Precision medicine: NGS variant analysis and interpretation for translational research

Exercise 3: Prioritization and variant filtering

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Additional annotations and prioritization

VEP_parser

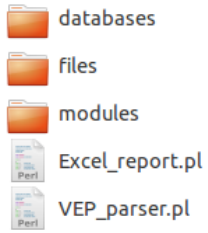
Adds annotations from additional data sources.
Change vcf format a tabular format.
Formatting and filtering annotations in which we are interested.

Parsing

Computes a score for each variant. Cancer oriented.

Execution: Command line and files

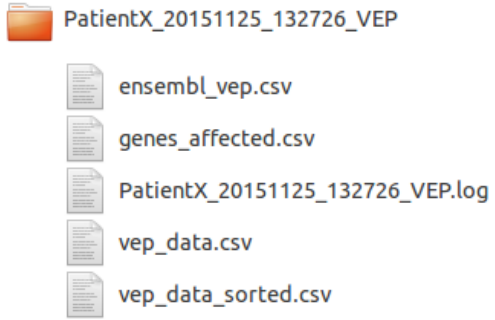
Directory structure



Command line execution

```
epineiro@epineiro:~/ExerciseDay2/VEP_parser$ perl VEP_parser.pl

--vepfile=filename or -f=filename              Input file containing results of VEP from Ensembl analysis. Mandatory.

--output=directory or -o=directory             Execution output dir. Default ./output.

--sample=code or -sp=code                      Tumor code. Mandatory.

--root=rootname or -r=rootname                 Root name for the PCDA output files. Default none.

--cfile=boolean or -v=boolean                  Use of filter consequences file VEP_conseq.csv or not. Default TRUE.

--databases=directory or -d=directory          Absolute path to databases directory. Mandatory.

i.e. VEP_parser.pl -f=file.vcf -o=/home/user/z13-222 -sp=z13-222 -r=analysis -v=TRUE -d=/home/user/VEP_parser/databases
```

Output files



vep_data_sorted.csv

chr	loc	mut	gene	feature	feature_type	consequence	principal	poly_effect	poly_score
3	178921553	T/A	ENSG00000121879	ENST00000468036	Transcript	downstream_gene_variant			
3	178921553	T/A	ENSG00000121879	ENST00000477735	Transcript	downstream_gene_variant			
3	178921553	T/A	ENSG00000121879	ENST00000263967	Transcript	missense_variant	PRINCIPAL:1	probably_damaging	1
4	1808898	-/C	ENSG00000068078	ENST00000469068	Transcript	downstream_gene_variant			
4	1808898	-/C	ENSG00000068078	ENST00000474521	Transcript	downstream_gene_variant			
4	1808898	-/C	ENSG00000068078	ENST00000507588	Transcript	downstream_gene_variant			
4	1808898	-/C	ENSG00000068078	ENST00000481110	Transcript	frameshift_variant		inferred	1
4	1808898	-/C	ENSG00000068078	ENST00000352904	Transcript	frameshift_variant		inferred	1
4	1808898	-/C	ENSG00000068078	ENST00000412135	Transcript	frameshift_variant		inferred	1
4	1808898	-/C	ENSG00000068078	ENST00000260795	Transcript	frameshift_variant	PRINCIPAL:3	inferred	1
4	1808898	-/C	ENSG00000068078	ENST00000440486	Transcript	frameshift_variant	PRINCIPAL:3	inferred	1
4	1808898	-/C	ENSG00000068078	ENST00000340107	Transcript	frameshift_variant	ALTERNATIVE:2	inferred	1

Excel generator

Selection of principal isoform:

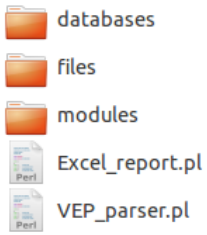
PRINCIPAL:1 - Transcript(s) expected to code for the main functional isoform based solely on the core modules in the APPRIS database
PRINCIPAL:2 - Where the APPRIS core modules are unable to choose a clear principal variant (approximately 25% of human protein coding genes), the database chooses two or 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))

Flag possible artifacts:

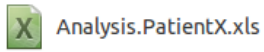
Germline variants defined as somatic - Filtered in normal sample
Very low coverage variants (especially with MuTect data)

Execution Excel generator

Directory structure



Output file



Command line execution

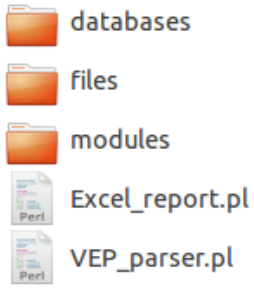
```
epineiro@epineiro:~/ExerciseDay2/VEP_parser$ perl Excel_report.pl

--output=directory       Execution output dir. Default ./output.

--case=name              Tumor case name. Mandatory.

--tsample=name           Tumor sample name. Mandatory.

--nsample=name           Normal sample name. Optional.

--vepfile1=path          VEP file absolute path. Mandatory.

--vepfile2=path          VEP file for bothTC calling absolute path. Optional.

--vepfile3=path          VEP file for MuTect calling absolute path. Optional.

--rubioseq=path          RUbioSeq output path. Mandatory.

--mutect=path            MuTect output path. Mandatory if --vepfile3.


i.e. perl IonTorrent_report.pl --output=/home/user/z13-222 --case=z13-222 --tsample=z13-222 --nsample=c13-222 --vepfile1=/home/user/z13-222/z13-222_20130826_104703_VEP/vep_data_sorted.csv --vepfile2=/home/
user/z13-222/z13-222-both_20130826_104703_VEP/vep_data_sorted.csv --vepfile3=/home/user/z13-222/z13-222-MuTect_20130826_104703_VEP/vep_data_sorted.csv --rubioseq=/home/user/z13-222/results35 --mutect=/home
/user/z13-222/MuTEct
```

Output file

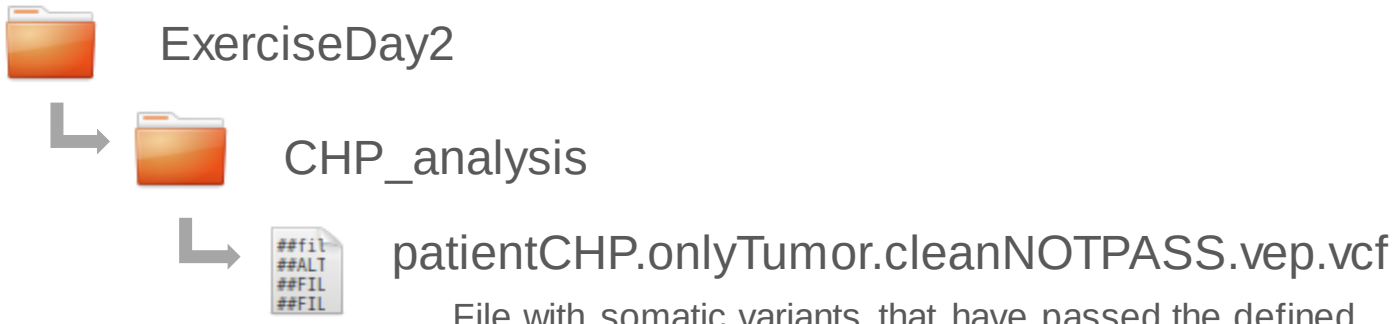
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	chr	loc	mut	gene_hgr	tumorpor	role_drive	zygosity	var_freq	coverage	gene	feature	conseque	functiona	cosmic_i
2	3	17892155	T/A	PIK3CA	PRAD:Ne	CGC:onco	Heterozyg	0.11	625	ENSG000	ENST000	missense	probably_	COSM754
3	4	1808898	-/C	FGFR3	NB:Near s	CGC:onco	Heterozyg	0.16	924	ENSG000	ENST000	frameshift	inferred/inferred/inferre	
4	4	1808898	-/C	LETM1			Heterozyg	0.16	924	ENSG000	ENST000	downstream_gene_variant		
5	5	14943359	T/G	HMGXB3			Heterozyg	0.94	158	ENSG000	ENST000	downstream_gene_variant		
6	5	14943359	T/G	CSF1R			Heterozyg	0.94	158	ENSG000	ENST000	3_prime_UTR_variant		
7	5	14943359	G/A	HMGXB3			Heterozyg	0.82	184	ENSG000	ENST000	downstream_gene_variant		
8	5	14943359	G/A	CSF1R			Heterozyg	0.82	184	ENSG000	ENST000	3_prime_UTR_variant		
9	12	25378562	C/T	KRAS	OV:Near s	CGC:onco	Heterozyg	0.25	581	ENSG000	ENST000	missense	possibly_	COSM194
10	12	25378562	C/T	AC087239.	1		Heterozyg	0.25	581	ENSG000	ENST000	upstream_gene_variant		

Things to do

Programs are in ExerciceDay2 folder (VEP_parser y el Excel_report)



Our input file is the vcf file annotated in the previous practice with the VEP.



Execute VEP_parser

- Without consequence filtering.
- Sample code and rootname = patientCHP
- Output folder= CHP_analysis
- Database directory= databases folder inside PracticeDay2 folder

```
perl VEP_parser.pl -f=/home/user/ExerciceDay2/CHP_analysis/patientCHP.onlyTumor.cleanNOTPASS.vep.vcf
-o=/home/user/ExerciceDay2/CHP_analysis/ -sp=patientCHP -r=patientCHP -v=FALSE -
d=/home/user/ExerciceDay2/VEP_parser/databases/
```

Check that all output files have been generated in the new folder in CHP_analysis.

Open the file vep_data_sorted.csv to check that it has been generated correctly.

Databases versions

- Cosmic Release v76 - hg19
- Pfam 29.0 (Nov 2015)
- UniProt release 2016_03 (12/04/2016)
- InterPro 56.0 (13/04/2016)
- Clinvar 1.36 (01/09/2016)
- CGC (Cosmic v76) → The corresponding assembly is GRCH38 (but we search at gene level)
- APPRIS (gen19.ensembl74 13/04/2016)
- ExAC 0.3 (Uses HG19 coordinates)
- KEGG (12/04/2016)

Execute Excel_report

- Output folder= CHP_analysis
- Tumor case name = patientCHP
- Tumor sample name = patientCHP-tumor
- Normal sample name = patientCHP-normal
- vepfile1 = vep_data_sorted.csv file from VEP_parser
- rubioseq = path to output file in RUbioSeq including the folder name

```
perl Excel_report.pl --output=/home/user/ExerciseDay2/ CHP_analysis/ --case=patientCHP --
tsample=patientCHP-tumor --nsample=patientCHP-normal --vepfile1=/home/user/ ExerciseDay2/
CHP_analysis/patientCHP_20160228_175748_VEP/vep_data_sorted.csv --
rubioseq=/home/user/ExerciseDay2/ CHP_analysis/RSresults/
```

Check that the output file has been generated

Play with results

Review variants in IGV

Open the Analysis.patientCHP.xls file and check the results

Have indels the same nomenclature in vcf file? What happens with the coordinates?

Which variants seem false positives? What annotations support this assumption?

Which variants seem more relevant in the pathology? Which annotations support this assumption?

Which threshold could be established for the vscore according to this data?