May 24, 2023

Investigation and identification of somatic and germline variants for colorectal cancer exomes using the NGS pipeline: a computational analysis perspective

DOI
https://dx.doi.org/10.17504/protocols.io.x54v9dqxqg3e/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.x54v9dqxqg3e/v1

Protocol Citation: Chandrashekar K, Anagha S Setlur, Vidya Niranjan 2023. Investigation and identification of somatic and germline variants for colorectal cancer exomes using the NGS pipeline: a computational analysis perspective. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9dqxqg3e/v1

Protocol status: Working
We use this protocol and it's working

Created: May 23, 2023

Last Modified: May 24, 2023

Protocol Integer ID: 82294

Keywords: Colorectal cancer exomes, quality controls, somatic & germline variants, annotation & post-processing, computational analysis, germline variants for colorectal cancer exome, colorectal cancer exome, cancer exome dataset, germline variant identification, colorectal cancer, identified variant, germline mutant, human reference genome, germline variant, identified mutant, potential mutations such as single nucleotide polymorphism, potential mutation, important gene, using snpeff, variant identification, single nucleotide polymorphism, germline, haplotype caller, early disease detection, process of early disease detection

Disclaimer

This protocol was run with colorectal cancer exome datasets. However, the SRR-IDs of the datasets have not been mentioned to make this protocol universal. The steps may be followed for any cancer exome, with the intent of investigating the somatic and germline mutations.

Abstract

A thorough analysis on colorectal cancer exomes reveals potential mutations such as single nucleotide polymorphisms that can be beneficial for early detection of the disease. Thus, through a comprehensive computational protocol that identifies, investigates and analyzes the identified variants, this process of early disease detection becomes much simpler. In brief, the cancer exome datasets were retrieved from publicly available databases, followed by performing quality control checks. The datasets that qualified the quality control checks were then aligned with the human reference genome. Somatic and germline mutants were then identified and called separately, with specific tools for each case. Haplotype Caller was employed for germline variant identification, and Mutect2 for somatic. The identified mutants were then normalized, annotated and post-processed using snpEFF, wANNOVAR and VEP. This protocol helps in garnering insights on the various alterations that might possibly lead to colorectal cancer and suggests the possibility of utilizing the most important genes identified for wet-lab experimentation.

Keywords: Colorectal cancer exomes, quality controls, somatic & germline variants, annotation & post-processing, computational analysis

Guidelines

The commands mentioned in the protocol can be run in the Linux terminal.
Enough storage space is recommended while running this protocol.

Materials

Tools mentioned in the current protocol must initially be installed in the system before running the commands for the NGS pipeline. These include: SRAtoolkit, Fastqc, Multiqc, Cutadapt, Bowtie2, Samtools, picard, GATK, and bcftools. wANNOVAR, VEP are available online and snpEFF is command line based.

Safety warnings

Nil

Ethics statement

The datasets used to arrive at this protocol were all from publicly available databases such as NCBI-SRA.

Before start

All the mentioned tools in the materials section must be installed and checked for appropriate installation.

COLORECTAL CANCER EXOME RETRIEVAL

1 **Retrieval of colorectal cancer exomes**

This protocol was run with colorectal cancer exome datasets. However, the SRR-IDs of datasets have not been mentioned to make this protocol universal. Therefore, NCBI-SRA database (https://www.ncbi.nlm.nih.gov/sra) was employed to retrieve the colorectal cancer exome datasets. The SRR-IDs were noted down for further use. The exome datasets were selected based on a set of criteria. The strategy used for the datasets must be whole exome sequenced and the exomes should be of genomic source. In the present protocol, all the selected sequences were of paired layout, with Illumina used for sequencing and acquiring the reads. Other criteria as desired by the user depending on the requirement can be employed for retrieving the cancer exomes.
Additionally, the tools mentioned in the current protocol must initially be installed in the system before running the commands for the NGS pipeline. These include: SRAtoolkit, Fastqc, Multiqc, Cutadapt, Bowtie2, Samtools, picard, GATK, and bcftools. wANNOVAR, VEP are available online and snpEFF is command line based.

QUALITY CONTROL

2 **Quality control assessments**

Prior to calling variants, the quality of raw data was assessed using the below-mentioned steps. The codes used for running the quality checks are provided in the following sections.

2.1 **Quality Check using FastQC and MultiQC (for multiple datasets)**

FastQC (**https://www.bioinformatics.babraham.ac.uk/projects/fastqc/**) and MultiQC (**https://multiqc.info/**) in the present study were used to effectively assess the sequencing data quality, to identify any possible issues with the raw sequence reads and generate informative reports. Scrutiny of the mean sequence quality per reading and per base, nucleotide content per base position, distribution of GC, etc were analyzed via FastQC. A cumulative report for all FastQC outcomes was obtained from MultiQC.

For multiple datasets, FastQC and MultiQC commands used were:

Command

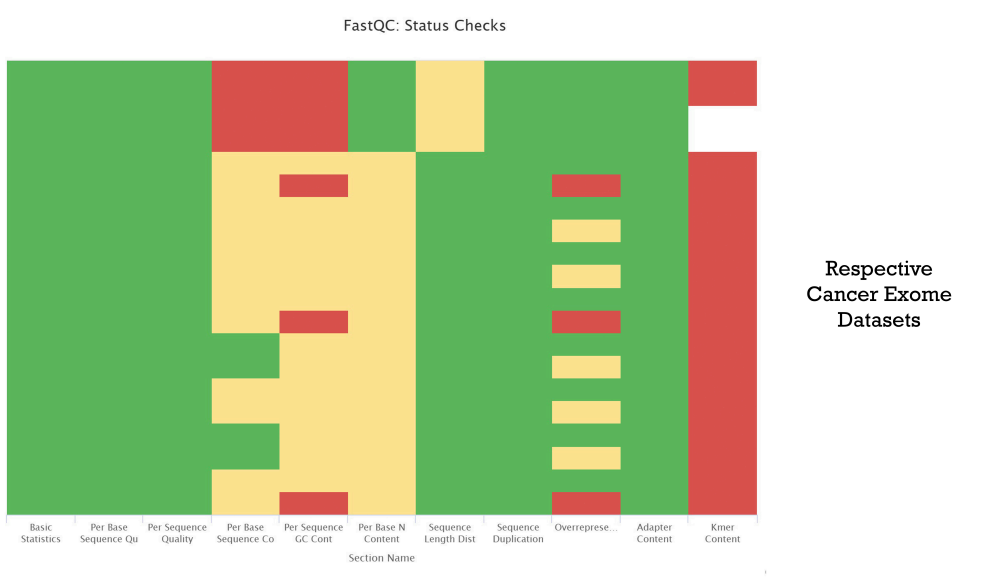
fastQC

```
$ ./fastqc
```

Command

multiQC

```
$ multiqc .
```



Expected multiQC heatchart

2.2 Adaptor sequence removal via Cutadapt

This tool identifies and eliminates the adaptor sequences from raw reads and improves the quality and accuracy of downstream analysis. All unwanted sequences was thus removed using Cutadapt (**https://cutadapt.readthedocs.io/en/stable/**) by running:

Command

Running Cutadapt

```
$ cutadapt -a adapter-sequence* -o SRRID_cut.fastq.gz SRRID.fastq.gz
```

ALIGNMENT WITH HUMAN REFERENCE GENOME

3 **Retrieval and indexing of human reference genome for analysis**

Indexing is a necessary step to pre-process the reference genome so the aligner can seek potential alignment locations for the reads. This is useful during alignment with Bowtie2 (https://bowtie-bio.sourceforge.net/bowtie2/index.shtml), where short read aligners are processed with the reference genome. The following codes were run in the terminal for obtaining Bowtie2 outputs.

Command

Unzipping the reference genome file

```
$ gunzip GRCh37_latest_genomic.fna.gz
```

Command

Indexing reference genome

```
$ bowtie2-build -f GRCh37_latest_genomic.fna.gz hg19
```

3.1 **Alignment of indexed human reference genome against exome datasets**

Once indexing is complete, alignment was performed using Bowtie2.

Command

Gapped alignment command with human reference genome (hg19 in this case)

```
$ bowtie2 -x hg19/38/37 -1 SRRID_1_cut.fastq.gz -2
SRRID_2_cut.fastq.gz -S SRRID.sam $ head SRRID.sam
```

Running Bowtie2 with the specified parameters allows alignment of paired-end sequencing reads to a reference genome, enabling downstream analysis such as variant calling, differential gene expression, or identification of genomic features specific to colorectal cancer.

SAM TO BAM CONVERSION

4 **SAM (Sequence Alignment/Map) to BAM (Binary Alignment/Map) conversion**

To obtain the alignment outcomes in a readable format and to act as appropriate input for the next steps, SAM to BAM (**http://www.htslib.org/**) conversion was performed. The code used was:

Command

SAM to BAM file conversion

```
$ samtools view -bS SRRID.sam > SRRID.bam
```

4.1 **Sorting BAM**

BAM sorting and merging was performed using command:

Command

BAM sorting

```
$ samtools sort SRRID.bam -o SRRID.sorted.bam
```

BAM sorting helps in supporting quick retrieval of alignments and has a compact size for further analysis.

4.2 **Identifying and marking duplicate reads in BAM file**

Duplicate reads may arise at times during sequencing due to several factors, inclusive of technical biases, PCR amplification artifacts and preparation of library protocols. These may skew the downstream analyses and impact the result accuracy. Therefore, marking duplicates is essential to address this issue, by using the “MarkDuplicates” function of Picard (**https://broadinstitute.github.io/picard/**) tool.

Command

Marking Duplicates

```
$ java -jar picard.jar MarkDuplicates INPUT=SRRID_sorted_reads.bam
OUTPUT=SRRID_dedup_reads.bam METRICS_FILE=SRRID_metrics.txt
```

4.3 **Sorting BAM files for deleted duplicate files**

To improve the compression efficiency, another round of BAM sorting was performed for the deleted duplicate files.

Command

BAM sorting for deleted duplicate files

```
$ samtools sort SRRID_dedup_reads.bam -o SRRID_sorted_dedup_reads.bam
```

4.4 **Computing and collecting alignment summary metrics from BAM file**

To obtain various statistics and metrics associated with alignment of sequencing reads to a reference genome, the “CollectAlignmentSummaryMetrics” function was used. The command used to run this function was:

Command

Collecting Alignment Summary Metrics

```
$ java -jar picard.jar CollectAlignmentSummaryMetrics R=ref.fa
I=SRRID_sorted_dedup_reads.bam O=SRRID_alignment_metrics.txt
```

ref.fa: fasta sequence file of the human reference genome

4.5 **Estimating and collecting the insert size distribution of paired-end reads from BAM file**

The primary purpose of collecting the insert sizes is to check the distribution and characteristics of the DNA fragment sizes, in a sequencing library. This enables the collection and visualization of the insert size metrics and distribution, that are useful for the quality scrutiny of the library, alignment of the reads, selection of the fragment size and quality control assessments in several genomic analyses. For this purpose, in the present work flow, the "CollectInsertSizeMetrics" function was used. The distribution of the paired end reads from BAM was analyzed using the command:

Command

Collecting Insert Size Metrics

```
$ java -jar picard.jar CollectInsertSizeMetrics
INPUT=SRRID_sorted_dedup_reads.bam OUTPUT=SRRID_insert_metrics.txt
HISTOGRAM_FILE=SRRID_insert_size_histogram.pdf
```

4.6 **Calculating coverage depth at each position in a sorted and duplicate-marked BAM file**

The "samtools depth" function was used to examine the depth of coverage at every position in the genome of reference depending on the aligned reads. Computation and estimation of depth of coverage important for assessing the sequence data

quality, calling of variants, structure and gene expression analysis and overall exploration of data becomes much easier with the samtools depth function. Thus, to better understand and obtain insights into the coverage depth, this function was performed via:

Command

Determining coverage depth of sorted, deleted, & duplicated BAMs

```
$ samtools depth -a SRRID_sorted_dedup_reads.bam > SRRID_depth_out.txt
```

4.7 Add or modify read group information in BAM file

To assign or update the read group information to the sequence reads in a BAM file, the add or replace read groups function was employed. This was carried out using "picard.jar AddOrReplaceReadGroups". Sample identification, tracking of the data, sequence data differentiation, integrity checks of data and overall sequence reads compatibility was obtained after this step was performed, ensuring that the reads were properly annotated and organized, facilitating meaningful data analyses.

This step was run using the following code in the terminal:

Command

Adding/modifying read group info

```
$ java -jar picard.jar AddOrReplaceReadGroups
I=SRRID_sorted_dedup_reads.bam O=SRRID_output.bam RGID=4 RGLB=lib1
RGPL=ILLUMINA RGPU=unit1 RGSM=20
```

4.8 Indexing BAM file- necessary before variant calling

Prior to variant calling, another indexing of the final BAM output file was carried out to allow faster retrieval of specific genomic regions, enhanced visualization, and to facilitate random access.

Command

Indexing BAM file

```
$ samtools index SRRID_output.bam
```

VARIANT CALLING - GERMLINE & SOMATIC

5 **Calling of germline variants**

The below sections detail preliminary steps followed for calling germline variants.
Mutations from the sequenced data were identified, processed and the variants were called using PICARD (**https://broadinstitute.github.io/picard/**) and GATK (The Genome Analysis Toolkit, https://github.com/broadinstitute/gatk). The Haplotype caller function was used to call the germline variants.
The purpose of using the "HaplotypeCaller" function is to identify and call genetic variants, including single nucleotide variants (SNVs), insertions, deletions, and complex variants, based on the sequencing data and the provided reference genome.
The following command was employed to call the variants:

Command

Germline variant calling

```
$ gatk HaplotypeCaller -R ref.fa -I SRRID_output.bam -O
SRRID_raw_variants.vcf
```

5.1 Selection of SNPs from raw variants

The "SelectVariants" function with the "-select-type SNP" option was utilized to filter and extract only the SNPs from the original VCF file. This step enabled filtering and data refining to focus on SNPs, simplified further analyses, optimized computational resources, and ensured compatibility with SNP-specific analysis tools or pipelines. For working with larger datasets, this function works best for SNP-centric analyses. Therefore, to select the SNPs, the code run was:

Command

Selection of variants from raw mutants

```
$ gatk SelectVariants -R ref.fa -V SRRID_raw_variants.vcf –select-
type-to-include SNP -O SRRID_raw_snps.vcf
```

5.2 Selection of INDELs from raw variants

The purpose of using the "SelectVariants" function with the "-select-type INDEL" option is to filter and extract only the INDELs from the original VCF file. Using the GATK "SelectVariants" with the "-select-type INDEL" option allows for the selection and extraction of INDELs from a VCF file. This step enables filtering and refining the data to focus on INDELs. This step was run prior to variant filtration.

Command

INDELs selection from raw mutants

```
$ gatk SelectVariants -R ref.fa -V SRRID_raw_variants.vcf –select-
type-to-include INDEL -o SRRID_raw_indels.vcf
```

5.3 Variant filtration

To remove potential false positives and retain only the high quality variants, a series of filters were applied to the raw SNPs using the following codes:

Command

Calling SNPs

```
$ gatk VariantFiltration -R ref.fa -V SRRID_raw_snps.vcf -O
SRRID_filtered_snps.vcf -filter-name "QD_filter" -filter "QD < 2.0" -
filter-name "FS_filter" -filter "FS > 60.0" -filter-name "MQ_filter" -
filter "MQ < 40.0" -filter-name "SOR_filter" -filter "SOR > 4.0" -
filter-name "MQRankSum_filter" -filter "MQRankSum < -12.5" -filter-
name "ReadPosRankSum_filter" -filter "ReadPosRankSum < -8.0"
```

Command

Calling INDELs

```
$ gatk VariantFiltration -R ref.fa -V SRRID_raw_indels.vcf -O
SRRID_filtered_indels.vcf -filter-name "QD_filter" -filter "QD < 2.0"
-filter-name "FS_filter" -filter "FS > 200.0" -filter-name
"SOR_filter" -filter "SOR > 10.0"
```

5.4 Excluding filtered variants

To filter out the variants that do not meet the specific quality (as defined by the user), the “--exclude-filtered" option in the "SelectVariants" step was used. This ensured removal of mutants that failed specific criteria and thereby enhanced quality of the data, prioritized the high-confidence variants only, decreased the number of false positives, allowed compatibility access between datasets and also optimized the

computational resources. This is a crucial step to be followed in the data refinement process.

Command

Excluding SNPs

```
$ gatk SelectVariants --exclude-filtered -V SRRID_filtered_snps.vcf -
O SRRID_bqsr_snps.vcf
```

Command

Excluding INDELs

```
$ gatk SelectVariants --exclude-filtered -V SRRID_filtered_indels.vcf
-O SRRID_bqsr_indels.vcf
```

5.5 Recalibration of base quality

To augment the accuracy and reliability of the called variants, a base recalibration was carried out by correcting the systematic errors, addressing biases, and providing more accurate base quality scores. This step is essential in ensuring that the sequencing data quality is maintained appropriately.

Command

Base recalibration- Step 1

```
$ gatk BaseRecalibrator -R ref.fa -I SRRID_output.bam --known-sites
SRRID_bqsr_snps.vcf --known-sites SRRID_bqsr_indels.vcf -O
SRRID_recal_data.table
```

Command

Base recalibration- step 2

```
$ gatk ApplyBQSR -R ref.fa -I SRRID_output.bam -bqsr
SRRID_recal_data.table -O SRRID_recal_reads.bam
```

Command

Base recalibration- step 3

```
$ gatk BaseRecalibrator -R ref.fa -I SRRID_recal_reads.bam --known-
sites SRRID_bqsr_snps.vcf --known-sites SRRID_bqsr_indels.vcf -O
SRRID_post_recal_data.table
```

Command

Base recalibration- step 4

```
$ gatk AnalyzeCovariates -before SRRID_recal_data.table -after
SRRID_post_recal_data.table -plots SRRID_recalibration_plots.pdf
```

Command

Base recalibration- step 5

```
$ gatk HaplotypeCaller -R ref.fa -I SRRID_recal_reads.bam -O
SRRID_raw_variants_recal.vcf
```

5.6 **Selection of variants from recalibrated VCF files**

To filter and extract only the SNPs from the recalibrated VCF files, functions "SelectVariants" along with "-selectType SNP" were used. This was useful since the data authors were working with were recalibrated variant sets.

Command

SNP selection

```
$ gatk SelectVariants -R ref.fa -V SRRID_raw_variants_recal.vcf
–select-type-to-include SNP -O SRRID_raw_snps_recal.vcf
```

Command

INDEL selection

```
$ gatk SelectVariants -R ref.fa -V SRRID_raw_variants.vcf –select-
type-to-include INDEL -O SRRID_raw_indels_recal.vcf
```

5.7 **Variant filtration step**

A series of filters were then applied to the raw SNPs to remove the potential false positives and retain high-quality variants. The following filters were applied:

Command

Filtration

```
$ gatk VariantFiltration -R ref.fa -V SRRID_raw_snps_recal.vcf -O
SRRID_filtered_snps_final.vcf -filter-name "QD_filter" -filter "QD <
2.0" -filter-name "FS_filter" -filter "FS > 60.0" -filter-name
"MQ_filter" -filter "MQ < 40.0" -filter-name "SOR_filter" -filter
"SOR > 4.0" -filter-name "MQRankSum_filter" -filter "MQRankSum <
-12.5" -filter-name "ReadPosRankSum_filter" -filter "ReadPosRankSum <
-8.0"
```

6 **Calling of somatic variants**

The below sections detail preliminary steps followed for calling somatic variants.

6.1 **Tumor samples with matched normal ones**

Somatic variants were called using Mutect2 (https://gatk.broadinstitute.org/hc/en-us/articles/360037593851-Mutect2), specific for calling these variants, part of the GATK pipeline.
The purpose of this step is to identify and call somatic variants, which are genetic variations specific to the tumor sample compared to the matched normal sample. This was first carried out via:

Command

Calling somatic variants

```
$ gatk Mutect2  -R ref.fa -I tumor.bam -I normal.bam  -normal
normal_sample_name  --germline-resource af-only-gnomad.vcf.gz --panel-
of-normals pon.vcf.gz  -O somatic.vcf.gz
```

To identify and call somatic variants for more than 2 samples (which are variations specific to the tumor samples compared to the matched normal ones).

Command

Somatic variant calling for multiple samples

```
$ gatk Mutect2 -R reference.fa -I tumor1.bam -I tumor2.bam -I
normal1.bam -I normal2.bam -normal normal1_sample_name -normal
normal2_sample_name --germline-resource af-only-gnomad.vcf.gz --panel-
of-normals pon.vcf.gz -O somatic.vcf.gz
```

6.2 Tumor only mode- without normal samples

To detect and call somatic variants particular to a tumor sample.

Command

Somatic variant calling for only tumor samples

```
$ gatk Mutect2 -R ref.fa -I sample.bam -O single_sample.vcf.gz
```

Additionally, to call somatic variants while applying filters depending on the population frequencies and a panel of normals, the following codes were run:

Command

Somatic variant calling with filters

```
$ gatk Mutect2 -R ref.fa -I sample.bam --germline-resource af-only-
gnomad.vcf.gz --panel-of-normals pon.vcf.gz -O single_sample.vcf.gz
```

6.3 Filtering of somatic variants

Filtering variants are an essential step before the VCF files are prepared, since it ensures that the obtained variants are more likely to be true somatic mutations. In the present work, the mutations were filtered that will further help in understanding the biology of colorectal cancer.

Command

Filtration of variants

```
$ gatk FilterMutectCalls -V raw_variants.vcf -O filtered_variants.vcf
```

The variant files obtained from both germline and somatic calling were then used for further analysis as described in the following steps.

VCF FILE PREPARATION, ANNOTATION, POST-PROCESSING

7 **VCF file preparation, annotation and post-processing of variants**

Once both germline and somatic mutations were identified and called, the VCF files for both these variants were prepared using bcftools for further analysis. Annotation and processing of these variants were then carried out using snpEFF (**https://pcingola.github.io/SnpEff/**), wANNOVAR (**https://wannovar.wglab.org/**) and VEF (Variant Effect Predictor) (https://asia.ensembl.org/Tools/VEP).

Command

VCF file prep- step 1

```
$ Vcf-validator SRRID_vcf_file.vcf
```

Command

VCF file prep- step 2

```
$ bcftools view -i 'DP>10' SRRID_vcf_file.vcf >
SRRID_filtered_vcf_file.vcf
```

Command

VCF file prep- step 3

```
$ bcftools norm -f ref.fasta SRRID_vcf_file.vcf -o
SRRID_normalized_vcf_file.vcf
```

```
#CHROM  POS  ID  REF  ALT  QUAL  FILTER  INFO  FORMAT  20
chr1  876499  .  A  G  308.06  PASS  AC=2;AF=1;AN=2;DP=13;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=42;QD=28.01;SOR=3.442  GT:AD:DP:GQ:PL  1/1:0,11:11:32:322,32,0
chr1  877831  .  T  C  375.06  PASS  AC=2;AF=1;AN=2;DP=15;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.21;QD=25;SOR=1.112  GT:AD:DP:GQ:PL  1/1:0,15:15:44:389,44,0
chr1  881627  .  G  A  570.06  PASS  AC=2;AF=1;AN=2;DP=21;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=40.97;QD=27.15;SOR=1.508  GT:AD:DP:GQ:PL  1/1:0,21:21:63:584,63,0
chr1  883625  .  A  G  428.06  PASS  AC=2;AF=1;AN=2;DP=17;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=42;QD=28.54;SOR=3.126  GT:AD:DP:GQ:PL  1/1:0,15:15:44:442,44,0
chr1  888639  .  T  C  650.06  PASS  AC=2;AF=1;AN=2;DP=23;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=40.4;QD=30.96;SOR=0.99  GT:AD:DP:GQ:PL  1/1:0,21:21:62:664,62,0
chr1  888659  .  T  C  365.06  MQ_filter  AC=2;AF=1;AN=2;DP=13;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=39.45;QD=33.19;SOR=2.494  GT:AD:DP:GQ:PL  1/1:0,11:11:33:379,33,0
chr1  889158  .  G  C  751.06  PASS  AC=2;AF=1;AN=2;DP=19;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=40.16;QD=28.2;SOR=1.371  GT:AD:DP:GQ:PL  1/1:0,17:17:51:765,51,0
chr1  889159  .  A  C  751.06  PASS  AC=2;AF=1;AN=2;DP=19;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=40.16;QD=25;SOR=1.371  GT:AD:DP:GQ:PL  1/1:0,17:17:51:765,51,0
chr1  894573  .  G  A  930.06  PASS  AC=2;AF=1;AN=2;DP=36;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.5;QD=29.06;SOR=2.765  GT:AD:DP:GQ:PL  1/1:0,32:32:95:944,95,0
chr1  897325  .  G  C  2278.06  PASS  AC=2;AF=1;AN=2;DP=73;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.74;QD=33.5;SOR=2.105  GT:AD:DP:GQ:PL  1/1:0,68:68:99:2292,204,0
chr1  897564  .  T  C  681.06  PASS  AC=2;AF=1;AN=2;DP=27;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.86;QD=28.38;SOR=1.27  GT:AD:DP:GQ:PL  1/1:0,24:24:72:695,72,0
chr1  909238  .  G  C  220.64  PASS  AC=1;AF=0.5;AN=2;BaseQRankSum=1.039;DP=12;ExcessHet=0;FS=0;MLEAC=1;MLEAF=0.5;MQ=41.84;MQRankSum=-0.431;QD=
18.39;ReadPosRankSum=-0.165;SOR=1.609  GT:AD:DP:GQ:PL  0/1:4,8:12:89:228,0,89
chr1  909768  .  A  G  1079.06  PASS  AC=2;AF=1;AN=2;DP=36;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=42;QD=32.7;SOR=2.833 GT:AD:DP:GQ:PL  1/1:0,33:33:99:1093,99,0
chr1  914876  .  T  C  411.06  PASS  AC=2;AF=1;AN=2;DP=13;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.7;QD=29.56;SOR=2.494  GT:AD:DP:GQ:PL  1/1:0,11:11:33:425,33,0
chr1  914940  .  T  C  261.64  PASS  AC=1;AF=0.5;AN=2;BaseQRankSum=-0.808;DP=30;ExcessHet=0;FS=3.453;MLEAC=1;MLEAF=0.5;MQ=41.87;MQRankSum=-1.548;QD=
9.02;ReadPosRankSum=-0.988;SOR=1.609  GT:AD:DP:GQ:PL  0/1:16,13:29:99:269,0,333
chr1  915227  .  A  G  626.06  MQ_filter  AC=2;AF=1;AN=2;DP=22;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=39.79;QD=28.46;SOR=1.609  GT:AD:DP:GQ:PL  1/1:0,22:22:66:640,66,0
chr1  916549  .  A  G  248.64  PASS  AC=1;AF=0.5;AN=2;BaseQRankSum=1.053;DP=18;ExcessHet=0;FS=0;MLEAC=1;MLEAF=0.5;MQ=41.89;MQRankSum=-0.717;QD=14.63;ReadPosRankSum=
0.44;SOR=0.33  GT:AD:DP:GQ:PL  0/1:7,10:17:99:256,0,178
```

Snapshot of results obtained from normalizing VCF files using bcftools

7.1 Annotation of variants

Command

Variant annotation

```
$ java -jar snpEff.jar GRChXX.86 SRRID_vcf_file.vcf >
SRRID_annotated_vcf_file.vcf $ java -jar snpEff.jar -v <snpeff_db>
SRRID_filtered_snps_final.vcf > SRRID_filtered_snps_final.ann.vcf
```

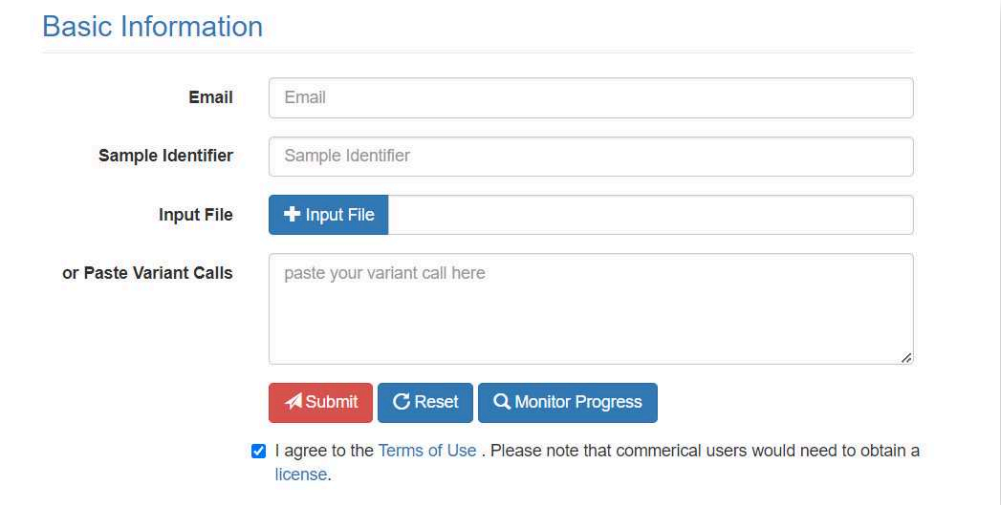
```
#CHROM  POS  ID  REF  ALT  QUAL  FILTER  INFO  FORMAT  20
chr1  876499  .  A  G  308.06  PASS  AC=2;AF=1;AN=2;DP=13;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=42;QD=28.01;SOR=3.442  GT:AD:DP:GQ:PL  1/1:0,11:11:32:322,32,0
chr1  877831  .  T  C  375.06  PASS  AC=2;AF=1;AN=2;DP=15;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.21;QD=25;SOR=1.112  GT:AD:DP:GQ:PL  1/1:0,15:15:44:389,44,0
chr1  881627  .  G  A  570.06  PASS  AC=2;AF=1;AN=2;DP=21;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=40.97;QD=27.15;SOR=1.508  GT:AD:DP:GQ:PL  1/1:0,21:21:63:584,63,0
chr1  883625  .  A  G  428.06  PASS  AC=2;AF=1;AN=2;DP=17;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=42;QD=28.54;SOR=3.126  GT:AD:DP:GQ:PL  1/1:0,15:15:44:442,44,0
chr1  888639  .  T  C  650.06  PASS  AC=2;AF=1;AN=2;DP=23;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=40.4;QD=30.96;SOR=0.99  GT:AD:DP:GQ:PL  1/1:0,21:21:62:664,62,0
chr1  888659  .  T  C  365.06  MQ_filter  AC=2;AF=1;AN=2;DP=13;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=39.45;QD=33.19;SOR=2.494  GT:AD:DP:GQ:PL  1/1:0,11:11:33:379,33,0
chr1  889158  .  G  C  751.06  PASS  AC=2;AF=1;AN=2;DP=19;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=40.16;QD=28.2;SOR=1.371  GT:AD:DP:GQ:PL  1/1:0,17:17:51:765,51,0
chr1  889159  .  A  C  751.06  PASS  AC=2;AF=1;AN=2;DP=19;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=40.16;QD=25;SOR=1.371  GT:AD:DP:GQ:PL  1/1:0,17:17:51:765,51,0
chr1  894573  .  G  A  930.06  PASS  AC=2;AF=1;AN=2;DP=36;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.5;QD=29.06;SOR=2.765  GT:AD:DP:GQ:PL  1/1:0,32:32:95:944,95,0
chr1  897325  .  G  C  2278.06  PASS  AC=2;AF=1;AN=2;DP=73;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.74;QD=33.5;SOR=2.105  GT:AD:DP:GQ:PL  1/1:0,68:68:99:2292,204,0
chr1  897564  .  T  C  681.06  PASS  AC=2;AF=1;AN=2;DP=27;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.86;QD=28.38;SOR=1.27  GT:AD:DP:GQ:PL  1/1:0,24:24:72:695,72,0
chr1  909238  .  G  C  220.64  PASS  AC=1;AF=0.5;AN=2;BaseQRankSum=1.039;DP=12;ExcessHet=0;FS=0;MLEAC=1;MLEAF=0.5;MQ=41.84;MQRankSum=-0.431;QD=
18.39;ReadPosRankSum=-0.165;SOR=1.609  GT:AD:DP:GQ:PL  0/1:4,8:12:89:228,0,89
chr1  909768  .  A  G  1079.06  PASS  AC=2;AF=1;AN=2;DP=36;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=42;QD=32.7;SOR=2.833 GT:AD:DP:GQ:PL  1/1:0,33:33:99:1093,99,0
chr1  914876  .  T  C  411.06  PASS  AC=2;AF=1;AN=2;DP=13;ExcessHet=0;FS=0;MLEAC=2;MLEAF=1;MQ=41.7;QD=29.56;SOR=2.494  GT:AD:DP:GQ:PL  1/1:0,11:11:33:425,33,0
chr1  914940  .  T  C  261.64  PASS  AC=1;AF=0.5;AN=2;BaseQRankSum=-0.808;DP=30;ExcessHet=0;FS=3.453;MLEAC=1;MLEAF=0.5;MQ=41.87;MQRankSum=-1.548;QD=
9.02;ReadPosRankSum=-0.988;SOR=1.609  GT:AD:DP:GQ:PL  0/1:16,13:29:99:269,0,333
```

Results obtained from snpEFF for variant annotation, using filtered SNPs as the input

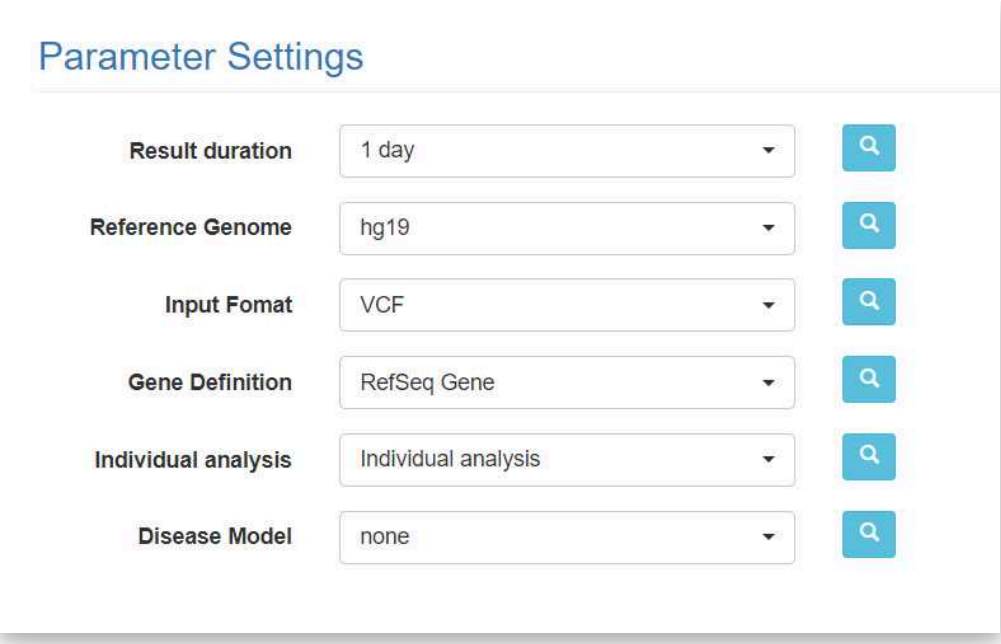
7.2 Post-processing of variants using wANNOVAR and VEP

Variant post processing was performed using two tools: wANNOVAR and VEP

Step 1- wANNOVAR:
This tool is accessible from: **https://wannovar.wglab.org/**.

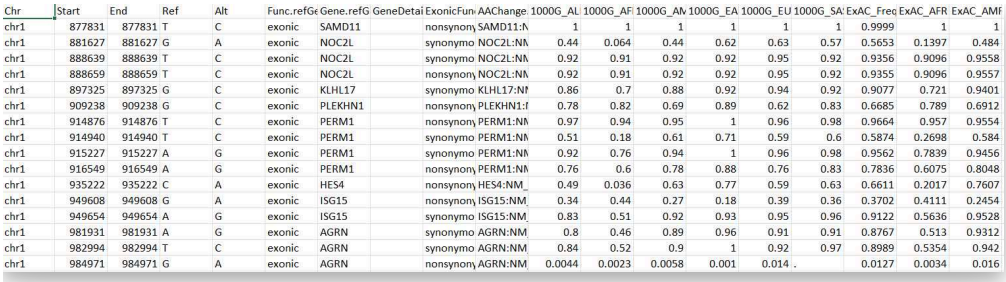


Input information to be filled in wANNOVAR to obtain the post-processing results using wANNOVAR

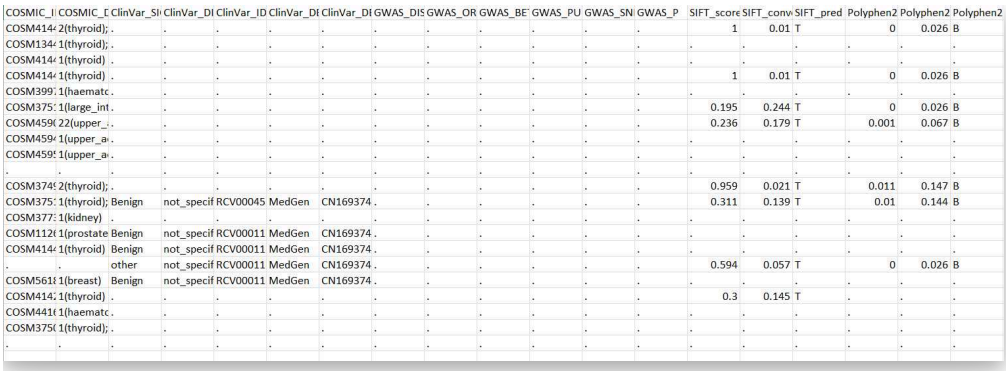


The default settings for running wANNOVAR

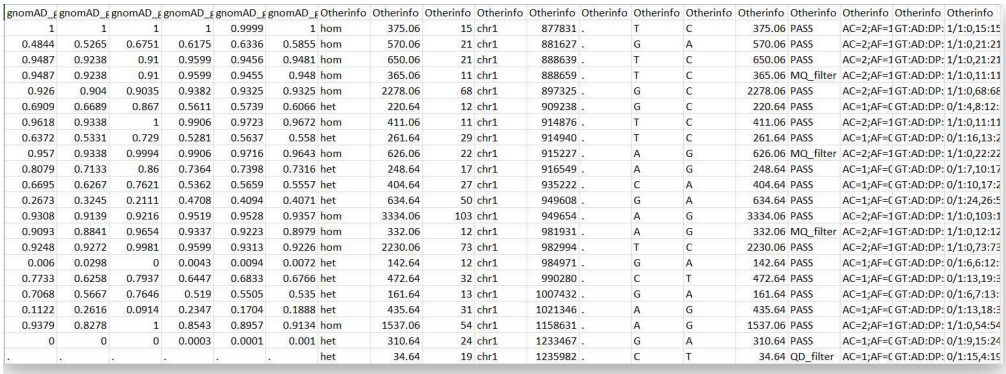
Some expected results for wANNOVAR are provided below:



Chr	Start	End	Ref	Alt	Func.refGe	Gene.refG	GeneDetai	ExonicFun	AAChange	1000G_AL	1000G_AF	1000G_AM	1000G_EA	1000G_EU	1000G_SA	ExAC_Freq	ExAC_AFR	ExAC_AMF
chr1	877831	877831	T	C	exonic	SAMD11		nonsynony	SAMD11:N	1	1	1	1	1	1	0.9999	1	1
chr1	881627	881627	G	A	exonic	NOC2L		synonymo	NOC2L:NM	0.44	0.064	0.44	0.62	0.63	0.57	0.5653	0.1397	0.484
chr1	888639	888639	T	C	exonic	NOC2L		synonymo	NOC2L:NM	0.92	0.91	0.92	0.92	0.95	0.92	0.9356	0.9096	0.9558
chr1	888659	888659	T	C	exonic	NOC2L		nonsynony	NOC2L:NM	0.92	0.91	0.92	0.92	0.95	0.92	0.9355	0.9096	0.9557
chr1	897325	897325	G	C	exonic	KLHL17		synonymo	KLHL17:NM	0.86	0.7	0.88	0.92	0.94	0.92	0.9077	0.721	0.9401
chr1	909238	909238	G	C	exonic	PLEKHN1		nonsynony	PLEKHN1:N	0.78	0.82	0.69	0.89	0.62	0.83	0.6685	0.789	0.6912
chr1	914876	914876	T	C	exonic	PERM1		nonsynony	PERM1:NM	0.97	0.94	0.95	1	0.96	0.98	0.9664	0.957	0.9554
chr1	914940	914940	T	C	exonic	PERM1		synonymo	PERM1:NM	0.51	0.18	0.61	0.71	0.59	0.6	0.5874	0.2698	0.584
chr1	915227	915227	A	G	exonic	PERM1		synonymo	PERM1:NM	0.92	0.76	0.94	1	0.96	0.98	0.9562	0.7839	0.9456
chr1	916549	916549	A	G	exonic	PERM1		nonsynony	PERM1:NM	0.76	0.6	0.78	0.88	0.76	0.83	0.7836	0.6075	0.8048
chr1	935222	935222	C	A	exonic	HES4		nonsynony	HES4:NM_	0.49	0.036	0.63	0.77	0.59	0.63	0.6611	0.2017	0.7607
chr1	949608	949608	G	A	exonic	ISG15		nonsynony	ISG15:NM	0.34	0.44	0.27	0.18	0.39	0.36	0.3702	0.4111	0.2454
chr1	949654	949654	A	G	exonic	ISG15		synonymo	ISG15:NM	0.83	0.51	0.92	0.93	0.95	0.96	0.9122	0.5636	0.9528
chr1	981931	981931	A	G	exonic	AGRN		synonymo	AGRN:NM	0.8	0.46	0.89	0.96	0.91	0.91	0.8767	0.513	0.9312
chr1	982994	982994	T	C	exonic	AGRN		synonymo	AGRN:NM	0.84	0.52	0.9	1	0.92	0.97	0.8989	0.5354	0.942
chr1	984971	984971	G	A	exonic	AGRN		nonsynony	AGRN:NM	0.0044	0.0023	0.0058	0.001	0.014	.	0.0127	0.0034	0.016



COSMIC_I	COSMIC_I	ClinVar_SI	ClinVar_DI	ClinVar_ID	ClinVar_DI	ClinVar_DI	GWAS_DIS	GWAS_OR	GWAS_BE	GWAS_PU	GWAS_SN	GWAS_P	SIFT_score	SIFT_conv	SIFT_pred	Polyphen2	Polyphen2	Polyphen2
COSM414	2(thyroid);	.	.	.	.	.	.	.	.	.	.	.	1	0.01	T	0	0.026	B
COSM134	1(thyroid);	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
COSM414	1(thyroid)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
COSM414	1(thyroid)	.	.	.	.	.	.	.	.	.	.	.	1	0.01	T	0	0.026	B
COSM399	1(haematc	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
COSM375	1(large_int	.	.	.	.	.	.	.	.	.	.	.	0.195	0.244	T	0	0.026	B
COSM459	22(upper_	.	.	.	.	.	.	.	.	.	.	.	0.236	0.179	T	0.001	0.067	B
COSM459	1(upper_a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
COSM459	1(upper_a	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
COSM374	2(thyroid);	.	.	.	.	.	.	.	.	.	.	.	0.959	0.021	T	0.011	0.147	B
COSM375	1(thyroid);	Benign	not_specif	RCV00045	MedGen	CN169374	.	.	.	.	.	.	0.311	0.139	T	0.01	0.144	B
COSM377	1(kidney)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
COSM112	1(prostate	Benign	not_specif	RCV00011	MedGen	CN169374	.	.	.	.	.	.	.	.	.	.	.	.
COSM414	1(thyroid)	Benign	not_specif	RCV00011	MedGen	CN169374	.	.	.	.	.	.	.	.	.	.	.	.
.	.	other	not_specif	RCV00011	MedGen	CN169374	.	.	.	.	.	.	0.594	0.057	T	0	0.026	B
COSM561	1(breast)	Benign	not_specif	RCV00011	MedGen	CN169374	.	.	.	.	.	.	.	.	.	.	.	.
COSM414	1(thyroid)	.	.	.	.	.	.	.	.	.	.	.	0.3	0.145	T	.	.	.
COSM441	1(haematc	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
COSM375	1(thyroid);	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

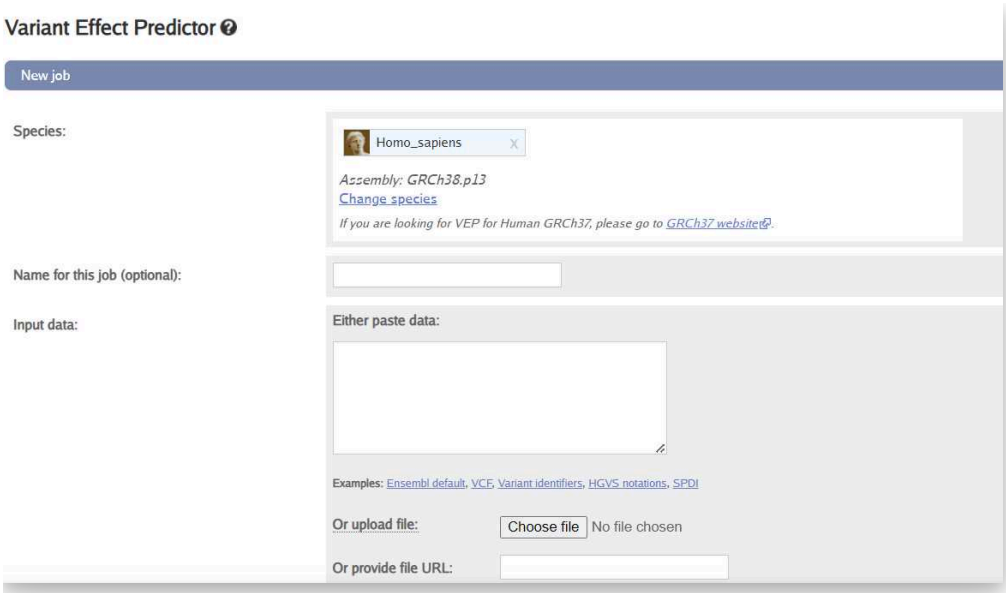


gnomAD_g	gnomAD_g	gnomAD_g	gnomAD_g	gnomAD_g	gnomAD_g	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo	Otherinfo
1	1	1	1	0.9999	1	hom	375.06	15	chr1	877831	.	T	C	375.06	PASS	AC=2;AF=1	GT:AD:DP:	1/1:0,15:15
0.4844	0.5265	0.6751	0.6175	0.6336	0.5855	hom	570.06	21	chr1	881627	.	G	A	570.06	PASS	AC=2;AF=1	GT:AD:DP:	1/1:0,21:21
0.9487	0.9238	0.91	0.9599	0.9456	0.9481	hom	650.06	21	chr1	888639	.	T	C	650.06	PASS	AC=2;AF=1	GT:AD:DP:	1/1:0,21:21
0.9487	0.9238	0.91	0.9599	0.9455	0.948	hom	365.06	11	chr1	888659	.	T	C	365.06	MQ_filter	AC=2;AF=1	GT:AD:DP:	1/1:0,11:11
0.926	0.904	0.9035	0.9382	0.9325	0.9325	hom	2278.06	68	chr1	897325	.	G	C	2278.06	PASS	AC=2;AF=1	GT:AD:DP:	1/1:0,68:68
0.6909	0.6689	0.867	0.5611	0.5739	0.6066	het	220.64	12	chr1	909238	.	G	C	220.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:4,8:12:
0.9618	0.9338	1	0.9906	0.9723	0.9672	hom	411.06	11	chr1	914876	.	T	C	411.06	PASS	AC=2;AF=1	GT:AD:DP:	1/1:0,11:11
0.6372	0.5331	0.729	0.5281	0.5637	0.558	het	261.64	29	chr1	914940	.	T	C	261.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:16,13:2
0.957	0.9338	0.9994	0.9906	0.9716	0.9643	hom	626.06	22	chr1	915227	.	A	G	626.06	MQ_filter	AC=2;AF=1	GT:AD:DP:	1/1:0,22:22
0.8079	0.7133	0.86	0.7364	0.7398	0.7316	het	248.64	17	chr1	916549	.	A	G	248.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:7,10:17
0.6695	0.6267	0.7621	0.5362	0.5659	0.5557	het	404.64	27	chr1	935222	.	C	A	404.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:10,17:2
0.2673	0.3245	0.2111	0.4708	0.4094	0.4071	het	634.64	50	chr1	949608	.	G	A	634.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:24,26:5
0.9308	0.9139	0.9216	0.9519	0.9528	0.9357	hom	3334.06	103	chr1	949654	.	A	G	3334.06	PASS	AC=2;AF=1	GT:AD:DP:	1/1:0,103:1
0.9093	0.8841	0.9654	0.9337	0.9223	0.8979	hom	332.06	12	chr1	981931	.	A	G	332.06	MQ_filter	AC=2;AF=1	GT:AD:DP:	1/1:0,12:12
0.9248	0.9272	0.9981	0.9599	0.9313	0.9226	hom	2230.06	73	chr1	982994	.	T	C	2230.06	PASS	AC=2;AF=1	GT:AD:DP:	1/1:0,73:73
0.006	0.0298	0	0.0043	0.0094	0.0072	het	142.64	12	chr1	984971	.	G	A	142.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:6,6:12:
0.7733	0.6258	0.7937	0.6447	0.6833	0.6766	het	472.64	32	chr1	990280	.	C	T	472.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:13,19:3
0.7068	0.5667	0.7646	0.519	0.5505	0.535	het	161.64	13	chr1	1007432	.	G	A	161.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:6,7:13:
0.1122	0.2616	0.0914	0.2347	0.1704	0.1888	het	435.64	31	chr1	1021346	.	A	G	435.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:13,18:3
0.9379	0.8278	1	0.8543	0.8957	0.9134	hom	1537.06	54	chr1	1158631	.	A	G	1537.06	PASS	AC=2;AF=1	GT:AD:DP:	1/1:0,54:54
0	0	0	0.0003	0.0001	0.001	het	310.64	24	chr1	1233467	.	G	A	310.64	PASS	AC=1;AF=(	GT:AD:DP:	0/1:9,15:24
.	.	.	.	.	.	het	34.64	19	chr1	1235982	.	C	T	34.64	QD_filter	AC=1;AF=(	GT:AD:DP:	0/1:15,4:19

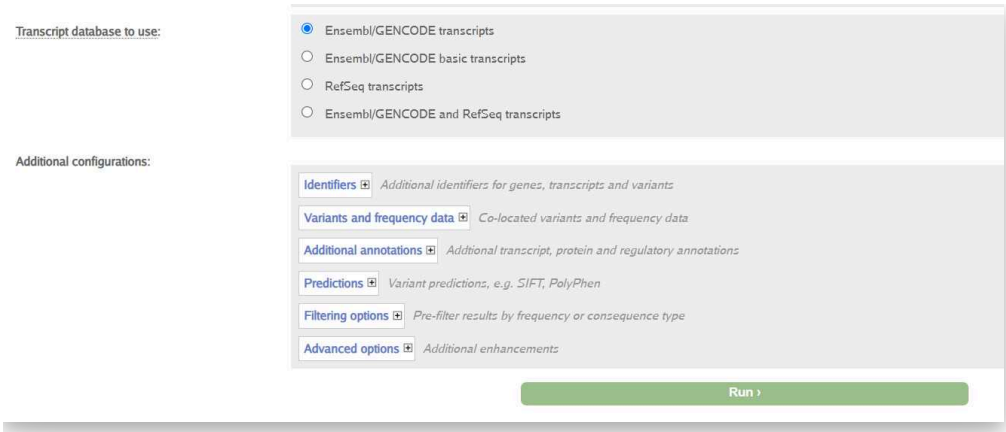
Snapshots of expected wANNOVAR results

Step 2- Variant Effect Predictor (VEP)

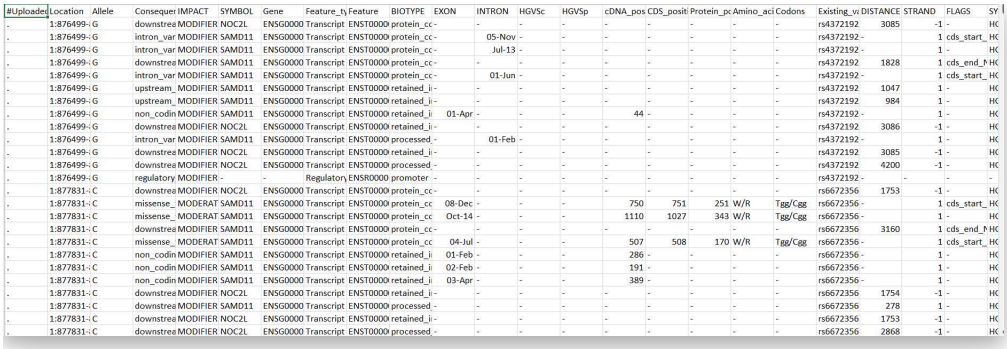
The VEP tool is accessible from: https://asia.ensembl.org/Tools/VEP



Input data settings for VEP



Additional configurations and the type of transcript database to be used for VEP analysis



#Uploaded	Location	Allele	Consequen	IMPACT	SYMBOL	Gene	Feature_ty	Feature	BIOTYPE	EXON	INTRON	HGVSc	HGVSp	cDNA_pos	CDS_positi	Protein_p	Amino_aci	Codons	Existing_v	DISTANCE	STRAND	FLAGS	SY
.	1:876499-/	G	downstrea	MODIFIER	NOC2L	ENSG0000	Transcript	ENST0000	protein_cc	-	-	-	-	-	-	-	-	-	rs4372192	3085	-1	-	HC
.	1:876499-/	G	intron_var	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	protein_cc	-	05-Nov	-	-	-	-	-	-	-	rs4372192	-	1	cds_start_	HC
.	1:876499-/	G	intron_var	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	protein_cc	-	Jul-13	-	-	-	-	-	-	-	rs4372192	-	1	-	HC
.	1:876499-/	G	downstrea	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	protein_cc	-	-	-	-	-	-	-	-	-	rs4372192	1828	1	cds_end_N	HC
.	1:876499-/	G	intron_var	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	protein_cc	-	01-Jun	-	-	-	-	-	-	-	rs4372192	-	1	cds_start_	HC
.	1:876499-/	G	upstream_	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	retained_i	-	-	-	-	-	-	-	-	-	rs4372192	1047	1	-	HC
.	1:876499-/	G	upstream_	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	retained_i	-	-	-	-	-	-	-	-	-	rs4372192	984	1	-	HC
.	1:876499-/	G	non_codin	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	retained_i	01-Apr	-	-	-	44	-	-	-	-	rs4372192	-	1	-	HC
.	1:876499-/	G	downstrea	MODIFIER	NOC2L	ENSG0000	Transcript	ENST0000	retained_i	-	-	-	-	-	-	-	-	-	rs4372192	3086	-1	-	HC
.	1:876499-/	G	intron_var	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	processed	-	01-Feb	-	-	-	-	-	-	-	rs4372192	-	1	-	HC
.	1:876499-/	G	downstrea	MODIFIER	NOC2L	ENSG0000	Transcript	ENST0000	retained_i	-	-	-	-	-	-	-	-	-	rs4372192	3085	-1	-	HC
.	1:876499-/	G	downstrea	MODIFIER	NOC2L	ENSG0000	Transcript	ENST0000	processed	-	-	-	-	-	-	-	-	-	rs4372192	4200	-1	-	HC
.	1:876499-/	G	regulatory	MODIFIER	-	-	Regulatory	ENSR0000	promoter	-	-	-	-	-	-	-	-	-	rs4372192	-	-	-	-
.	1:877831-/	C	downstrea	MODIFIER	NOC2L	ENSG0000	Transcript	ENST0000	protein_cc	-	-	-	-	-	-	-	-	-	rs6672356	1753	-1	-	HC
.	1:877831-/	C	missense_	MODERAT	SAMD11	ENSG0000	Transcript	ENST0000	protein_cc	08-Dec	-	-	-	750	751	251	W/R	Tgg/Cgg	rs6672356	-	1	cds_start_	HC
.	1:877831-/	C	missense_	MODERAT	SAMD11	ENSG0000	Transcript	ENST0000	protein_cc	Oct-14	-	-	-	1110	1027	343	W/R	Tgg/Cgg	rs6672356	-	1	-	HC
.	1:877831-/	C	downstrea	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	protein_cc	-	-	-	-	-	-	-	-	-	rs6672356	3160	1	cds_end_N	HC
.	1:877831-/	C	missense_	MODERAT	SAMD11	ENSG0000	Transcript	ENST0000	protein_cc	04-Jul	-	-	-	507	508	170	W/R	Tgg/Cgg	rs6672356	-	1	cds_start_	HC
.	1:877831-/	C	non_codin	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	retained_i	01-Feb	-	-	-	286	-	-	-	-	rs6672356	-	1	-	HC
.	1:877831-/	C	non_codin	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	retained_i	02-Feb	-	-	-	191	-	-	-	-	rs6672356	-	1	-	HC
.	1:877831-/	C	non_codin	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	retained_i	03-Apr	-	-	-	389	-	-	-	-	rs6672356	-	1	-	HC
.	1:877831-/	C	downstrea	MODIFIER	NOC2L	ENSG0000	Transcript	ENST0000	retained_i	-	-	-	-	-	-	-	-	-	rs6672356	1754	-1	-	HC
.	1:877831-/	C	downstrea	MODIFIER	SAMD11	ENSG0000	Transcript	ENST0000	processed	-	-	-	-	-	-	-	-	-	rs6672356	278	1	-	HC
.	1:877831-/	C	downstrea	MODIFIER	NOC2L	ENSG0000	Transcript	ENST0000	retained_i	-	-	-	-	-	-	-	-	-	rs6672356	1753	-1	-	HC
.	1:877831-/	C	downstrea	MODIFIER	NOC2L	ENSG0000	Transcript	ENST0000	processed	-	-	-	-	-	-	-	-	-	rs6672356	2868	-1	-	HC

Expected result for VEP

CONCLUSIONS AND FUTURE PERSPECTIVES

8 Colorectal cancers have several diagnostic and treatment options to combat it, however, a delay in disease detection is life-threatening. Therefore, this protocol emphasizes on the identification and analysis of somatic and germline variants for colorectal cancer exome datasets, retrieved from publicly available databases such as NCBI-SRA. These steps describe the various tools required to run this pipeline, with the codes required to run each tool. Moreover, although this protocol is described for colorectal cancer exomes, the same set of codes and steps may be followed for the overall analysis of any cancer exome, when the desired outcome is to obtain and analyze somatic and germline variants separately. This computational pipeline requires further in-vitro and in-vivo studies, however, the outcomes will offer prospects for similar such studies that are crucial for designing a cure, prognosis or a treatment strategy for colorectal cancers, based on the scrutinized variants.

The outcomes from this protocol can be carried forward to building and designing decision support systems using artificial intelligence and machine learning (AI/ML), so that the identified somatic and germline mutants can be used for early colorectal cancer prediction through a prediction model. This will aid clinicians/ researchers/diagnosticians to help take better decisions for treatment strategies.

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