GULBENKIAN CIÊNCIA

Annotation and filtering: hands on

Precision Oncology Course

SPANISH NATIONAL CANCER RESEARCH CENTRE

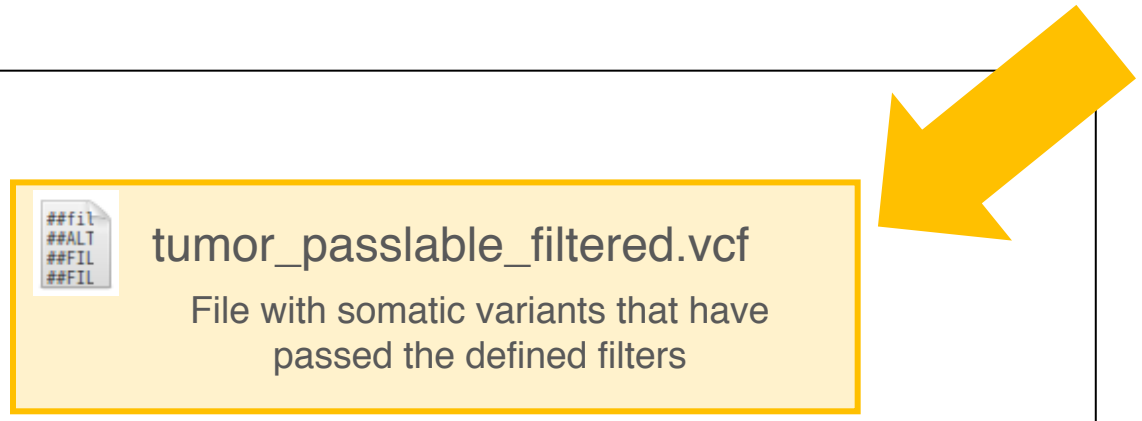
CNIO **BIOINFORMATICS** UNIT

Coral Fustero Torre
Bioinformatics Unit,
Structural Biology Programme.
cfustero@cnio.es | bioinformatics.cnio.es

Exercise 1: Annotations with VEP

Tumor type: hepatic met – colon adenocarcinoma

- Sequencing: Illumina
- Panel: HiSeq 2500
- Tumor – control paired sample
- File with somatic variants: variants detected in tumor sample but not in the corresponding control
- Assembly version: hg19



Link to exercise directory: https://drive.google.com/drive/folders/1T4PLkI4wgzxdjf-USfvv7gI8W5gW4Ypx?usp=sharing

Exercise 1: Annotations with VEP

Execution of VEP through the web page

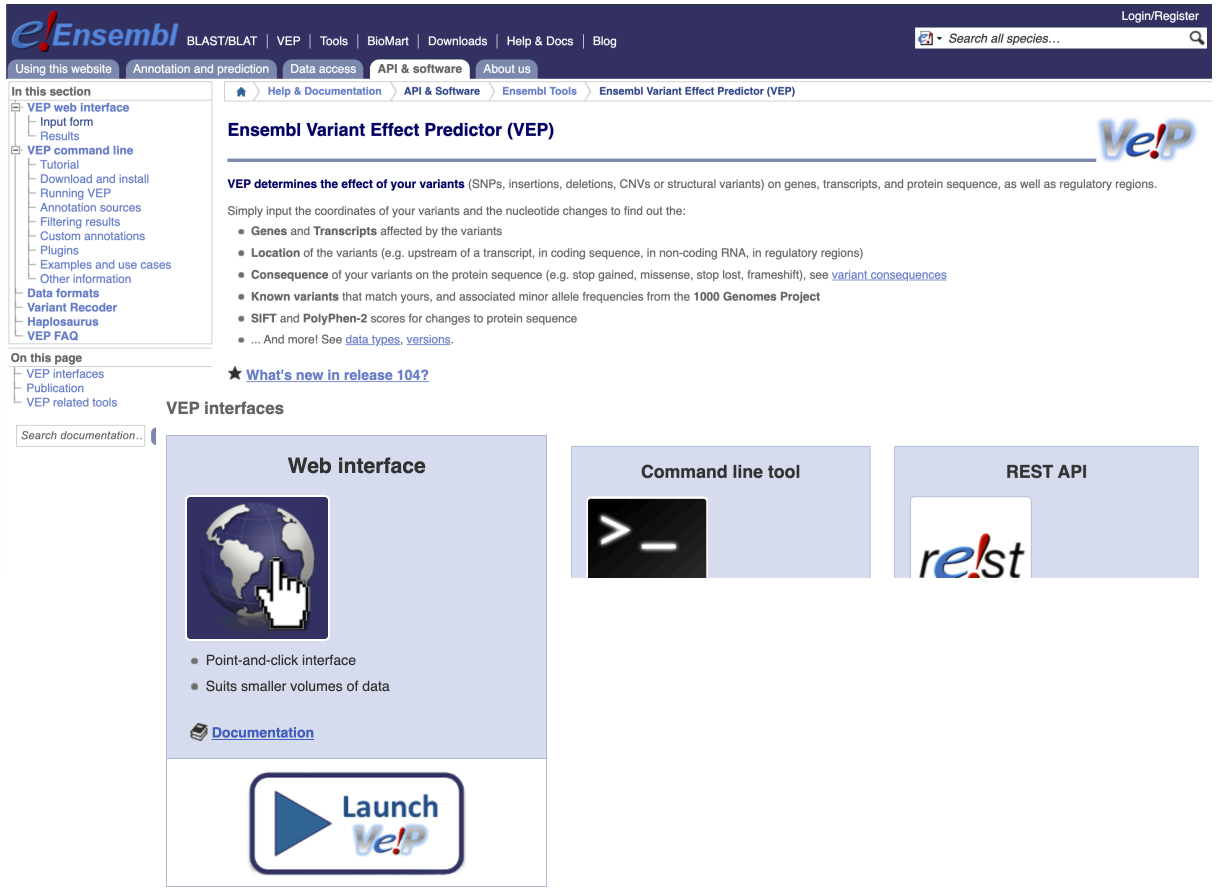
1. Go to:
http://www.ensembl.org/info/docs/tools/vep/index.html

2. We want to obtain the annotations using the ensembl transcript set

3. We want in the output the following annotations:

- HUGO gene symbol
- The HGVS identifiers for coding DNA and protein
- The Global Minor Allele Frequency of 1000 genomes project
- gnomAD frequencies
- SIFT and PolyPhen prediction and score
- Condel prediction and score

4. You can choose any other parameters to explore the results



HINTS: Remember to use the same assembly used in the variant detection.
Further info: http://www.ensembl.org/info/docs/tools/vep/online/input.html

Exercise 1: Annotations with VEP

Answer the following questions

- How many variants were in the input file?
- How many of them are not known in the database?
- How many genes and transcripts are affected by the variants?
- Is there any regulatory region overlapping some variant?
- Which is the most represented consequence category?
- Which is the most represented coding sequence consequence?
- How many variants fall in a coding region in some gene?
- What do the HGVS identifiers mean in each case?
- Does the prediction tool agree in the prediction of functional impact?
- Is there any clear polymorphism within the data?

… and explore the results

Exercise 1: Annotations with VEP

Download the file

1. Save the file in vcf format

2. Check that the following annotations have been added to the INFO field:

- Consequence
- Existing_variation
- Feature
- PolyPhen
- Condel
- SIFT
- SYMBOL
- Protein_position
- Amino_acids
- HGVSc
- HGVSp
- AF
- CDS_position
- Allele
- Gene
- Feature_type
- cDNA_position
- Codons
- gnomAD_AF
- gnomAD_NFE_AF
- ExAC

Exercise 2: Understanding the annotations

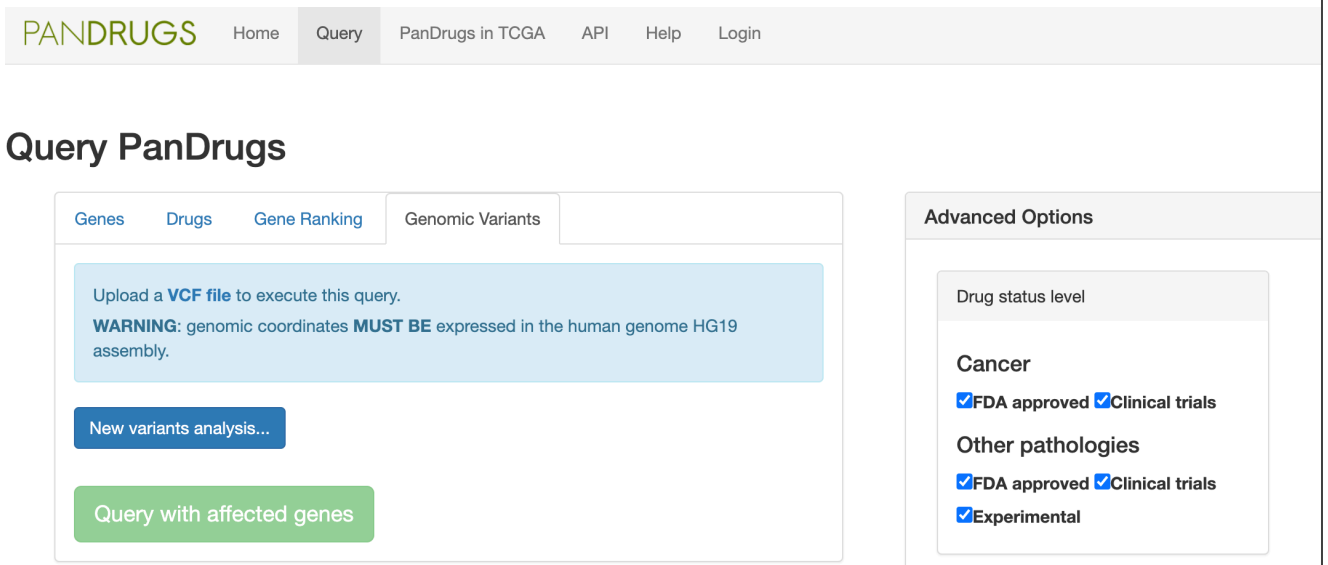
Tumor type: hepatic met – colon adenocarcinoma

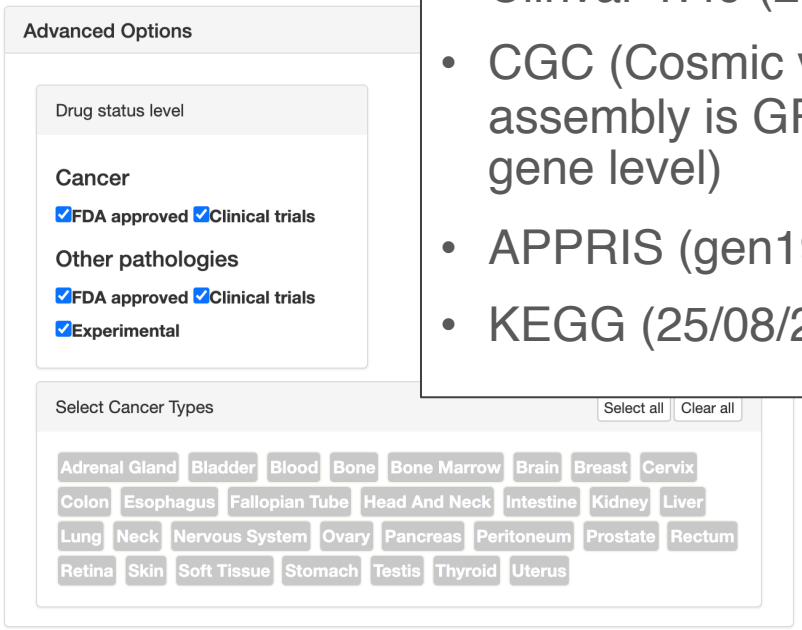
- Sequencing: Illumina
- Panel: HiSeq 2500
- Tumor – control paired sample
- File with somatic variants: variants detected in tumor sample but not in the corresponding control
- Assembly version: hg19

Link to exercise file:
https://fundacioncnio-my.sharepoint.com/:x:/g/personal/epineiro_cnio_es/EfnzQT8TszRAibwh_tK21MUBe7zTZ8Uah-Tr6qD5EU_I4Q?e=BQJiaw

Exercise 2: Understanding the annotations

1. Go to: pandrugs.org/
2. Upload the vcf file @ Genomic variants section
3. Download the results
This might take some time…





Databases versions

- Cosmic Release v82 - hg19
- Pfam 31.0 (Mar 2017)
- UniProt release 2017_07 (28/08/2017)
- InterPro 64.0 (28/08/2017)
- Clinvar 1.49 (26/08/2017)
- CGC (Cosmic v82) → The corresponding assembly is GRCH38 (but we search at gene level)
- APPRIS (gen19.ensembl74 29/08/2017)
- KEGG (25/08/2017)

Exercise 2: Understanding the annotations

Selection of principal isoform:

PRINCIPAL:1 - Transcript(s) expected to code for the main functional isoform based solely on the core modules in the APPRIS database
PRINCIPAL:2 - Where the APPRIS core modules are unable to choose a clear principal variant (approximately 25% of human protein coding genes), the database chooses two or more of the CDS variants as "candidates" to be the principal variant
PRINCIPAL:3 - Where the APPRIS core modules are unable to choose a clear principal variant and more than one of the variants have distinct CCDS identifiers, APPRIS selects the variant with lowest CCDS identifier as the principal variant
PRINCIPAL:4 - Where the APPRIS core modules are unable to choose a clear principal CDS and there is more than one variant with a distinct (but consecutive) CCDS identifiers, APPRIS selects the longest CCDS isoform as the principal variant
PRINCIPAL:5 - Where the APPRIS core modules are unable to choose a clear principal variant and none of the candidate variants are annotated by CCDS, APPRIS selects the longest of the candidate isoforms as the principal variant
REST (ALTERNATIVE:1 (Candidate transcript(s) models that are conserved in at least three tested non-primate species), ALTERNATIVE:2 (Candidate transcript(s) models that appear to be conserved in fewer than three tested non-primate species), NO LABEL (Non-candidate transcripts are not flagged and are considered as "MINOR" transcripts))

Reduced to relevant isoforms if PRINCIPAL

Exercise 2: Understanding the annotations

Open the results file and check the results

Try to answer the following questions:

- Which fields indicate polymorphisms?
- Which fields have information about the effect in the sequence?
- Which fields have information about the effect in the protein?
- Which fields give specific information about the pathology under study?
- In which processes are involved APC and FBXW7 gene?
- Is the gene KRAS frequently mutated in the same tumor type?
- Which variant has been reported more times in tumors?
- Should ATM gene be inhibited?
- Name 3 candidates as relevant variants in the disease

Thanks!

Credits for many class materials to:

Héctor Tejero: htejero@cnio.es
Elena Piñeiro: epineiro@cnio.es
Javier Perales-Patón: jperales@cnio.es

CNIO **BIOINFORMATICS** UNIT