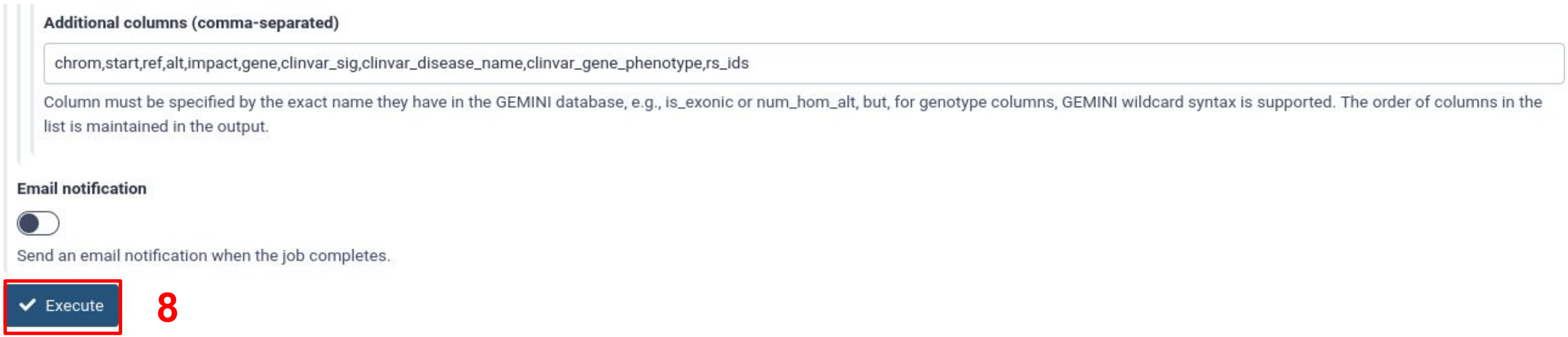
Additional columns (comma-separated)

chrom,start,ref,alt,impact,gene,clinvar_sig,clinvar_disease_name,clinvar_gene_phenotype,rs_ids

7

Column must be specified by the exact name they have in the GEMINI database, e.g., is_exonic or num_hom_alt, but, for genotype columns, GEMINI wildcard syntax is supported. The order of columns in the list is maintained in the output.

Variant reporting - Querying



Additional columns (comma-separated)

chrom,start,ref,alt,impact,gene,clinvar_sig,clinvar_disease_name,clinvar_gene_phenotype,rs_ids

Column must be specified by the exact name they have in the GEMINI database, e.g., is_exonic or num_hom_alt, but, for genotype columns, GEMINI wildcard syntax is supported. The order of columns in the list is maintained in the output.

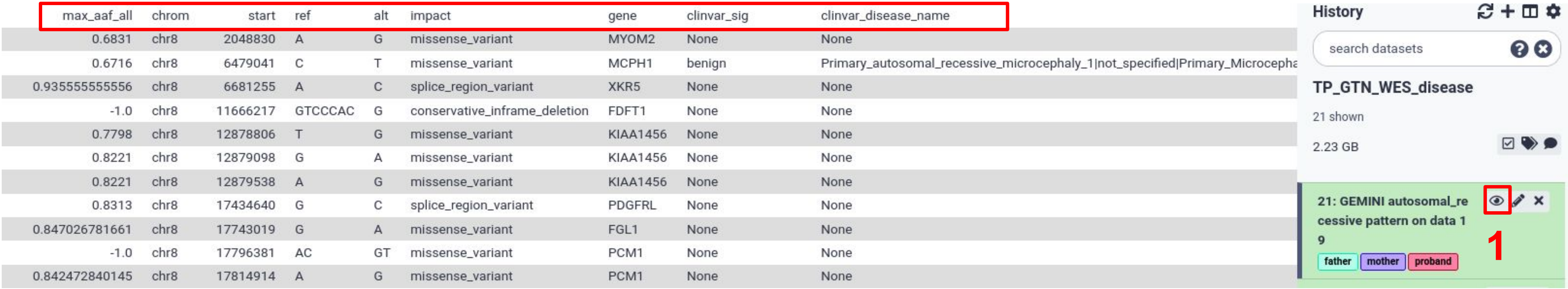
Email notification

○

Send an email notification when the job completes.

8

Variant reporting - Results



max_aaf_all	chrom	start	ref	alt	impact	gene	clinvar_sig	clinvar_disease_name
0.6831	chr8	2048830	A	G	missense_variant	MYOM2	None	None
0.6716	chr8	6479041	C	T	missense_variant	MCPH1	benign	Primary_autosomal_recessive_microcephaly_1|not_specified|Primary_Microcepha
0.935555555556	chr8	6681255	A	C	splice_region_variant	XKR5	None	None
-1.0	chr8	11666217	GTCCCAC	G	conservative_inframe_deletion	FDFT1	None	None
0.7798	chr8	12878806	T	G	missense_variant	KIAA1456	None	None
0.8221	chr8	12879098	G	A	missense_variant	KIAA1456	None	None
0.8221	chr8	12879538	A	G	missense_variant	KIAA1456	None	None
0.8313	chr8	17434640	G	C	splice_region_variant	PDGFRL	None	None
0.847026781661	chr8	17743019	G	A	missense_variant	FGL1	None	None
-1.0	chr8	17796381	AC	GT	missense_variant	PCM1	None	None
0.842472840145	chr8	17814914	A	G	missense_variant	PCM1	None	None

History

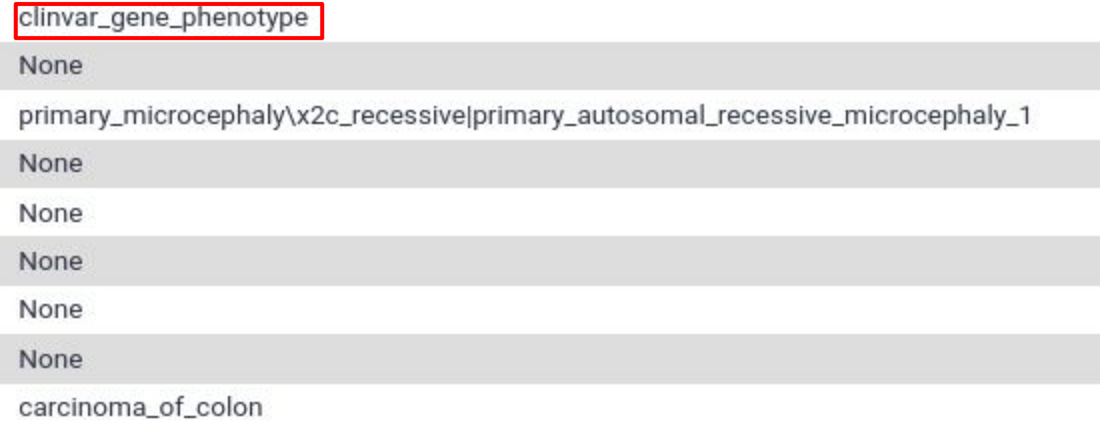
TP_GTN_WES_disease

21 shown

2.23 GB

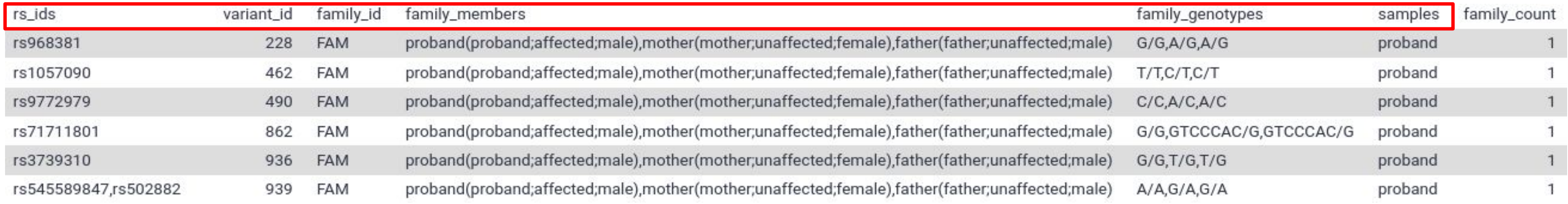
21: GEMINI autosomal_recessive pattern on data 19

Variant reporting - Results



clinvar_gene_phenotype
None
primary_microcephaly\x2c_recessive|primary_autosomal_recessive_microcephaly_1
None
None
None
None
None
carcinoma_of_colon

Variant reporting - Results

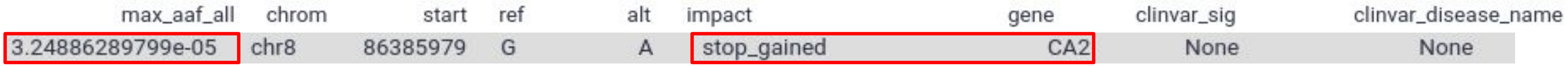


rs_ids	variant_id	family_id	family_members	family_genotypes	samples	family_count
rs968381	228	FAM	proband(proband;affected;male),mother(mother;unaffected;female),father(father;unaffected;male)	G/G,A/G,A/G	proband	1
rs1057090	462	FAM	proband(proband;affected;male),mother(mother;unaffected;female),father(father;unaffected;male)	T/T,C/T,C/T	proband	1
rs9772979	490	FAM	proband(proband;affected;male),mother(mother;unaffected;female),father(father;unaffected;male)	C/C,A/C,A/C	proband	1
rs71711801	862	FAM	proband(proband;affected;male),mother(mother;unaffected;female),father(father;unaffected;male)	G/G,GTCCCAC/G,GTCCCAC/G	proband	1
rs3739310	936	FAM	proband(proband;affected;male),mother(mother;unaffected;female),father(father;unaffected;male)	G/G,T/G,T/G	proband	1
rs545589847,rs502882	939	FAM	proband(proband;affected;male),mother(mother;unaffected;female),father(father;unaffected;male)	A/A,G/A,G/A	proband	1

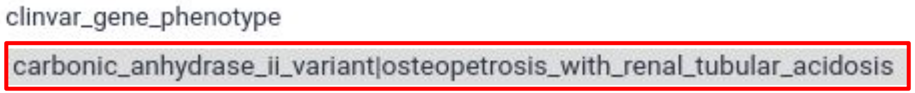
Variant reporting - Results

Most likely variant candidate for child's disease ?

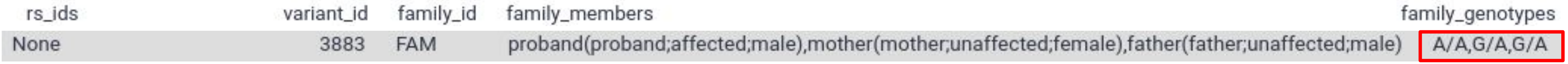
Variant reporting - Results



max_aaf_all	chrom	start	ref	alt	impact	gene	clinvar_sig	clinvar_disease_name
3.24886289799e-05	chr8	86385979	G	A	stop_gained	CA2	None	None



clinvar_gene_phenotype
carbonic_anhydrase_ii_variant|osteopetrosis_with_renal_tubular_acidosis



rs_ids	variant_id	family_id	family_members	family_genotypes
None	3883	FAM	proband(proband;affected;male),mother(mother;unaffected;female),father(father;unaffected;male)	A/A,G/A,G/A