Galaxy for virologist training Exercise 7: Illumina Variant Annotation 101

Title	Galaxy
Training dataset:	PRJEB43037 - In August 2020, an outbreak of West Nile Virus affected 71 people with meningoencephalitis in Andalusia and 6 more cases in Extremadura (south-west of Spain), causing a total of eight deaths. The virus belonged to the lineage 1 and was relatively similar to previous outbreaks occurred in the Mediterranean region. Here, we present a detailed analysis of the outbreak, including an extensive phylogenetic study. This is one of the outbreak samples.
Questions:	• Which effects have variants in the genome?
Objectives:	• Understand the importance of variants effect significance.
Estimated time:	1h

Table of Contents

- 1. Description
- 2. Upload data to galaxy
  - Training dataset
  - Create new history
  - Upload data
- 3. Preprocess our reads.
- 4. Map our reads against our reference genome.
- 5. Variant Calling.
- 6. Variants annotation
  - Load annotation file for West Nile genome.
  - Snpeff build
  - Snpeff eff
  - SnpSift: transfrom vcf snpeff to table.

1. Description

After performing variant calling, we want to know which is the importance of the variants in the viral genome. In order to give sense to the variants, we need to know in which gene they are, and which are their effects.

2. Upload data to galaxy

Training dataset

- Experiment info: PRJEB43037, WGS, Illumina MiSeq, paired-end
- Fastq R1: ERR5310322_1 - url : `ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR531/002/ERR5310322/ERR5310322_1.fastq.gz`
- Fastq R2: ERR5310322_2 url : `ftp://ftp.sra.ebi.ac.uk/vol1/fastq/ERR531/002/ERR5310322/ERR5310322_2.fastq.gz`
- Reference genome NC_009942.1: fasta -- gff

Create new history

- Click the + icon at the top of the history panel and create a new history with the name `mapping 101 tutorial` as explained here

Upload data

Follow the same instructions here

3. Preprocess our reads.

Follow instructions here

4. Map our reads against our reference genome.

Follow instructions here

5. Variant Calling.

Follow instructions here

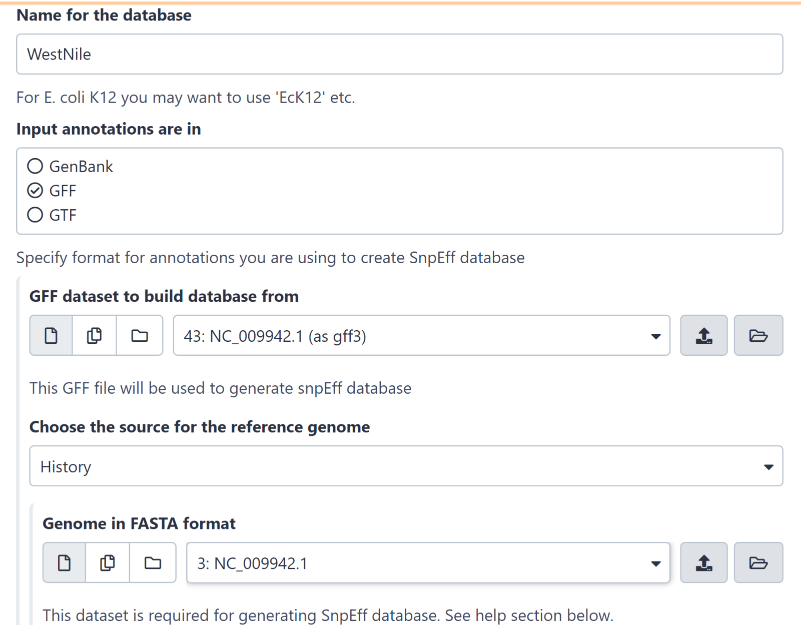
6. Variants annotation

Load annotation file for West Nile genome.

1. Load gff file
2. Upload file
3. Paste/Fetch data: gff
4. Rename dataset: NC_009942.1.

Snpeff build

1. Search `snpeff build` in the search toolbox.
2. Name of the database: WestNile.
3. Input annotations are in: GFF
4. GFF dataset to build database from: NC_009942.1 gff
5. Choose the source of the reference genome: History. NC_009942.1 fasta.
6. Click execute and wait.



Name for the database

WestNile

For E. coli K12 you may want to use 'EcK12' etc.

Input annotations are in

○ GenBank
☑ GFF
○ GTF

Specify format for annotations you are using to create SnpEff database

GFF dataset to build database from

43: NC_009942.1 (as gff3)

This GFF file will be used to generate snpEff database

Choose the source for the reference genome

History

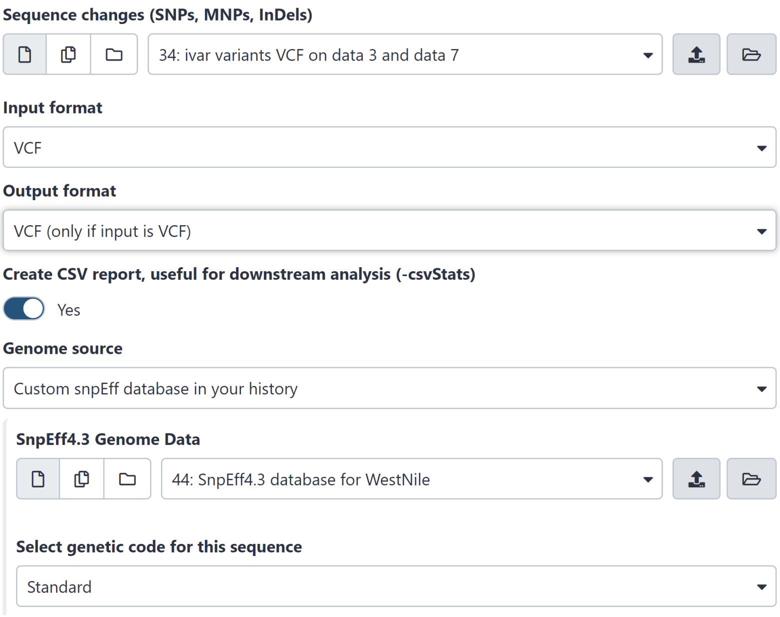
Genome in FASTA format

3: NC_009942.1

This dataset is required for generating SnpEff database. See help section below.

Snpeff eff

1. Search `snpeff eff` in the search toolbox.
2. Sequence changes (SNPs, MNPs, InDels): ivar vcf file
3. Genome source: Custom snpEff database in your history. Snpeff build output.
4. Create CSV report, useful for downstream analysis (-csvStats): Yes.
5. Click execute and wait.



Sequence changes (SNPs, MNPs, InDels)

34: ivar variants VCF on data 3 and data 7

Input format

VCF

Output format

VCF (only if input is VCF)

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

Yes

Genome source

Custom snpEff database in your history

SnpEff4.3 Genome Data

44: SnpEff4.3 database for WestNile

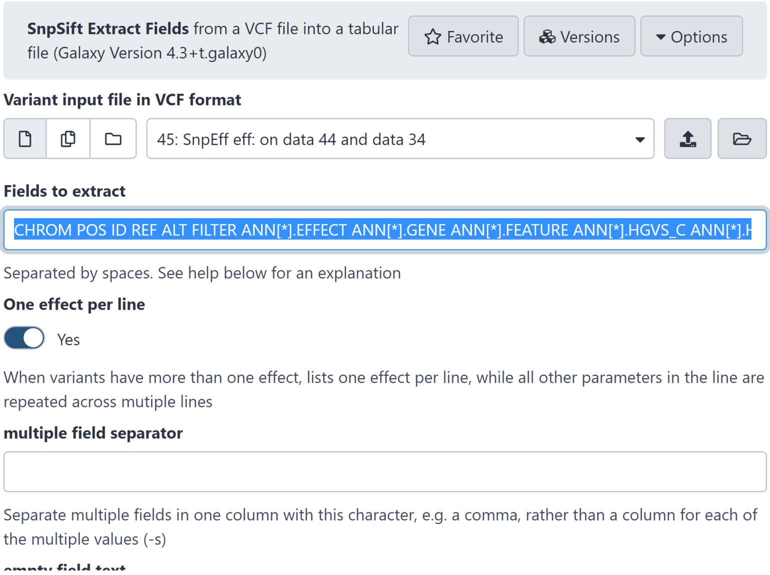
Select genetic code for this sequence

Standard

1. Click the :eye: icon in the SnpEff html output and check the results.

SnpSift: transfrom vcf snpeff to table.

1. Search `SnpSift ExtractFields` in the search toolbox.
2. Variant input file in VCF format: snpeff eff vcf output.
3. Fields to extract: CHROM POS ID REF ALT FILTER ANN[*].EFFECT ANN[*].GENE ANN[*].FEATURE ANN[*].HGVS_C ANN[*].HGVS_P
4. One effect per line: Yes.
5. Click execute and wait.
6. Click the :eye: icon in the snpsift output and check the results.



SnpSift Extract Fields from a VCF file into a tabular file (Galaxy Version 4.3+t.galaxy0)

Variant input file in VCF format

45: SnpEff eff: on data 44 and data 34

Fields to extract

CHROM POS ID REF ALT FILTER ANN[*].EFFECT ANN[*].GENE ANN[*].FEATURE ANN[*].HGVS_C ANN[*].H

Separated by spaces. See help below for an explanation

One effect per line

● Yes

When variants have more than one effect, lists one effect per line, while all other parameters in the line are repeated across mutiple lines

multiple field separator

Separate multiple fields in one column with this character, e.g. a comma, rather than a column for each of the multiple values (-s)

> Galaxy history for this exercise: https://usegalaxy.eu/u/smonzon/h/variant-calling-101-tutorial