GULBENKIAN CIÊNCIA

PO: Precision Oncology Course

Variant annotation, filtering and prioritization

BU CNIO BIOINFORMATICS UNIT

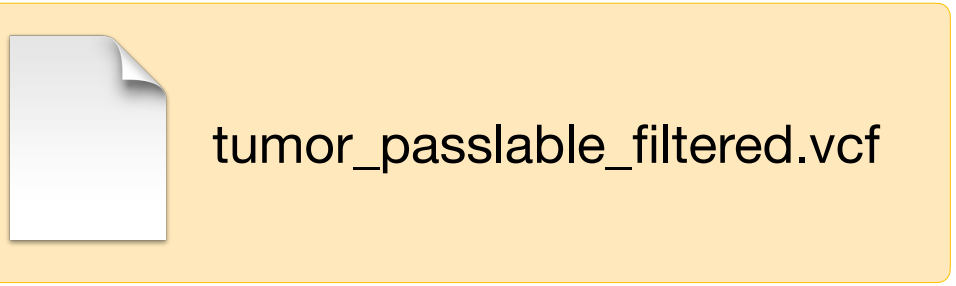
cnio stop cancer

Exercise

Annotation of the panel 1 using PanDrugs

Study case

Panel 1



Tumor type: Patient with Colon Adenocarcinoma

Sequencing platform: Illumina HiSeq2500

Type of data: Sequencing panel (paired). Ion Ampliseq Cancer Hotspot Panel v2 (46 genes)

Samples: Tumor with matched healthy tissue

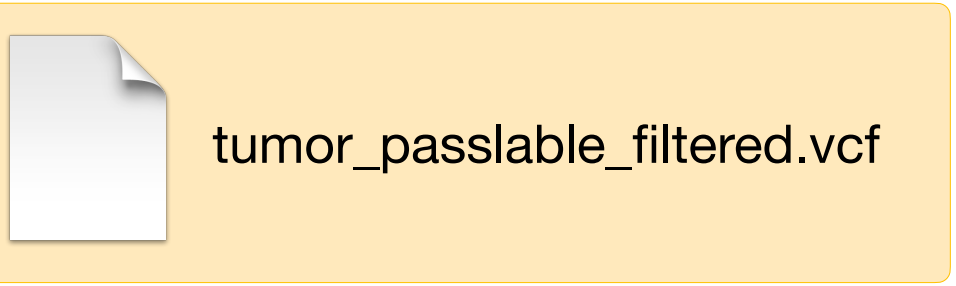
File with somatic variants from Mutect2: Variants detected in tumor sample but not in the corresponding control

Data: https://drive.google.com/drive/folders/1d1lRuevln_rL9yx2oYUppt9ylJRCtnJ7?usp=sharing

Reference genome: hg19

Study case

Panel 1



The VCF has only **5 somatic mutations** and all of them have a **PASS label** in the column FILTER.

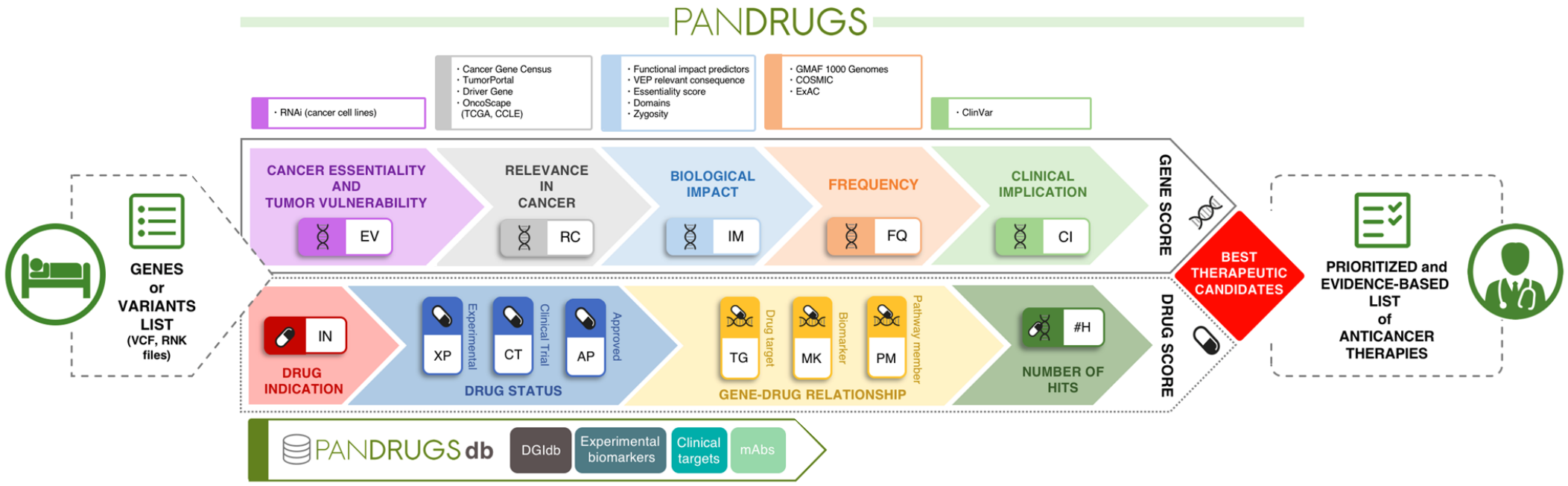
```
##normal_sample=normal
##source=FilterMutectCalls
##source=Mutect2
##source=VariantFiltration
##tumor_sample=tumor
#CHROM   POS      ID       REF      ALT      QUAL     FILTER   INFO     FORMAT   normal   tumor
chr3     178952085        .        A        G        .        PASS
CONTQ=93;DP=2498;ECNT=1;GERMQ=93;MBQ=20,20;MFRL=187,193;MMQ=60,60;MPOS=31;NALOD=2.04;NLOD=358.52;POPAF=6.00;SEQQ=93;STRANDQ=93;TLOD=876.40
GT:AD:AF:DP:F1R2:F2R1:SB  0/0:1219,2:1.536e-03:1221:1145,1:0,0:802,417,0,2   0/1:745,448:0.373:1193:694,418:0,0:499,246,300,148
chr4     153245446        .        G        A        .        PASS
CONTQ=93;DP=1949;ECNT=1;GERMQ=93;MBQ=23,31;MFRL=191,180;MMQ=60,60;MPOS=36;NALOD=2.89;NLOD=229.94;POPAF=6.00;SEQQ=93;STRANDQ=93;TLOD=2194.74
GT:AD:AF:DP:F1R2:F2R1:SB  0/0:776,1:1.282e-03:777:725,0:0,0:616,160,1,0      0/1:317,799:0.720:1116:288,747:0,0:249,68,621,178
chr5     112175423        .        C        T        .        PASS
CONTQ=93;DP=5354;ECNT=1;GERMQ=93;MBQ=32,31;MFRL=203,198;MMQ=60,60;MPOS=30;NALOD=2.88;NLOD=713.02;POPAF=6.00;SEQQ=93;STRANDQ=93;TLOD=3699.09
GT:AD:AF:DP:F1R2:F2R1:SB  0/0:2410,4:7.317e-04:2414:2250,2:0,0:995,1415,3,1  0/1:1274,1484:0.536:2758:1200,1390:0,0:509,765,773,711
chr11    108117798        .        C        T        .        PASS
CONTQ=93;DP=1223;ECNT=1;GERMQ=93;MBQ=32,33;MFRL=186,189;MMQ=60,60;MPOS=35;NALOD=2.78;NLOD=177.83;POPAF=6.00;SEQQ=93;STRANDQ=93;TLOD=721.32
GT:AD:AF:DP:F1R2:F2R1:SB  0/0:597,0:1.663e-03:597:566,0:0,0:478,119,0,0      0/1:320,281:0.468:601:301,275:0,0:252,68,224,57
chr12    25398281 .       C        T        .        PASS
CONTQ=93;DP=2926;ECNT=1;GERMQ=93;MBQ=20,20;MFRL=189,188;MMQ=60,60;MPOS=31;NALOD=2.56;NLOD=106.16;POPAF=6.00;SEQQ=93;STRANDQ=93;TLOD=3583.42
GT:AD:AF:DP:F1R2:F2R1:SB  0/0:358,1:2.746e-03:359:334,1:0,0:238,120,0,1      0/1:1015,1446:0.590:2461:950,1359:0,0:658,357,944,502
```

So we can proceed to **annotate** these 5 variants **using PanDrugs.**

PanDrugs

Annotation and prioritization of variants

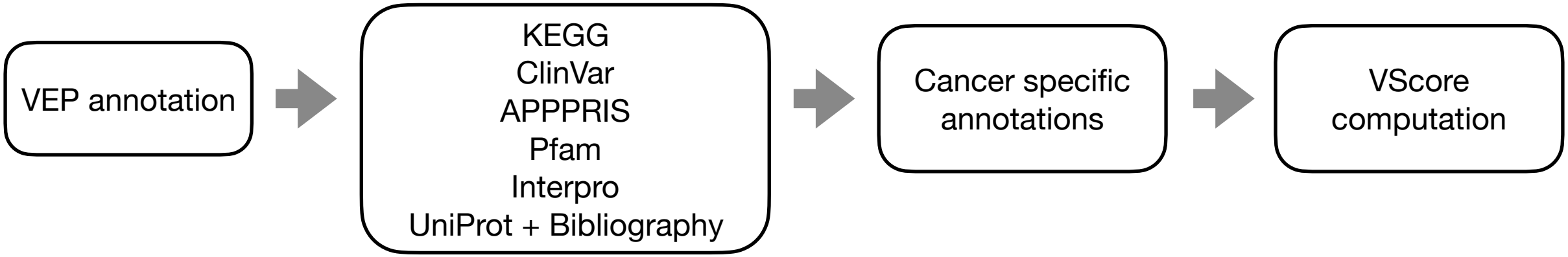
PanDrugs database unifies several resources for variant annotation. When given a VCF, PanDrugs annotates the variants and provides a Variant Score or **VScore (0 to 1)** which reflects their implication in cancer.



PanDrugs

Annotation and prioritization of variants

PanDrugs database unifies several resources for variant annotation. When given a VCF, PanDrugs annotates the variants and provides a Variant Score or **VScore (0-1)** which reflects their implication in cancer.



PanDrugs

Annotation and prioritization of variants

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)

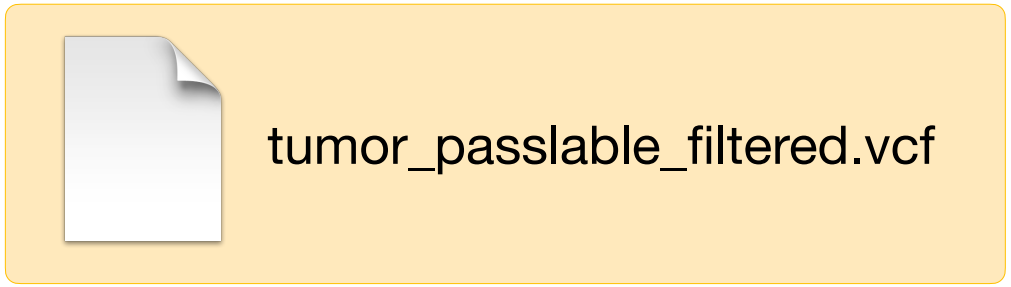
APPRIS (gen19.ensembl74 29/08/2017)

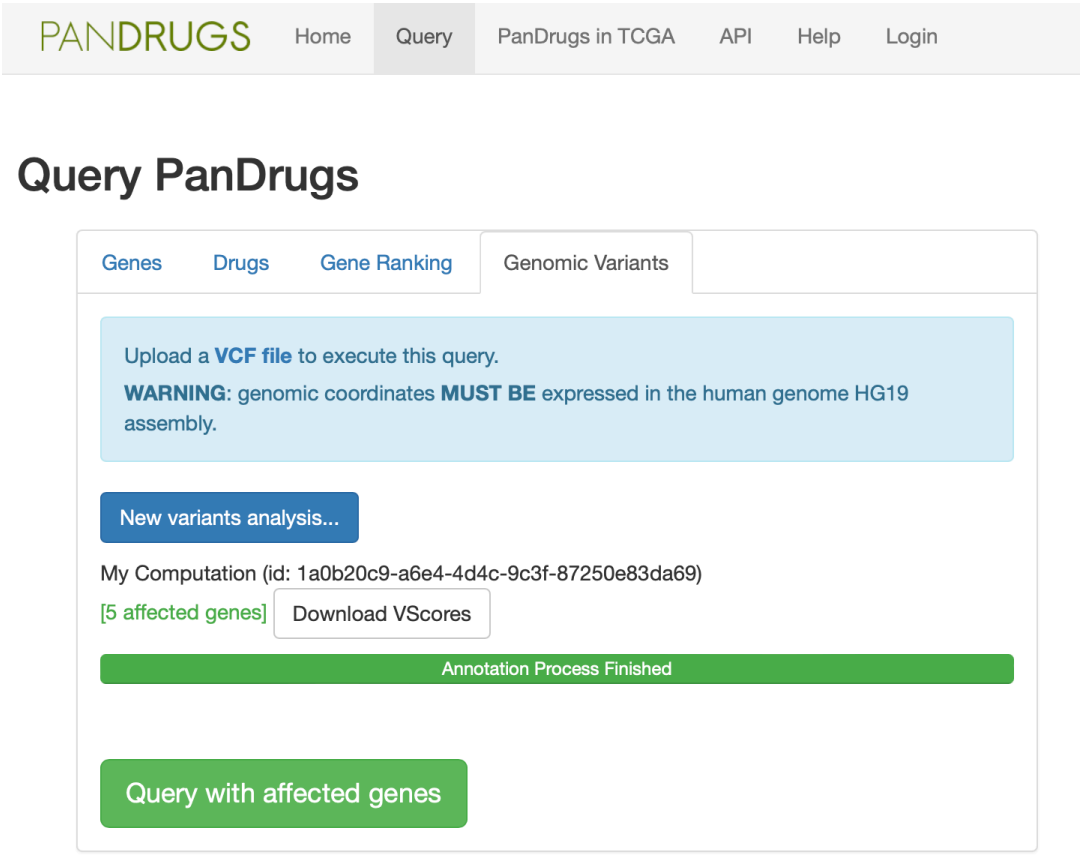
KEGG (25/08/2017)

Steps

Run PanDrugs from the web

1. Go to: https://www.pandrugs.org/#!/
2. Click in “Query” and “Genomic Variants”
3. Upload the VCF
4. Download the results clicking in “Download VScores”





Steps

Summarize PanDrugs annotation

Execute SummaryGenerator.py

```
$ python2 SummaryGenerator.py <input> <output>
```

The <input> is the output of PanDrugs.
This scripts filters the rows of <input> based on **APPRIS** annotation.

The script was created to work with the specific data used in these exercises.

APPRIS annotation

Annotation of splice isoforms

Selection of the 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)).

APPRIS annotation

Keep the annotations with the most reliable isoforms

Selection of the 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)).

Questions

30 min

- **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?**

Questions

30 min

- **In which processes are involved *APC* and *FBXW7* genes?**
- **Is the gene *KRAS* frequently mutated in the same tumor types?**
- **Which variant has been reported more times in tumors**
- **Should *ATM* gene be inhibited?**
- **Name 3 candidates as relevant variants in the disease.**


GULBENKIAN
CIÊNCIA

Thanks!


BU
CNIO BIOINFORMATICS UNIT


cnio
stop cancer