# Omics in human diseases

- Omics data and Biological databases
- NGS methods
- <u>NGS data analysis</u>
- Prediction and interpretation of pathogenic variants
- Protein-protein interaction networks

#### Course organization 2022/2023

Frontal lecture/ guided practical activity

How to pass the exam: multiple choice quiz (50%) + results from practical activities (50%) + Bonus points, e.g. summary of previous lecture (up to 10%)

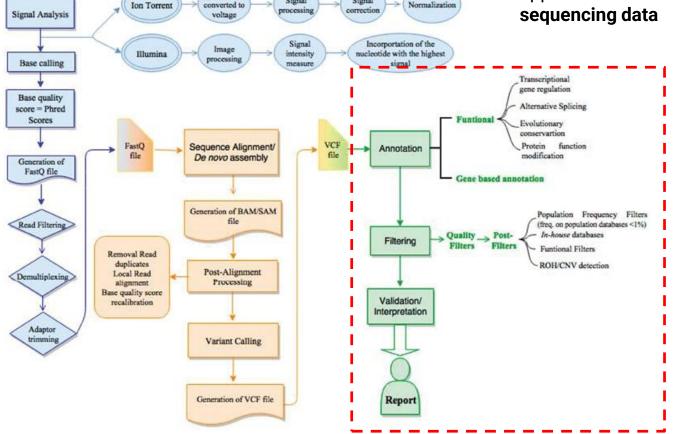
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#### NGS analysis workflow

The same data analysis tools used for WGS can be applied to **exome**sequencing data

lioComputin



pH change

Signal

Signal

#### **BCF tools**

- BCFtools is a set of utilities that manipulate variant calls in the Variant Call Format (VCF) and its binary counterpart BCF
- All commands work transparently with both VCFs and BCFs, both uncompressed and BGZF-compressed
- Several commands can thus be combined with Unix pipes.

annotate .. edit VCF files, add or remove annotations
concat .. concatenate VCF/BCF files from the same set of samples
convert .. convert VCF/BCF to other formats and back
filter .. filter VCF/BCF files using fixed thresholds
gtcheck .. check sample concordance, detect sample swaps and contamination
head .. view VCF/BCF file headers
isec .. intersections of VCF/BCF files
merge .. merge VCF/BCF files files from non-overlapping sample sets
plugin .. run user-defined plugin
query .. transform VCF/BCF into user-defined formats
stats .. produce VCF/BCF stats (former vcfcheck)



#### Standard pipelines for NGS analysis

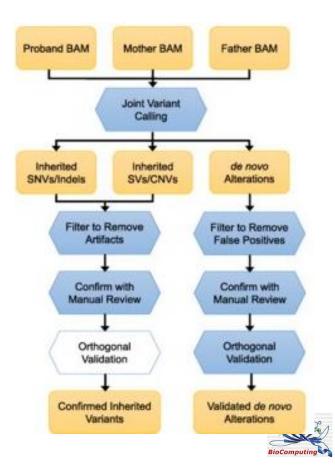
Individual versus joint variant calling

#### Individual

- Perform variant calling in each sample
- Merge VCF files with **BCFtools**
- The VCF contains entries only for positions that are variants in a particular sample

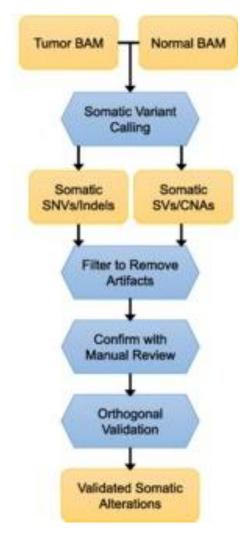
#### Joint

- Perform variant calling in all samples simultaneously
- Called genotypes for every sample at **all variant positions**
- Differentiates among match with reference sequence and low coverage positions
- For trios, direct inference of variant **phase** (cis or trans)
- Increase sensitivity of variant calling in low-coverage regions



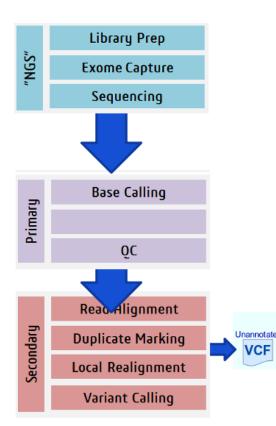
#### Somatic variant calling

- 10% of cancer patients harbor **germline** predisposition variants
- Clinical tumor sequencing aims to search somatic variants
- Sequencing DNA from a tumor sample and a matched control sample from the same patient
- Specific variant callers for somatic variants (MuTect2, Strelka, VarScan2) use simultaneous alignment from tumor and normal samples
- **Challenges**: Tumor purity, low frequency of somatic variants, type of specifment (formalin fixed, paraffin embedded)
- An Ensemble approach that combines the results of two or more callers offer the best balance of sensitivity and specificity





#### is this a pathogenic variant?

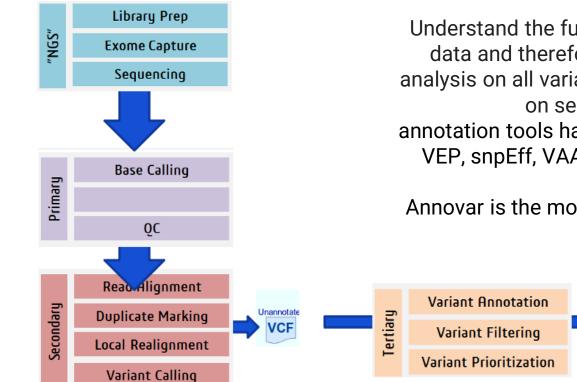


|                | AAT= <id=gp,numb+< td=""><td></td><td></td><td></td><td></td><td></td><td>mitochondrial contig''&gt;<br/>babilitu''&gt;</td><td></td></id=gp,numb+<>      |               |            |          |              |          | mitochondrial contig''><br>babilitu''>       |  |
|----------------|---|---------------|------------|----------|--------------|----------|--|--|
|                | R= <id=imp,descrip< td=""><td></td><td></td><td></td><td></td><td>0.9pc110</td><td>Eability ?</td><td></td></id=imp,descrip<>                             |               |            |          |              | 0.9pc110 | Eability ?                                   |  |
|                | R= <id=boosted,d< td=""><td></td><td></td><td></td><td>==1"&gt;</td><td></td><td></td><td></td></id=boosted,d<>   |               |            |          | ==1">        |          |  |  |
| ##FILTE        | R= <id=lowdp,des< td=""><td>cription="Set</td><td>if GQ&gt;20 s</td><td>nd 10&lt;=D</td><td>P&lt;=20"&gt;</td><td></td><td></td><td></td></id=lowdp,des<> | cription="Set | if GQ>20 s | nd 10<=D | P<=20">      |          |  |  |
|                | R= <id=low@,dese< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td></id=low@,dese<>   |               |            |          |              |          |  |  |
|                | R= <id=notvalida< td=""><td></td><td></td><td></td><td></td><td></td><td>c range"&gt;</td><td></td></id=notvalida<>                                       |               |            |          |              |          | c range">                                    |  |
|                | AAT= <id=gl,numbe< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td></id=gl,numbe<>   |               |            |          |              |          |  |  |
|                |   |               |            |          |              |          |  | JESTITUTION, MNV, COMPLEX                          |
|                |   |               |            |          |              |          | observed STR sequence length                 | STR-contraction, STR-proximal">                    |
|                |   |               |            |          |              |          | eriod for STR variants">                     |  |
|                |   |               |            |          |              |          | tition for STR variants">                    |  |
|                | ne=helix-v2.6.1   |               |            |          |              |          |  |  |
| #CHRON         | POS ID  | REF           | ALT        | QUAL     | FILTER       | INFO     | FORMAT                                       | PC-TA537BFRZC6B332GAQD                             |
| chr1           | 55039879 .  | A             | ACTG       | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47805173 .  | G             | A          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:99:SNV                            |
| chr2           | 47799169 .  | с             | G          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr13          | 32319070 .  | т             | A,TA       | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr19          | 11113686 .  | <u>^</u>      | G          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:99:SNV                            |
| chr2           | 21011802 .  | ç             | T          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr?           | 5977709 .<br>43094795 .   | T<br>A        | c<br>c     | 35<br>35 | PASS<br>PASS |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr17<br>chr13 | 43034135 .  | Ĝ             | Ă          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE<br>GT:AD:DP:GQ:VAR_TYPE | 0/1:500,500:1000:33:SNV<br>0/1:500,500:1000:33:SNV |
| chri3          | 6003794 .   | т             | â          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr3           | 37028782  | ÅG            | ĉc         | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47798826  | A             | AAC        | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr7           | 5987451   | стт           | c          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500.500:1000:33:SNV                            |
| chr13          | 32340378  |               | ATGCTG     | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 21038086 .  | с             | A          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr19          | 11129669 .  | с             | A          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr1           | 55039330 .  | G             | GGAGGA     | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 21010226 .  | CTCA          | С          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr7           | 6009018 .   | A             | G          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr17          | 43124094 .  | G             | GCCT       | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:99:SNV                            |
| chr17          | 43124097 .  | π             | т          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr17          | 43045679 .  | ç             | G          | 35<br>35 | PASS<br>PASS |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr13<br>chr7  | 32398769 .<br>5973402 .   | A<br>CTGA     | AT<br>C    | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE<br>GT:AD:DP:GQ:VAR_TYPE | 0/1:500,500:1000:33:SNV<br>0/1:500,500:1000:33:SNV |
| chr2           | 47806206 .  | A             | G          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr19          | 11128084  | ĉ             | Ť          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47806452  | Ğ             | GGGG       | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47801152 .  | ΠGG           | т          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:99:SNV                            |
| chr19          | 11120166  | c             | Ť          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500.500:1000:33:SNV                            |
| chr19          | 11111506 .  | т             | с          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47805601 .  | A             | AT,ATT     | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47805601 .  | AT            | A          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr19          | 11128142 .  | с             | т          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:99:SNV                            |
| chr17          | 43059469 .  | с             | CACA       | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47806751 .  | CTT           | C,CT       | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr17          | 43125260 .  | G             | A          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:99:SNV                            |
| chr17          | 43124135 .  | с             | CAT        | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr17          | 43124745 .  | ளாா           | G          | 35<br>35 | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr17<br>chr2  | 43044346 .<br>47806983 .  | C<br>A        | AGTTC      | 35       | PASS<br>PASS |          | GT:AD:DP:GQ:VAR_TYPE<br>GT:AD:DP:GQ:VAR_TYPE | 0/1:500,500:1000:33:SNV<br>0/1:500,500:1000:33:SNV |
| chr19          | 11133511  | πA            | T          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 21038086  | c             | ÷.         | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr13          | 11113534  | Ğ             | Ä          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 21012365  | Å             | ĉ          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:39:SNV                            |
| chr7           | 5337333   | Ĝ             | Ă          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr19          | 11120188  | Ť             | G          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500.500:1000:33:SNV                            |
| chr19          | 11116988 .  | ċ             | Ť          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:99:SNV                            |
| chr2           | 47805638  | G             | Ä          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr1           | 55057514 .  | G             | A          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47799092 .  | т             | с          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47799601 .  | c             | т          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |
| chr2           | 47806206 .  | A             | G          | 35       | PASS         |          | GT:AD:DP:GQ:VAR_TYPE                         | 0/1:500,500:1000:33:SNV                            |





#### is this a pathogenic variant?



Understand the functional content within the data and therefore perform prioritization analysis on all variants for functional follow-up on selected variants annotation tools have been developed, such as VEP, snpEff, VAAST, AnnTools, ANNOVAR

Annovar is the most common annotation tool

Annotated

VCF



## **ANNOVAR (ANNOtate VARiation)**

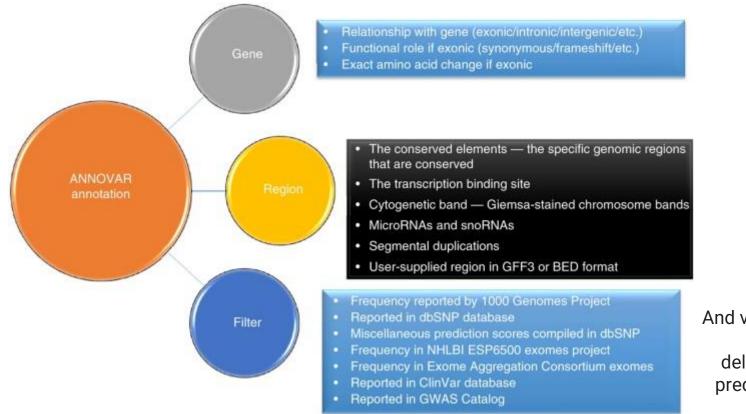
#### http://annovar.openbioinformatics.org/

- It is a command-line tool written in the Perl programming language, which can be executed on a variety of operating systems with a Perl interpreter installed.
- VCF files as input
- Outputs an annotated variant file in several different formats (such as annotated VCF file, tabdelimited text file or comma-delimited text file) with annotations for each variant in the input file
- Download ANNOVAR package with databases for gene- and region-based annotations and filtering
- User-contributed datasets
  - regSNP intron: machine learning algorithm to prioritize the disease-causing probability of intronic SNVs
  - LoFtool score: gene loss-of-function score percentiles
  - RVIS-ESV score: RVIS score measures genetic intolerance of genes to functional mutations
  - SPIDEX: Machine-learning prediction on how genetic variants affect RNA splicing.



#### **ANNOVAR (ANNOtate VARiation)**

http://annovar.openbioinformatics.org/



And various types of variantdeleteriousness prediction scores



| #VCF/CFV | CF/POS VCF/ID      | VCF/REF   | VCF/ALT VCF/ | /QU/ VCF/FILT      | CAVA/GI        | CAVA/TRANSCRIP             |   | IMPACT : AVA/LO      | CAVA       | VCI CADD_p_MetaSVN | Mutation | PROVEA   | VEST4_s B          | IOR::GN I   | BIOR::GN B       | BIOR::GN E | IOR::GN CAVA/TF CAV                | A/T1 BIOR::0 | CL BIOR::CL B | OR::CL B | BIOR::CL | BIOR::CL BIO | R::CL BIOR::CL BIOR::CL                              |
|----------|--------------------|-----------|--------------|--------------------|----------------|----------------------------|---|----------------------|------------|--------------------|----------|----------|--------------------|-------------|------------------|------------|------------------------------------|--------------|---------------|----------|----------|--------------|--|
| 1        | 6E+07 .            | A         | ACTG         | 35 PASS            |                | NM_174936.3                | c.63_65dupGCT_p.Leu23dup                        | 2 Ex1                | IF         |                    |          |          |                    |             |                  |            | +/25.4kb/ Inse                     |              | 7 2           | 0        | 0        | 0            | 1 criteria pr no assert                              |
| 2        | SE+07 .            |           | A            | 35 PASS            | MSH6           | NM_000173.2                | c.3556+146G>A                                   | 3 In6/7              | INT        | ·                  | -        | ÷        | · ·                |             |                  |            | +/23.9kb+ Sub                      |              | 0 0           | 0        | 0        | 0            | 0 reviewed reviewed                                  |
| 13       | 5E+07 .<br>3E+07 . |           | G<br>A,TA    | 35 PASS<br>35 PASS | MSH6<br>BRCA2  | NM_000179.2<br>NM_000059.3 | c.1186C>G_p.Leu386Val<br>c.68-4dupA             | 2 Ex4<br>3 ln2/3     | NSY<br>SS  | 21.7 T             | D        | N        | 0.32               |             | 276930<br>274652 | 0.0056 .   | +/23.9kb+ Sub<br>+/84.2kb+ Inse    |              | 16 2<br>3 12  | 0        | 0        | 0            | O reviewed no assert<br>O reviewed reviewed          |
| 13       | 3E+07 .            | ÷         | ATA          | 35 PASS            | BRCA2          | NM_000053.3                | c.68-4dupA                                      | 3 In2/3              | \$\$       |                    |          |          |                    |             | 274652           | 0.0023 .   | +/84.2kbi Inse                     |              | 3 12          | 3        | ŏ        | ŏ            | 0 reviewed reviewed                                  |
| 19       | 1E+07 .            | à.        | G            | 35 PASS            | LDLR           | NM_000527.4                | c.1510A>G_p.Lys504Glu                           | 2 Ex10               | NSY        | 21.8 T             | D.       | Ň        | 0.347              |             | 246252           | 2E-05      | 0 +/44.5kb/ Sub                    |              | 1 1           | ž        | ŏ        | ĭ            | 1 criteria pr criteria p                             |
| 2        | 2E+07 .            | ĉ         | Ť            | 35 PASS            | APOB           | NM_000384.2                | c.5066G>A_p.Arg1689His                          | 2 Ex26               | NSY        | 25.9 T             | D        | D        | 0.718              |             | 276904           | 0.0014 .   | -/42.6kb/ Sub                      |              | 0 3           | 5        | ō        | Ó            | 1 criteria pr criteria p                             |
| 7        | 6E+06 .            | т         | с            | 35 PASS            | PMS2           | NM_000535.6                | c.2324A>G_p.Asn775Ser                           | 2 Ex14               | NSY        | 23.2 T             | D        | D        | 0.391              | 88          | 275058           | 0.0003 .   | -/38.2kb/ Sub                      |              | 14 1          | 1        | 0        | 0            | 0 reviewed criteria p                                |
| 17       | 4E+07 .            |           | c            | 35 PASS            | BRCA1          | NM_007294.3                | c.736T>G_p.Leu246Val                            | 2 Ex10               | NSY        | 11.52 D            | D        | N        | 0.358              |             | 276040           | 0.0003 .   | -/81.2kb/: Sub                     |              | 3 6           | 5        | 0        | 0            | O reviewed no assert                                 |
| 19       | 1E+07 .            |           | Ŷ.           | 35 PASS            | LDLR           | NM_000527.4                | c.313+1G>A                                      | 1 In3/4              | ESS        | 24.8 .             | D        |          |                    |             | 246208           | 3E-05      | 0 +/44.5kb/ Sub                    |              | 0 1           | 0        | 3        | 16           | 1 criteria pr criteria p                             |
| Ĩ        | 6E+06 .<br>4E+07 . |           | A<br>CC      | 35 PASS<br>35 PASS | PMS2<br>MLH1   | NM_000535.6<br>NM_000249.3 | c.251-2A>T<br>c.1410-2_1410-1delinsCC           | 1 ln3/4<br>1 ln12/13 | ESS<br>ESS | 27.                | D        |          |                    | 1           | 239164           | 4E-06      | 0 -/38.2kb/ Sub<br>+/57.5kb/* Con  |              | 0 0           | 0        | 2        | 8            | 1 criteria pr criteria p<br>1 criteria pr criteria p |
| 3        | 4E+07 .            |           | cc           | 35 PASS            | MLH1           | NM_000243.3                | c.1410-2_1410-1delinsCC                         |                      |            |                    |          |          |                    |             |                  |            | +/57.5kb/* Con                     |              | ň ň           | ň        | ÷        |              | 1 criteria pr criteria p                             |
| 2        | 5E+07 .            |           | AAC          | 35 PASS            | MSH6           | NM_000173.2                | c.843_844insAC                                  | 1 Ex4                | FS         |                    |          |          |                    | 1           | 245844           | 4E-06      | 0 +/23.3kbi Inse                   |              | õ õ           | ŏ        | ó        | ó            | 0  |
| 7        | 6E+06 .            | CTT       | с            | 35 PASS            | PMS2           | NM_000535.6                | c.1312_1313delAA                                | 1 Ex11               | FS         |                    |          |          |                    |             |                  |            | -/38.2kb/ Dek                      |              | 0 0           | 0        | 0        | 0            | 0  |
| 13       | 3E+07 .            |           | ATGCTG       | 35 PASS            | BRCA2          | NM_000059.3                | c.6024_6035delinsTGCTGTT+                       | 1 Ex11               | FS         |                    |          |          |                    |             |                  |            | +/84.2kb; Con                      |              | 0 0           | 0        | 0        | 0            | 0  |
| 2        | 2E+07 .            | ç         | ė.           | 35 PASS            | APOB           | NM_000384.2                | c.409G>T_p.Glu137X                              | 1 Ex5                | SG         | 34 .               | A.       |          | 0.796 .            |             |                  |            | -/42.6kb/ Sub                      |              | 0 0           | 0        | 0        | 2            | 0 criteria pr criteria p                             |
| 2        | 2E+07 .<br>2E+07 . | -         | ÷.           | 35 PASS<br>35 PASS | APOB<br>APOB   | NM_000384.2                | c.409G>T_p.Glu137X                              | 1 Ex5<br>1 Ex5       | SG<br>SG   | 34.<br>34.         | ÷.       |          | 0.796 .            |             |                  |            | -/42.6kb/ Sub                      |              | 0 0           | 0        | 0        | 2            | 0 criteria pr criteria p<br>0 criteria pr criteria p |
| 13       | 1E+07 .            |           | A<br>A       | 35 PASS<br>35 PASS | LDLR           | NM_000384.2<br>NM_000527.4 | c.409G>T_p.Glu137X<br>c.2546C>A_p.Ser849X       | 1 Ex5                | SG         | 42.                | A<br>D   |          | 0.736              |             |                  |            | -/42.6kb/ Sub<br>+/44.5kb/ Sub     |              | 0 0           | 0        | 1        | 2            | 1 criteria pr criteria p                             |
| 1        | 6E+07 .            |           | GGAGGA       | 35 PASS            | PCSK9          | NM_174936.3                | c.101_106dup6_p.Glu34_Asp(                      | 2 Ex1                | iF         | 4c .               |          | :        |                    | 19          | 175140           | 0.0001     | •r44.5Kbr Sub<br>0 +/25.4kb/ Inse  |              | 0 I           | ŏ        | ó        | 0            | 0 criteria pr criteria p                             |
| 2        | 2E+07 .            |           | C            | 35 PASS            | APOB           | NM_000384.2                | c.6639_6641delTGA_p.Asp2;                       | 2 Ex26               | IF         |                    |          |          |                    |             |                  | 0.0052 .   | -/42.6kb/ Dele                     |              | 3 6           | 4        | ŏ        | ō            | 1 criteria pr criteria p                             |
| 2        | 2E+07 .            |           | č            | 35 PASS            | APOB           | NM_000384.2                | c.6633_6641defTGA_p.Asp2;                       | 2 Ex26               | IF         |                    |          |          |                    | 1266        | 245070           | 0.0052 .   | -/42.6kb/ Dek                      | tion         | 3 6           | 4        | ō        | 0            | 1 criteria pr criteria p                             |
| 7        | 6E+06 .            |           | G            | 35 PASS            | PMS2           | NM_000535.6                | c.2T>C_p.Met1?                                  | 2 Ex1                | IM         | 22.7 D             | D        | N        | 0.353              | 1           | 245322           | 4E-06      | 0 -/38.2kb/ Sub                    |              | 0 0           | 0        | 2        | 2            | 1 criteria pr criteria p                             |
| 17       | 4E+07 .            |           | GCCT         | 35 PASS            |                | NM_007294.3                | c.2_3insAGG_p.Met1?                             | 2 Ex2                | IM         |                    | -        |          |                    |             |                  |            | -/81.2kb/: Inse                    |              | 0 0           | 0        | 0        | 0            | 0  |
| 17       | 4E+07 .<br>4E+07 . |           | T<br>G       | 35 PASS<br>35 PASS | BRCA1<br>BRCA1 | NM_007294.3                | c.1delA_p.Met1?                                 | 1 Ex2<br>2 Ex23      | FS<br>SL   | 11.18              | N        |          | 0.431              |             |                  |            | -/81.2kb/; Dek<br>-/81.2kb/; Sub   |              | 0 0           | 0        | 0        | 0            | 0  |
| 13       | 3E+07 .            | Ă         | AT           | 35 PASS            | BRCA2          | NM_007294.3<br>NM_000059.3 | c.5591G>C_p.X1864SerextX35<br>c.10256_10257insT | 2 Ex23<br>1 Ex27     | FS         | 11.10              | 14       |          | 0.451.             |             |                  |            | +/84.2kbi Inse                     |              | 0 0           | Ň        | ő        | ő            | 0  |
| 7        | 6E+06 .            |           | c            | 35 PASS            | PMS2           | NM_000535.6                | c.2583_2585delGAA_p.Gln86                       | 2 Ex15               | IF         |                    |          |          |                    |             |                  |            | -/38.2kb/ Dek                      |              | ŏ ŏ           | ŏ        | ŏ        | ŏ            | 0.   |
| 2        | 5E+07 .            |           | G            | 35 PASS            | MSH6           | NM_000179.2                | c.3649A>G_p.Arg1217Gly                          | 2 Ex8                | EE         | 33 D               | D        | D        | 0.937              | 1           | 30978            | 3E-05      | 0 +/23.3kbi Sub                    |              | 0 0           | 5        | ō        | ō            | 0 criteria pr criteria p                             |
| 2        | 5E+07 .            |           | G            | 35 PASS            | MSH6           | NM_000173.2                | :.3649A>G_p.Arg1217Gly                          | 2 Ex8                | EE         | 33 D               | D        | D        | 0.937              | 1           | 30978            | 3E-05      | 0 +/23.3kbi Sub                    |              | 0 0           | 5        | 0        | 0            | 0 criteria pr criteria p                             |
| 19       | 1E+07 .            |           | т            | 35 PASS            | LDLR           | NM_000527.4                | c.2388C>T_p.                                    | 2 Ex16               | EE         |                    |          |          |                    | 11          | 277170           | 4E-05 .    | +/44.5kb/ Sub                      |              | 0 2           | 1        | 0        | 0            | 1 criteria pr criteria p                             |
| 2        | 5E+07 .            |           | GGGG         | 35 PASS            | MSH6           | NM_000179.2                | c.3802_3803insGGG_p.Met1;                       | 2 Ex9                | EE         |                    |          |          |                    | ,           |                  |            | +/23.9kb+ Inse                     |              | 0 0           | 0        | 0        | 0            | 0  |
| 13       | 5E+07 .<br>1E+07 . | TTGG<br>C | T            | 35 PASS<br>35 PASS | MSH6<br>LDLR   | NM_000179.2<br>NM_000527.4 | c.3170_3172delTGG_p.Leu105"<br>c.1920C>T_p.     | 2 Ex4<br>3 Ex13      | EE<br>SY   | · ·                | -        |          |                    | 1179        | 277244           | 0.0042     | +/23.3kb+ Dek<br>+/44.5kb/ Sub     |              | 0 0<br>5 2    | 0        | 0        | 0            | 0.<br>1 criteria pr criteria p                       |
| 13       | 1E+07 .            |           | ċ            | 35 PASS            | LDLR           | NM_000527.4                | c.1020C01_p.<br>c.1061-8T>C                     | 3 In7/8              | SS         |                    |          |          |                    |             | 276844           |            | +/44.5kb/ Sub                      |              | 2 2           | ě        | ő        | 0            | 1 criteria pr criteria p<br>1 criteria pr criteria p |
| 2        | 5E+07 .            | Å         | AT, ATT      | 35 PASS            | MSH6           | NM_000173.2                | c.3557-5_3557-4dupTT                            | 3 In6/7              | ss         |                    |          |          |                    |             |                  | 0.00000 .  | +/23.3kbi Inse                     |              | 3 1           | ŏ        | ŏ        | ŏ            | 0 reviewed criteria p                                |
| 2        | 5E+07 .            |           | A            | 35 PASS            | MSH6           | NM_000179.2                | c.3557-4deff                                    | 3 In6/7              | SS         |                    |          |          |                    |             |                  |            | •/23.3kbi Dek                      |              | 2 2           | ō        | 0        | ō            | 1 criteria pr criteria p                             |
| 19       | 1E+07 .            |           | т            | 35 PASS            | LDLR           | NM_000527.4                | c.2389+57C>T                                    | 3 In16/17            | INT        |                    |          |          |                    | 189         | 30354            | 0.0061     | 1 +/44.5kb/ Sub                    |              | 0 1           | 0        | 0        | 0            | 0 criteria pr criteria p                             |
| 17       | 4E+07 .            |           | CACA         | 35 PASS            | BRCA1          | NM_007294.3                | c.5194-2337_5194-2335dupT(                      | 3 In18/19            | INT        |                    | -        |          |                    |             |                  |            | -/81.2kb/: Inse                    |              | 1 0           | 0        | 0        | 0            | 0 reviewed reviewed                                  |
| 2        | SE+07 .            |           | C,CT         | 35 PASS            | MSH6<br>BRCA1  | NM_000179.2                | c.4002-10deff                                   | 3 In9/10             | INT        |                    |          |          |                    |             |                  |            | +/23.9kb+ Dek                      |              | 0 0           | !        | 0        | 0            | 0 criteria pr criteria p                             |
| 17       | 4E+07 .<br>4E+07 . |           | A<br>CAT     | 35 PASS<br>35 PASS | BRCA1          | NM_007294.3<br>NM_007294.3 | c20+11C>T<br>c13-2213-21dupAT                   | 3 ln1/2<br>3 ln1/2   | SPU<br>SPU |                    |          |          |                    | 63          | 156718           | 0.0004 .   | -/81.2kb/; Sub<br>-/81.2kb/; Inse  |              | 2 2           | 1        | 0        | ů.           | 1 criteria pr no assert<br>O criteria pr criteria p  |
| 17       | 4E+07 .            |           | G            | 35 PASS            | BRCAI          | NM_007294.3                | c20+52120+525del5                               | 3 In1/2              | SPU        |                    |          |          |                    | 95          | 30914            | 0.0031     | 0 -/81.2kb/; Dele                  |              | o 1           | ŏ        | ŏ        | ŏ            | 0 no asserti no assert                               |
| 17       | 4E+07 .            | c         | T            | 35 PASS            |                | NM_007294.3                | c.+1332G>A                                      | 3 SUTR               | 3PU        |                    |          | :        | :                  | 1496        |                  | 0.0094 .   | -/81.2kb/; Sub                     |              | ĭ 0           | ŏ        | ŏ        | ŏ            | 0 reviewed reviewed                                  |
| 19       | 1E+07 .            | TTA       | т            | 35 PASS            | LDLR           | NM_000527.4                | c.+2210_+2211delTA                              | 3 SUTR               | 3PU        |                    |          |          |                    |             |                  |            | +/44.5kb/ Dek                      |              | 0 1           | Ó        | Ó        | ō            | 0 criteria pr criteria p                             |
| 2        | 2E+07 .            | с         | Α            | 35 PASS            | APOB           | NM_000384.2                | c.409G>T_p.Glu137X                              | 1 Ex5                | SG         | 34 .               | A        |          | 0.796 .            |             |                  |            | -/42.6kb/ Sub                      |              | 0 0           | 0        | 0        | 2            | 0 criteria pr criteria p                             |
| 19       | 1E+07 .            | -         | A            | 35 PASS            | LDLR           | NM_000527.4                | c.1359-1G>Å                                     |                      | ESS        | 24.8 .             | D        |          |                    |             | 245718           |            | 0 +/44.5kb/ Sub                    |              | 0 0           | 0        | 3        | 10           | 1 criteria pr criteria p                             |
| 2        | 2E+07 .            |           | c .          | 35 PASS<br>35 PASS | APOB<br>PMS2   | NM_000384.2                | c.4503T>G_p.Tyr1501X                            | 1 Ex26               | SG         | 27.5 .             | Å        |          | 0.862              | 1           | 246156           | 4E-06      | 0 -/42.6kb/ Sub                    |              | 0 0           | 0        | 0        | 1            | 0 criteria pr criteria p                             |
|          | 6E+06 .<br>6E+06 . | -         | Å            | 35 PASS<br>35 PASS | PMS2<br>PMS2   | NM_000535.6<br>NM_000535.6 | c.730C>T_p.Gin244X<br>c.730C>T_p.Gin244X        | 1 Ex7<br>1 Ex7       | SG<br>SG   | 43.<br>43.         | Å        |          | 0.865              |             |                  |            | -/38.2kb/ Sub<br>-/38.2kb/ Sub     |              | 0 0           | 0        | 0        | 1            | 0 criteria pr criteria p<br>0 criteria pr criteria p |
| 19       | 1E+07 .            |           | Ĝ            | 35 PASS            | LDLR           | NM_000527.4                | c.1342T>G_p.Ser648Ala                           | 2 Ex13               | NSY        | 21.2 D             | Ñ        | Ň        | 0.401              | 4           | 277228           | 1E-05      | +/44.5kb/ Sub                      |              | o o           | ŏ        | 1        | ó            | 0 criteria pr criteria p                             |
| 19       | 1E+07 .            |           | Ť            | 35 PASS            | LDLR           | NM_000527.4                | c.1835C>T_p.Ala612Val                           | 2 Ex12               | NSY        | 26.5 D             | D        | N        | 0.511              |             | 277194           | 7E-06      | +/44.5kb/ Sub                      |              | ŏŏ            | ŏ        | ó        | 1            | 0 no asserti no assert                               |
| 19       | 1E+07 .            | č         | Ť            | 35 PASS            | LDLR           | NM_000527.4                | c.1835C>T_p.Ala612Val                           | 2 Ex12               | NSY        | 26.5 D             | D        | N        | 0.511              |             | 277194           | 7E-06 .    | +/44.5kb/ Sub                      | tituti       | õ õ           | Ó        | ŏ        | 1            | 0 no asserti no assert                               |
| 2        | 5E+07 .            |           | A            | 35 PASS            | MSH6           | NM_000179.2                | c.3577G>A_p.Glu1193Lys                          | 2 Ex7                | NSY        | 33 D               | D        | D        | 0.987 .            |             |                  |            | +/23.3kb+ Sub                      |              | 0 0           | 0        | 2        | 0            | 0 criteria pr criteria p                             |
| 1        | 6E+07 .            | -         | <u>^</u>     | 35 PASS            | PCSK9          | NM_174936.3                | c.1180G>A_p.Gly394Ser                           | 2 Ex7                | EE         | 27.1 D             | D        | D        | 0.824              | 26          | 236966           | 0.0001     | 0 +/25.4kb/ Sub                    |              | 0 0           | 4        | 0        | 1            | 0 criteria pr criteria p                             |
| 2        | 5E+07 .<br>5E+07 . | T<br>C    | c<br>T       | 35 PASS<br>35 PASS | MSH6<br>MSH6   | NM_000179.2                | c.1109T>C_p.Leu370Ser                           | 2 Ex4<br>2 Ex4       | NSY<br>NSY | 25.1 D<br>25 D     | D        | D        | 0.669 .<br>0.785 . |             |                  |            | +/23.9kb, Sub                      |              | 0 0           | 2        | 3        | 1            | 1 criteria pr criteria p                             |
| 2        | 5E+07 .            | c c       | ÷            | 35 PASS<br>35 PASS | PCSK9          | NM_000173.2<br>NM_174936.3 | c.1618C>T_p.Leu540Phe<br>c.1405C>T_p.Arg468Trp  | 2 Ex4<br>2 Ex9       | NSY        | 25 U<br>22.4 T     | N        | N        | 0.785 .            | 240         | 277130           | 0.0009     | +/23.9kb+ Sub<br>+/25.4kb/ Sub     |              | 1 2           |          | 0        | 1            | 0 criteria pr criteria p<br>1 criteria pr criteria p |
| 3        | 4E+07 .            | -         | ÷.           | 35 PASS            |                | NM_000249.3                | c.1217G>A_p.Ser406Asn                           | 2 Ex12               | NSY        | 13.46 T            | N        | N        | 0.31               |             | 276804           | 0.0003 .   | +/57.5kb/* Sub                     |              | 6 4           | 1        | ŏ        | ó            | 0 reviewed no assert                                 |
| 3        | 4E+07 .            | Ğ         | A            | 35 PASS            | MLH1           | NM_000249.3                | c.1217G>A_p.Ser406Asn                           | 2 Ex12               | NSY        | 13.46 T            | N        | N        | 0.31               |             | 276804           | 0.0009 .   | +/57.5kb/* Sub                     |              | 6 4           | i        | ŏ        | ō            | 0 reviewed no assert                                 |
| 7        | 6E+06 .            | т         | с            | 35 PASS            | PMS2           | NM_000535.6                | c.52A>G_p.lle18Val                              | 2 Ex2                | NSY        | 24.6 D             | D        | N        | 0.656              | 2457        | 271244           | 0.0091 .   | -/38.2kb/ Sub                      | stituti      | 15 6          | 1        | 0        | 0            | 0 reviewed criteria p                                |
| 17       | 4E+07 .            | G         | Α            | 35 PASS            |                | NM_007294.3                | c86C>T  | 3 5UTR               | 5PU        |                    |          |          |                    | 113         | 154744           | 0.0007 .   | -/81.2kb/; Sub                     |              | 1 0           | 3        | 0        | 0            | 0 criteria pr criteria p                             |
| 3        | 4E+07 .            | ç         | ė.           | 35 PASS            | MLH1           | NM_000249.3                | c.307-29C>A                                     | 3 In3/4              | INT        | ·                  | -        | <u>:</u> |                    | 1437        | 277100           | 0.0054 .   | +/57.5kb/* Sub                     |              | 0 4           | 0        | 0        | 0            | 0 reviewed reviewed                                  |
| 13       | 3E+07 .<br>4E+07 . |           | A<br>G       | 35 PASS<br>35 PASS | BRCA2<br>MLH1  | NM_000053.3<br>NM_000243.3 | c.8851G>A_p.Ala2951Thr<br>c.791-79A>G           | 2 Ex22<br>3 In9/10   | NSY<br>INT | 26.9 T             | U        | N        | 0.518              | 2486<br>150 | 276544<br>30350  | 0.009 .    | */84.2kbi Sub<br>0 */57.5kb/: Sub  |              | 6 4<br>0 0    | 0        | 0        | 0            | 0 reviewed no assert                                 |
| 19       | 4E+07 .<br>1F+07   | â         | Å            | 35 PASS<br>35 PASS | IDIR           | NM_000527.4                | c.131-13A3G<br>_c.1043G3A_n Are350Gls           | 2 Fy7                | NSY        |                    | N        | N        | 0 176              |             | 245590           | 2E-05      | 0 +/51.5Kb/ Sub<br>0 +/44.5kb/ Sub |              | 0 0           | ő        | 0        | 1            | 0 no asserti no assert<br>0 no asserti no assert     |
| ,        | 4,200              |           | a ,          |                    |                |                            |   | ,                    | - 000.     | ee,0               |          |          |                    |             | · arrent         |            | THE PARTY AND                      |              |               |          | 0        | 0.000        | II no asserti no asserti                             |



| #VCF/CFV | CF/POS V           | CF/ID V  | CF/REF V | CF/ALT V | CF/QU/ VCF/FIL     | T CAVA/G      | CAVA/TRANSCRIP             |   | IMPACT : AVA/LO   | CAVA        | /CI CADD_p_Me     | taSVN Mutat | ion' PROV | EA VEST4_¢I       | BIOR::GN    | BIOR::GN         | BIOR::GN        | BIOR::GN CAVA/TF                                   | CAVA/T` BIO | R::CL BI | IOR::CL BIOR::C | L BIOR::CI | L BIOR::CL BIOR: | CL BIOR::CL BIOR::CI                                 |
|----------|--------------------|----------|----------|----------|--------------------|---------------|----------------------------|---|-------------------|-------------|-------------------|-------------|-----------|-------------------|-------------|------------------|-----------------|--|-------------|----------|-----------------|------------|------------------|--|
| 1        | 6E+07 .            | A        |          | CTG      | 35 PASS            |               | NM_174936.3                | c.63_65dupGCT_p.Leu23dup                                | 2 Ex1             | IF          |                   |             |           |                   |             |                  |                 | . +/25.4kb/  |             | 7        | 2 (             |            |                  | 1 criteria pr no assert                              |
| 2        | 5E+07 .            | G        | A        |          | 35 PASS            | MSH6          | NM_000179.2                | c.3556+146G>A   | 3 In6/7           | INT         |                   | -           |           |                   |             |                  |                 | . +/23.3kb   |             | 0        | 0 0             |            |                  | 0 reviewed reviewed                                  |
| 2        | SE+07 .            | с        | G        |          | 35 PASS            | MSH6          | NM_000179.2                | c.1186C>G_p.Leu396Val                                   | 2 Ex4             | NSY         | 21.7 T            | D           | N         | 0.32              |             |                  |                 |  |             | 16       | 2 (             |            |                  | O reviewed no assert                                 |
| 13       | 3E+07 .            | Ţ        |          | TA.      | 35 PASS            | BRCA2         | NM_000053.3                | c.68-4dupA  | 3 ln2/3           | SS          |                   |             |           |                   | 787         | 274652           | 0.0029          | . +/84.2kb   |             | 9        | 12 3            |            |                  | 0 reviewed reviewed                                  |
| 13       | 3E+07 .            | Ţ        |          | TA       | 35 PASS<br>35 PASS | BRCA2<br>LDLR | NM_000059.3                | c.68-4dupA  | 3 In2/3           | \$\$<br>Nov | <br>21.8 T        |             |           |                   |             |                  | 0.0023          | . •/84.2kb   |             | 3        | 12 3            |            | · ·              | 0 reviewed reviewed                                  |
| 2        | 1E+07 .<br>2E+07 . | A<br>C   | G        |          | 35 PASS<br>35 PASS | APOB          | NM_000527.4<br>NM_000384.2 | c.1510A>G_p.Lys504Glu<br>c.5066G>A_p.Arg1689His         | 2 Ex10<br>2 Ex26  | NSY<br>NSY  | 21.0 T            | D           | D         | 0.347             |             | 246252<br>276304 | 2E-05<br>0.0014 | 0 +/44.5kb/<br>-/42.6kb/                           |             |          | 3 1             |            |                  | 1 criteria pr criteria p<br>1 criteria pr criteria p |
| - 7      | 6E+06 .            | Ť        | ċ        |          | 35 PASS            | PMS2          | NM_000535.6                | c.2324A>G_p.Asn775Ser                                   | 2 Ex14            | NSY         | 23.2 T            | Ď           | 6         | 0.391             | 88          | 275058           | 0.0003          | /38.2kb/   |             | 14       |                 | i          |                  | 0 reviewed criteria p                                |
| 17       | 4E+07 .            | Å        | -        |          | 35 PASS            | BRCA1         | NM_007294.3                | c.736T>G_p.Leu246Val                                    | 2 Ex10            | NSY         | 11.52 D           | Ď           | N         | 0.358             | 82          |                  | 0.0003          | /81.2kb/;  |             | 9        | 6 9             | s à        |                  | O reviewed no assert                                 |
| 19       | 1E+07 .            | Ğ        |          |          | 35 PASS            | LDLR          | NM_000527.4                | c.313+1G>A  | 1 ln3/4           | ESS         | 24.8 .            | ñ           |           |                   | 7           | 246208           | 3E-05           | 0 +/44.5kb/  |             | ŏ        | i i             |            |                  | 1 criteria pr criteria p                             |
| 7        | 6E+06 .            | т        | A        |          | 35 PASS            | PMS2          | NM_000535.6                | c.251-2A>T  | 1 In3/4           | ESS         | 27 .              | D           |           |                   | 1           | 239164           | 4E-06           | 0 -/38.2kb/  |             | ō        | 0 0             | ) 2        | 8                | 1 criteria pr criteria p                             |
| 3        | 4E+07 .            | Â        | G C      | .c       | 35 PASS            | MLH1          | NM_000243.3                | c.1410-2_1410-1delinsCC                                 | 1 In12/13         | ESS         |                   |             |           |                   |             |                  |                 | +/57.5kb/*   |             | 0        | 0 0             | )          | 1 1              | 1 criteria pr criteria p                             |
| 3        | 4E+07 .            | A        | G C      | C C      | 35 PASS            | MLH1          | NM_000249.3                | c.1410-2_1410-1delinsCC                                 | 1 In12/13         | ESS         |                   |             |           |                   |             |                  |                 | +/57.5kb/*   |             | 0        | 0 0             | )          | 1 1              | 1 criteria pr criteria p                             |
| 2        | SE+07 .            | A        |          | AC       | 35 PASS            | MSH6          | NM_000173.2                | c.843_844insAC  | 1 Ex4             | FS          |                   |             |           |                   | 1           | 245844           | 4E-06           | 0 +/23.3kb   |             | 0        | 0 0             |            |                  | 0  |
| 7        | 6E+06 .            |          | п с      |          | 35 PASS            | PMS2          | NM_000535.6                | c.1312_1313delAA  | 1 Ex11            | FS          |                   |             |           |                   |             |                  |                 | /38.2kb/   |             | 0        | 0 0             |            |                  | 0  |
| 13       | 3E+07 .            |          | GCAAG A  | TGCTG    | 35 PASS            | BRCA2         | NM_000059.3                | c.6024_6035delinsTGCTGTT+                               | 1 Ex11            | FS          |                   |             |           |                   |             |                  |                 | . +/84.2kb   |             | 0        | 0 0             |            |                  | 0  |
| 2        | 2E+07 .            | c c      |          | ۱.       | 35 PASS            | APOB          | NM_000384.2                | c.409G>T_p.Glu137X                                      | 1 Ex5             | SG          | 34 .              | <u>^</u>    |           | 0.796             |             |                  |                 | /42.6kb/   |             | 0        | 0 0             |            |                  | 0 criteria pr criteria p                             |
| 2        | 2E+07 .            | ç        |          |          | 35 PASS            | APOB          | NM_000384.2                | c.409G>T_p.Glu137X                                      | 1 Ex5             | SG          | 34 .              | Ŷ.          |           | 0.796 .           |             |                  |                 | /42.6kb/   |             | 0        | 0 0             |            |                  | 0 criteria pr criteria p                             |
| 2        | 2E+07 .<br>1E+07 . | ç        |          |          | 35 PASS<br>35 PASS | APOB<br>LDLR  | NM_000384.2<br>NM_000527.4 | c.409G>T_p.Glu137X<br>c.2546C>A_p.Ser843X               | 1 Ex5<br>1 Ex17   | SG<br>SG    | 34.               | Ê           |           | 0.796 .<br>0.71 . |             |                  |                 | /42.6kb/   |             | 0        | 0 0             |            | 2                | 0 criteria pr criteria p                             |
| 10       | 1E+07 .            | G        |          | GAGGA    | 35 PASS<br>35 PASS | PCSK9         | NM_000527.4<br>NM_174936.3 | c.2546U3A_p.Ser643X<br>c.101_106dup6_p.Glu34_Asp(       | 2 Ex1             | SIG<br>IF   | 42 .              | 0           |           | v.n .             | 19          | 175140           | 0.0001          | <ul> <li>+/44.5kb/</li> <li>0 +/25.4kb/</li> </ul> |             | 0        | 1 0             |            | 1 2              | 1 criteria pr criteria p<br>0 criteria pr criteria p |
|          | 2E+07 .            |          | TCA C    |          | 35 PASS<br>35 PASS | APOB          | NM_000384.2                | c.6639_6641delTGA_p.Asp2;                               | 2 Ex1<br>2 Ex26   | IF          |                   |             |           |                   | 1266        | 245070           | 0.0001          | /42.6kb/   |             | 3        | 6 4             |            |                  | 1 criteria pr criteria p                             |
| 2        | 2E+07 .            |          | TCA C    |          | 35 PASS            | APOB          | NM_000384.2                | c.6633_6641defTGA_p.Asp2;                               | 2 Ex26            | IF          |                   |             |           |                   | 1266        | 245070           | 0.0052          | /42.6kb/   |             | š        | 6 4             | i          |                  | 1 criteria pr criteria p                             |
| 7        | 6E+06              | Ă        |          |          | 35 PASS            | PMS2          | NM_000535.6                | c.2T>C_p.Met1?  | 2 Ex1             | iM          | 22.7 D            | Ď           | Ň         | 0.953             | 1           | 245322           | 4E-06           | 0 -/38.2kb/  |             | ŏ        | ŏ               |            |                  | 1 criteria pr criteria p                             |
| 17       | 4E+07 .            | G        |          | CCT      | 35 PASS            | BRCA1         | NM_007294.3                | c.2_3insAGG_p.Met1?                                     | 2 Ex2             | ID-0        |                   |             |           |                   |             |                  |                 | -/81.2kb/;   |             | ō        | 0 0             | ) (        | 0 0              | 0  |
| 17       | 4E+07 .            | T        | гт       |          | 35 PASS            | BRCA1         | NM_007294.3                | c.1delA_p.Met1?   | 1 Ex2             | FS          |                   |             |           |                   |             |                  |                 | -/81.2kb/:   | Deletion    | 0        | 0 0             | ) (        | 0 0              | 0  |
| 17       | 4E+07 .            | с        | G        | i        | 35 PASS            | BRCA1         | NM_007294.3                | c.5531G>C_p.X1864SerextX35                              | 2 Ex23            | SL          | 11.18 .           | N           |           | 0.431 .           |             |                  |                 | /81.2kb/:  | Substituti  | 0        | 0 0             | ) (        | 0 0              | 0  |
| 13       | 3E+07 .            | A        |          |          | 35 PASS            | BRCA2         | NM_000059.3                | c.10256_10257in#T                                       | 1 Ex27            | FS          |                   |             |           |                   |             |                  |                 | . +/84.2kb   | Insertion   | 0        | 0 0             |            | · ·              | 0  |
| 7        | 6E+06 .            |          | rga c    |          | 35 PASS            | PMS2          | NM_000535.6                | c.2583_2585delGAA_p.Gln86                               | 2 Ex15            | IF          | · · ·             |             |           |                   |             |                  |                 | /38.2kb/   |             | 0        | 0 0             |            |                  | 0  |
| 2        | 5E+07 .            | . A      |          |          | 35 PASS            | MSH6          | NM_000173.2                | c.3649A>G_p.Arg1217Gly                                  | 2 Ex8             | EE          | 33 D              | D           | D         | 0.937             | 1           | 30978            | 3E-05           | 0 +/23.3kb   |             | 0        | 0               | 5 (        |                  | 0 criteria pr criteria p                             |
| 2        | 5E+07 .            | A        |          | i .      | 35 PASS            | MSH6          | NM_000173.2                | :.3649A>G_p.Arg1217Gly                                  | 2 Ex8             | EE          | 33 D              | D           | D         | 0.937             | 1           | 30978            | 3E-05           | 0 +/23.9kb   |             | 0        | 0 9             |            |                  | 0 criteria pr criteria p                             |
| 13       | 1E+07 .<br>5E+07 . | CG       |          | GGG      | 35 PASS<br>35 PASS | LDLR<br>MSH6  | NM_000527.4<br>NM_000179.2 | c.2388C>T_p.  | 2 Ex16<br>2 Ex3   | EE<br>EE    |                   |             |           |                   | 11          | 277170           | 4E-05           |  |             | 0        | 2 (             | 1 (        |                  | 1 criteria pr criteria p<br>0.                       |
| 2        | 5E+07 .            |          | IGG T    |          | 35 PASS<br>35 PASS | MSH6          | NM_000179.2                | c.3802_3803insGGG_p.Met1;<br>c.3170_3172defTGG_p.Leu105 | 2 Ex3<br>2 Ex4    | EE          |                   |             |           |                   |             |                  |                 | . +/23.9kbi<br>. +/23.9kbi                         |             | ő        | n n             |            |                  | 0  |
| 19       | 1E+07 .            | ċ        |          |          | 35 PASS            | LDLR          | NM_000527.4                | c.1920C)T_p.  | 2 Ex4<br>3 Ex13   | SY          |                   | -           |           |                   | 117.9       | 277244           | 0.0043          | . +/44.5kb/  |             | ŝ        | 2 2             | -          | · ·              | 1 criteria pr criteria p                             |
| 19       | 1E+07 .            | Ť        | ċ        |          | 35 PASS            | LDLR          | NM_000527.4                | c.1061-8T>C   | 3 ln7/8           | SS          |                   |             |           |                   |             |                  |                 |  |             | 12       | 2 0             |            |                  | 1 criteria pr criteria p                             |
| 2        | 5E+07 .            | Å        | Ā        | T,ATT    |                    |               | NM_000173.2                | c.3557-5_3557-4dupTT                                    | 3 In6/7           | SS          |                   |             |           |                   |             |                  |                 | +/23.3kb   |             | 3        | 1 6             |            |                  | 0 reviewed criteriap                                 |
| 2        | 5E+07 .            | Ä        |          |          |                    |               | NM_000179.2                | c.3557-4delT  | 3 In6/7           | ss          |                   |             |           |                   |             |                  |                 | . +/23.3kb   |             | 2        | 2 0             | j          | ò õ              | 1 criteria pr criteria p                             |
| 19       | 1E+07 .            | c        |          |          |                    | <b>г</b>      | NM_000527.4                | c.2389+57C>T  | 3 In16/17         | INT         |                   |             |           |                   | 189         | 30354            | 0.0061          | 1 +/44.5kb/  |             | 0        | 1 (             | ) (        | 0 0              | 0 criteria pr criteria p                             |
| 17       | 4E+07 .            | c        |          | ACA      | GEN                | E             | NM_007294.3                | c.5194-2337_5194-2335dupT(                              | 3 In18/19         | INT         |                   |             |           |                   |             |                  |                 | -/81.2kb/:   | Insertion   | 1        | 0 0             | ) (        | 0 0              | 0 reviewed reviewed                                  |
| 2        | SE+07 .            | c        |          | CT CT    |                    | _             | NM_000179.2                | c.4002-10deff   | 3 In9/10          | INT         |                   |             |           |                   |             |                  |                 | . +/23.3kb+  |             | 0        | 0               | 1 (        |                  | 0 criteria pr criteria p                             |
| 17       | 4E+07 .            | G        |          |          |                    |               | NM_007294.3                | c20+11C>T   | 3 In1/2           | SPU         |                   |             |           |                   | 63          | 156718           | 0.0004          | /81.2kb/;  |             | 2        | 2               | 1 (        |                  | 1 criteria pr no assert                              |
| 17       | 4E+07 .            | с        |          | AT       | 35 PASS            | BRCA1         | NM_007294.3                | c19-2219-21dupAT  | 3 In1/2           | 5PU         |                   |             |           |                   |             | •                |                 | /81.2kb/:  |             | 1        | 1 (             |            |                  | 0 criteria pr criteria p                             |
| 17       | 4E+07 .            |          | mm g     | 1        | 35 PASS            | BRCA1         | NM_007294.3                | c20+52120+525del5                                       | 3 ln1/2           | SPU         |                   |             |           |                   | 95          |                  | 0.0031          | 0 -/81.2kb/:                                       |             | 0        | 1 (             |            |                  | 0 no asserti no assert                               |
| 11       | 4E+07 .            | <u>c</u> |          |          | 35 PASS            | BRCA1         | NM_007294.3                | c.+1332G>A  | 3 SUTR            | 3PU         |                   |             |           |                   | 1496        | 159758           | 0.0094          | /81.2kb/:  |             | 1        | 0 0             |            |                  | 0 reviewed reviewed                                  |
| 19       | 1E+07 .<br>2E+07 . |          | FA T     |          | 35 PASS<br>35 PASS | LDLR<br>APOB  | NM_000527.4<br>NM_000384.2 | c.+2210_+2211deITA<br>c.409G>T_p.Glu137X                | 3 3UTR<br>1 Ex5   | SPU<br>SG   | 34 .              | Å           |           | 0.796             |             |                  |                 | <ul> <li>+/44.5kb/</li> <li>-/42.6kb/</li> </ul>   |             | 0        | 1 0             |            |                  | 0 criteria pr criteria p<br>0 criteria pr criteria p |
| 19       | 2E+07 .            | G        |          | •        | 35 PASS<br>35 PASS | LDLR          | NM_000527.4                | c.1359-1G>A   | 1 Ex5<br>1 In3/10 | ESS         | 24.8 .            | Ê           |           | 0.136             |             | 245718           |                 | /42.0KD/<br>0 +/44.5kb/                            |             | 0        | 0 0             |            |                  | 1 criteria pr criteria p                             |
| 2        | 2E+07 .            | Ä        |          | •        | 35 PASS            | APOB          | NM_000384.2                | c.4503T>G_p.Tyr1501X                                    | 1 Ex26            | SG          | 27.5 .            | Ă           |           | . 0.862           |             | 246156           |                 | 0 -/42.6kb/  |             | ŏ        | ŏ               |            |                  | 0 criteria pr criteria p                             |
| 1        | 6E+06 .            | Ĝ        |          |          | 35 PASS            | PMS2          | NM_000535.6                | c.730C>T_p.Gln244X                                      | 1 Ex7             | SG          | 43 .              | â           | :         | 0.865             |             |                  | 46-00           | /38.2kb/   |             | ŏ        | ŏ ŭ             |            |                  | 0 criteria pr criteria p                             |
| i        | 6E+06 .            | Ğ        | Ä        |          | 35 PASS            | PMS2          | NM_000535.6                | c.730C>T_p.Gln244X                                      | 1 Ex7             | SG          | 43 .              | Ä           |           | 0.865             |             |                  |                 | /38.2kb/   |             | ŏ        | ŏ               |            |                  | 0 criteria pr criteria p                             |
| 19       | 1E+07 .            | Ť        | G        |          | 35 PASS            | LDLR          | NM_000527.4                | c.1342T>G_p.Ser648Ala                                   | 2 Ex13            | NSY         | 21.2 D            | N           | Ň         | 0.401             | 4           | 277228           | 1E-05           |  |             | ō        | ŏ               |            | 1 0              | 0 criteria pr criteria p                             |
| 19       | 1E+07 .            | ċ        | т        |          | 35 PASS            | LDLR          | NM_000527.4                | c.1835C>T_p.Ala612Val                                   | 2 Ex12            | NSY         | 26.5 D            | D           | N         | 0.511             |             | 277194           | 7E-06           | +/44.5kb/  |             | 0        | 0 0             | 5 0        | ) 1              | O no asserti no assert                               |
| 19       | 1E+07 .            | ċ        |          |          | 35 PASS            | LDLR          | NM_000527.4                | c.1835C>T_p.Ala612Val                                   | 2 Ex12            | NSY         | 26.5 D            | D           | N         | 0.511             |             | 277194           | 7E-06           | . +/44.5kb/  | Substituti  | 0        | 0 0             | ) (        | ) 1              | 0 no asserti no assert                               |
| 2        | 5E+07 .            | G        |          |          | 35 PASS            | MSH6          | NM_000173.2                | c.3577G>A_p.Glu1193Lys                                  | 2 Ex7             | NSY         | 33 D              | D           | D         | 0.987 .           |             |                  |                 | . +/23.9kb   |             | 0        | 0 0             |            |                  | 0 criteria pr criteria p                             |
| 1        | 6E+07 .            | G        |          |          | 35 PASS            | PCSK9         | NM_174936.3                | c.1180G>A_p.Gly394Ser                                   | 2 Ex7             | EE          | 27.1 D            | D           | D         | 0.824             | 26          | 236966           | 0.0001          | 0 +/25.4kb/  |             | 0        | 0 4             |            |                  | 0 criteria pr criteria p                             |
| 2        | 5E+07 .            | T        | <u> </u> | -        | 35 PASS            | MSH6          | NM_000173.2                | c.1109T>C_p.Leu370Ser                                   | 2 Ex4             | NSY         | 25.1 D            | D           | D         | 0.663             |             |                  |                 | . +/23.3kb   |             | 0        | 0 2             |            |                  | 1 criteria pr criteria p                             |
| 2        | 5E+07 .            | ç        | Ţ        |          | 35 PASS            | MSH6          | NM_000173.2                | c.1618C>T_p.Leu540Phe                                   | 2 Ex4             | NSY         | 25 D              | D           | N         | 0.785 .           |             |                  |                 | . +/23.3kb   |             | 0        | 0               | 1 0        |                  | 0 criteria pr criteria p                             |
|          | 6E+07 .            | ŝ        | . T      |          | 35 PASS            | PCSK9         | NM_174936.3                | c.1405C>T_p.Arg469Trp                                   | 2 Ex9             | NSY         | 22.4 T            | N           | N         | 0.709             |             |                  |                 | . +/25.4kb/  |             | 1        | 3 0             |            |                  | 1 criteria pr criteria p                             |
| 3        | 4E+07 .            | G        |          |          | 35 PASS            | MLH1<br>MLH1  | NM_000249.3<br>NM_000249.3 | c.1217G>A_p.Ser406Asn                                   | 2 Ex12            | NSY         | 13.46 T           | N           | PN N      | 0.31              |             |                  | 0.0003          |  |             | ě        | 4               | 1 0        |                  | O reviewed no assert                                 |
| 3        | 4E+07 .<br>6E+06 . | G<br>T   | Â        |          | 35 PASS<br>35 PASS | PMS2          | NM_000535.6                | c.1217G>A_p.Ser406Asn<br>c.52A>G_p.IIe18Val             | 2 Ex12<br>2 Ex2   | NSY<br>NSY  | 13.46 T<br>24.6 D | N<br>D      | N         | 0.31              | 243<br>2457 | 276804 271244    | 0.0009          | <ul> <li>+/57.5kb/*</li> <li>-/38.2kb/*</li> </ul> |             | 15       | 4               | 1 0        |                  | O reviewed no assert<br>O reviewed criteria p        |
| 17       | 4E+07 .            | Ġ        |          | ,        | 35 PASS            | BRCA1         | NM_007294.3                | c86C>T  | 3 SUTR            | SPU         | 24.0 D            | 0           |           | 0.050             | 113         | 154744           | 0.0007          |  |             | 1        | ñ :             |            |                  | 0 criteria pr criteria p                             |
| 3        | 4E+07 .            | č        | Â        |          | 35 PASS            | MLH1          | NM_000249.3                | c.307-29C>A   | 3 In3/4           | INT         |                   |             |           |                   | 14.97       | 277100           | 0.0054          | . +/57.5kb/  |             | ò        | 4 0             |            |                  | 0 reviewed reviewed                                  |
| 13       | 3E+07 .            | Ğ        | Ä        |          | 35 PASS            | BRCA2         | NM_000059.3                | c.8851G>A_p.Ala295fThr                                  | 2 Ex22            | NSY         | 26.9 T            | Ď           | Ň         | 0.518             | 2486        |                  | 0.009           | . +/84.2kb   |             | 26       | 4 0             |            |                  | 0 reviewed no assert                                 |
| 3        | 4E+07 .            | Ā        | G        | i i      | 35 PASS            | MLH1          | NM_000249.3                | c.791-79A>G   | 3 In9/10          | INT         |                   | -           |           |                   | 150         |                  | 0.0048          | 0 +/57.5kb/  |             | 0        | 0 0             | i i        | ) Ö              | 0 no asserti no assert                               |
| 19       | 1E+07              | 6        | A        |          | 35 PASS            | IDIR          | NM_000527.4                | c 1043G>A in Arn350GIn                                  | 2 Fy7             | NSY         | 22 N              | N           | N.        | 0.176             | 4           | 245530           | 2F-05           | 0 +/44 Skb/  |             | Ő.       | 0_0             | <u> </u>   | 1                | 0 no asserti no assert                               |
|          |                    |          |          |          |                    |               |                            |   |                   |             |                   |             |           |                   |             |                  |                 |  |             |          |                 |            |                  | 100  |



| #VCF/CFV | CF/POS VCF/ID      | VCF/REF  | VCF/ALT V   | CF/QU/ VCF/FIL     | CAVA/G         | CAVA/TRANSCR               |   | IMPACT : | VA/U        | O CAV      | A/CI CADD_p MetaS | VA Mutat | tion" PROV | EA VEST4_0 B | BIOR::GN     | BIOR::GN         | BIOR::GA        | BIOR::GN CAVA/TF (                | AVA/T` BIOR:   | CL BIOR::CL | BIOR::C | L BIOR::C  | L BIOR::CL BIOR | CL BIOR::CL BIOR::C   |
|----------|--------------------|----------|-------------|--------------------|----------------|----------------------------|---|----------|-------------|------------|-------------------|----------|------------|--------------|--------------|------------------|-----------------|-----------------------------------|--|-------------|---------|------------|-----------------|---|
| 1        | 6E+07 .            |          | ACTG        | 35 PASS            |                | NM_174936.3                | c.63_65dupGCT_p.Leu23dup                        | 2        | ix1         | IF         |                   |          |            |              |              |                  |                 | . +/25.4kb/ li                    |  | 7 2         | 0       |            |                 | 1 criteria pr no asse   |
| 2        | SE+07 .            |          | Α           | 35 PASS            | MSH6           | NM_000179.2                | c.3556+146G>A                                   | 3        | 16/7        | INT        |                   |          |            |              |              |                  |                 | . +/23.9kbi S                     |  | 0 0         |         | 0 1        |                 | 0 reviewed reviewe  |
| 2        | SE+07 .            |          | G           | 35 PASS            | MSH6           | NM_000179.2                | c.1186C>G_p.Leu396Val                           | 2        | x4          | NSY        | 21.7 T            | D        | N          | 0.32         | 1556         |                  | 0.0056          | . +/23.3kb, S                     |  | 16 2        |         |            |                 | O reviewed no asse  |
| 13       | 3E+07 .            |          | A,TA        | 35 PASS            |                | NM_000053.3                | c.68-4dupA                                      | 3        | 12/3        | SS         |                   |          |            |              | 787          | 274652           | 0.0029          | . +/84.2kbi li                    |  | 9 12        |         |            |                 | 0 reviewed reviewe  |
| 13       | 3E+07 .            |          | A,TA        | 35 PASS            |                | NM_000059.3                | c.68-4dupA                                      | 3        | 12/3        | \$\$       | ·                 | -        | ÷          |              | 787          |                  | 0.0029          | . •/84.2kbi li                    |  | 9 12        |         | 3 1        | · ·             | 0 reviewed reviewe  |
| 19       | 1E+07 .<br>2E+07 . |          | G           | 35 PASS<br>35 PASS | LDLR<br>APOB   | NM_000527.4<br>NM_000384.2 | c.1510A>G_p.Lys504Glu                           |          | x10<br>x26  | NSY<br>NSY | 21.8 T<br>25.9 T  | D        | D          | 0.347        | 396          | 246252<br>276304 | 2E-05<br>0.0014 | 0 +/44.5kb/ \$<br>/42.6kb/ \$     |  | 1 1         |         | 5 1        |                 | 1 criteria pr criteria<br>1 criteria pr criteria                      |
| 2        | 6E+06 .            | -        | c .         | 35 PASS            | PMS2           | NM_000535.6                | c.5066G>A_p.Arg1689His<br>c.2324A>G_p.Asn775Ser |          | x14         | NSY        | 23.2 T            | Ď        | D          | 0.391        | 336          |                  | 0.0003          | -/38.2kb/ S                       |  |             |         | 1 1        |                 | 0 reviewed criteria   |
| 17       | 4E+07 .            |          | c           | 35 PASS            | BRCA1          | NM_007294.3                | c.736T>G_p.Leu246Val                            | 5        | x14         | NSY        | 11.52 D           | Ď        | Ň          | 0.358        | 82           |                  | 0.0003          | /81.2kb/: S                       |  | 9 6         |         | s i        |                 | 0 reviewed criteria<br>0 reviewed no asse                             |
| 19       | 1E+07 .            |          | Ă           | 35 PASS            | LDLR           | NM_000527.4                | c.313+1G>A                                      | 1        | 3/4         | ESS        | 24.8 .            | Ď        |            | 0.000        | 7            | 246208           | 3E-05           | 0 +/44.5kb/ S                     |  | ñ 1         | i       |            |                 | 1 criteria pr criteria  |
| ĩ        | 6E+06 .            | _        | Ä           | 35 PASS            | PMS2           | NM_000535.6                | c.251-2A>T                                      | i        | 3/4         | ESS        | 27                | Ď        |            |              | i            | 239164           | 4E-06           | 0 -/38.2kb/ S                     |  | ŏ o         | i i     | -          |                 | 1 criteria pr criteria  |
| 3        | 4E+07 .            |          | ĊC          | 35 PASS            | MLH1           | NM_000249.3                | c.1410-2_1410-1delinsCC                         |          | 12/13       |            |                   |          |            |              |              |                  |                 | . +/57.5kb/* 0                    |  | ō ō         | ć       |            | 1 1             | 1 criteria pr criteria  |
| 3        | 4E+07 .            |          | čč          | 35 PASS            | MLH1           | NM_000249.3                | c.1410-2_1410-1delinsCC                         |          | 12/13       |            |                   |          |            |              |              |                  |                 | +/57.5kb/* 0                      |  | õ õ         | - i     | 5          | i i             | 1 criteria pr criteria  |
| 2        | SE+07 .            |          | AAC         | 35 PASS            | MSH6           | NM_000179.2                | c.843_844insAC                                  | 1        | x4          | FS         |                   |          |            |              | 1            | 245844           | 4E-06           | 0 +/23.3kbi li                    |  | 0 0         |         | 0 1        | 0 0             | 0   |
| 7        | 6E+06 .            | CTT      | с           | 35 PASS            | PMS2           | NM_000535.6                | c.1312_1313delAA                                | 1        | x11         | FS         |                   |          |            |              |              |                  |                 | /38.2kb/ D                        | leletion   | 0 0         |         | 0 1        | 0 0             | 0   |
| 13       | 3E+07 .            | AGCAAG.  | ATGCTG      | 35 PASS            | BRCA2          | NM_000053.3                | c.6024_6035delinsTGCTGTT+                       | 1        | 1x11        | FS         |                   |          |            |              |              |                  |                 | . +/84.2kbi 0                     | omplex   | 0 0         |         |            |                 | 0   |
| 2        | 2E+07 .            | c .      | Α           | 35 PASS            | APOB           | NM_000384.2                | c.409G>T_p.Glu137X                              |          | x5          | SG         | 34 .              | A        |            | 0.796 .      |              |                  |                 | /42.6kb/ S                        |  | 0 0         |         |            |                 | 0 criteria pr criteria  |
| 2        | 2E+07 .            |          | ė.          | 35 PASS            | APOB           | NM_000384.2                | c.409G>T_p.Glu137X                              | 1        | :x5         | SG         | 34 .              | <u>^</u> |            | 0.796 .      |              |                  |                 | /42.6kb/ S                        |  | 0 0         | 9       | 0 1        |                 | 0 criteria pr criteria  |
| 2        | 2E+07 .            | -        | ė.          | 35 PASS            | APOB           | NM_000384                  |   |          |             | SG         | 34 .              | <u>A</u> |            | 0.796 .      |              |                  |                 | /42.6kb/ S                        |  | 0 0         |         |            | 2               | 0 criteria pr criteria  |
| 19       | 1E+07 .            |          | A           | 35 PASS            | LDLR           | NM_000527                  |   |          | 7           | SG         | 42 .              | D        |            | 0.71 .       |              |                  |                 | . +/44.5kb/ S                     |  | 0 0         |         | ~          | 1 2             | 1 criteria pr criteria  |
|          | 6E+07 .<br>2E+07 . |          | GGAGGA<br>C | 35 PASS<br>35 PASS | PCSK9<br>APOB  | NM_174936<br>NM_000384     |   |          |             |            |                   |          |            |              | 19<br>1266   | 175140<br>245070 | 0.0001          | 0 +/25.4kb/ k<br>-/42.6kb/ D      |  | 0 1         |         |            |                 | 0 criteria pr criteria  |
| 2        | 2E+07 .<br>2E+07 . |          | c<br>c      | 35 PASS<br>35 PASS | APOB           | NM_000384<br>NM_000384     | Mutation ty                                     | Inc      | 6           | IF         | · ·               | -        |            |              | 1266         | 245070           | 0.0052          | /42.6kb/ L<br>/42.6kb/ D          |  | 3 6         |         |            |                 | 1 criteria pr criteria<br>1 criteria pr criteria                      |
| 7        | 6E+06 .            |          | G           | 35 PASS<br>35 PASS | PMS2           | NM_000535                  |   | yhe.     | 0           | 154        | 22.7 D            | 'n       | N          | . 0.353      | 1200         | 245010           | 4E-06           | 0 -/38.2kb/ S                     |  | 0 0         |         |            |                 | 1 criteria pr criteria  |
| 17       | 4E+07 .            |          | GCCT        | 35 PASS            | BRCA1          | NM_007294                  | -   |          |             | 154        | 22.1 0            | 0        | 14         | 0.033        |              | 243022           | 42-06           | /81.2kb/; li                      |  | ňů          | - 2     | , ,<br>, , |                 | 1 criteria pr criteria  |
| 17       | 4E+07 .            | π        | T           | 35 PASS            | BRCA1          | NM_007294                  | and locati                                      | ∩n       |             | FS         |                   |          |            |              |              |                  |                 | /81.2kb/; D                       |  | õ õ         |         | 5 1        |                 | 0   |
| 17       | 4E+07 .            |          | G           | 35 PASS            | BRCA1          | NM_007294                  | and locati                                      |          | 3           | SL         | 11.18             | Ň        |            | 0.431        |              | :                |                 | /81.2kb/: S                       |  | õ õ         | i i     | i i        |                 | ŏ   |
| 13       | 3E+07 .            | Ā .      | ĀT          | 35 PASS            | BRCA2          | NM_000053                  |   |          | 7           | FS         |                   |          |            |              |              |                  |                 | +/84.2kbi li                      |  | ō 0         | - i     | 5 1        | ò ò             | ō   |
| 7        | 6E+06 .            | CTGA     | с           | 35 PASS            | PMS2           | NM_000535                  |   |          | 5           | IF         |                   |          |            |              |              |                  |                 | -/38.2kb/ D                       |  | 0 0         |         | 0 1        | 0 0             | 0   |
| 2        | 5E+07 .            | A 1      | G           | 35 PASS            | MSH6           | NM_000173.2                | cooson/a_p.nrgizitaly                           | ۲        |             | EE         | 33 D              | D        | D          | 0.937        | 1            | 30978            | 3E-05           | 0 +/23.3kbi S                     | ubstituti  | 0 0         |         | 5 1        | 0 0             | 0 criteria pr criteria  |
| 2        | 5E+07 .            | A 1      | G           | 35 PASS            | MSH6           | NM_000173.2                | :.3649A>G_p.Arg1217Gly                          |          | x8          | EE         | 33 D              | D        | D          | 0.937        | 1            | 30978            | 3E-05           | 0 +/23.3kbi \$                    |  | 0 0         |         | 5 1        | 0 0             | 0 criteria pr criteria  |
| 19       | 1E+07 .            | ~        | т           | 35 PASS            | LDLR           | NM_000527.4                | c.2388C>T_p.                                    | 2        | x16         | EE         |                   |          |            |              | 11           | 277170           | 4E-05           | . +/44.5kb/ S                     |  | 0 2         |         | 1 1        |                 | 1 criteria pr criteria  |
| 2        | 5E+07 .            |          | GGGG        | 35 PASS            | MSH6           | NM_000179.2                | c.3802_3803insGGG_p.Met1;                       | 2        | хЭ          | EE         |                   |          |            |              |              |                  |                 | . +/23.9kbi li                    |  | 0 0         |         |            |                 | 0   |
| 2        | 5E+07 .            |          | Ţ           | 35 PASS            | MSH6           | NM_000179.2                | c.3170_3172defTGG_p.Leu105"                     | 2        | x4          | EE         |                   |          |            |              |              | ·                |                 | . +/23.9kb+ E                     |  | 0 0         |         |            |                 | 0   |
| 19       | 1E+07 .            | <u> </u> | T           | 35 PASS            | LOLR           | NM_000527.4                | c.1920C>T_p.                                    | 3        | x13         | SY         |                   |          |            |              |              | 277244           |                 | . +/44.5kb/ S                     |  | 5 2         |         |            |                 | 1 criteria pr criteria  |
| 19       | 1E+07 .<br>5E+07 . |          |             | 35 PASS            | LULR           | NM_000527.4<br>NM_000179.2 | c.1061-8T>C                                     | 3        | 6/7         | SS<br>SS   |                   |          |            |              | 1525         | 276844           | 0.0055          | . +/44.5kb/ S<br>. +/23.9kb- h    |  | 12 2        |         |            |                 | 1 criteria pri criteria<br>0 reviewed criteria                        |
| 2        | SE+07 .            |          | AT,ATT      |                    |                | NM_000179.2                | c.3557-5_3557-4dupTT<br>c.3557-4defT            | 3        | 6/7         | 55         | · ·               | -        |            |              |              |                  |                 | . •/23.3kbi li<br>. •/23.3kbi D   |  | 3 1         |         |            |                 | 1 criteria pri criteria   |
| 19       | 1E+07 .            |          | î 🗌         | ~                  |                | NM_000527.4                | c.2389+57C>T                                    | ~        | 16/17       |            |                   |          |            |              | 189          | 30954            | 0.0061          | 1 +/44.5kb/ S                     |  | 6 6<br>1    |         | 5 1        |                 | 0 criteria pr criteria  |
| 17       | 4E+07 .            |          | CACA        | Gen                | e 🛛            | NM_007294.3                | c.5194-2337_5194-2335dupT(                      | 3        | 18/19       |            |                   |          |            |              | 100          | 00004            | 0.0001          | /81.2kb/: li                      |  | 1 0         | - 2     |            |                 | 0 reviewed reviewe  |
| 2        | 5E+07 .            |          | C,CT        | 0011               | Ч              | NM_000179.2                | c.4002-10delT                                   | 3        | 3/10        |            |                   |          |            |              |              |                  |                 | +/23.9kb+ D                       |  | o õ         |         | 1 1        | ō õ             | 0 criteria pr criteria  |
| 17       | 4E+07 .            |          | A .         |                    |                | NM_007294.3                | c20+11C>T                                       | 3        | 1/2         | 5PU        |                   |          |            |              | 69           | 156718           | 0.0004          | -/81.2kb/; \$                     |  | 2 2         |         | 1 1        | ō õ             | 1 criteria pr no asse   |
| 17       | 4E+07 .            | с        | CAT         | 35 PASS            | BRCA1          | NM_007294.3                | c13-2213-21dupAT                                | 3        | 1/2         | 5PU        |                   |          |            |              |              |                  |                 | -/81.2kb/; li                     |  | 1 1         |         | 0 1        | 0 0             | 0 criteria pr criteria  |
| 17       | 4E+07 .            | GTTTT    | G           | 35 PASS            | BRCA1          | NM_007294.3                | c20+52120+525del5                               | 3        | 1/2         | SPU        |                   |          |            |              | 95           |                  | 0.0031          | 0 -/81.2kb/: D                    |  | 0 1         |         |            |                 | 0 no asserti no asse  |
| 17       | 4E+07 .            | c ·      | т           | 35 PASS            | BRCA1          | NM_007294.3                | c.+1332G>A                                      | 3        | UTR         |            |                   |          |            |              | 1496         | 159758           | 0.0094          | /81.2kb/: \$                      |  | 1 0         | (       |            |                 | 0 reviewed reviewe  |
| 19       | 1E+07 .            | TTA .    | т           | 35 PASS            | LDLR           | NM_000527.4                | c.+2210_+2211deITA                              | 3        | UTR         |            |                   | -        |            |              |              |                  |                 | . +/44.5kb/ D                     |  | 0 1         | 0       |            |                 | 0 criteria pr criteria  |
| 2        | 2E+07 .            | с .      | <u>^</u>    | 35 PASS            | APOB           | NM_000384.2                | c.409G>T_p.Glu137X                              | 1        | .x5         | SG         | 34 .              | <u>^</u> |            | 0.796 .      | _            |                  |                 | /42.6kb/ S                        |  | 0 0         |         |            |                 | 0 criteria pr criteria  |
| 19       | 1E+07 .            |          | A .         | 35 PASS            | LDLR           | NM_000527.4                | c.1359-1G>A                                     |          | 19/10       |            | 24.8 .            | D        |            |              | 0            |                  | 15 00           | 0 +/44.5kb/ \$                    |  | 0 0         |         |            |                 | 1 criteria pr criteria  |
| 2        | 2E+07 .            |          | ç           | 35 PASS<br>35 PASS | APOB<br>PMS2   | NM_000384.2                | c.4503T>G_p.Tyr1501X<br>c.730C>T_p.Gin244X      | 1        | x26         | SG<br>SG   | 27.5 .            | Å        | •          | 0.862        | 1            | 246156           | 4E-06           | 0 -/42.6kb/ \$                    |  | 0 0         |         |            |                 | 0 criteria pri criteria<br>0 criteria pri criteria                    |
| 2        | 6E+06 .<br>6E+06 . | -        | Å           | 35 PASS<br>35 PASS | PMIS2<br>PMIS2 | NM_000535.6<br>NM_000535.6 | c.730C>T_p.Gin244X<br>c.730C>T_p.Gin244X        |          |             | SG         | 43.<br>43.        | â        |            | 0.865 .      |              |                  |                 | /38.2kb/ S<br>/38.2kb/ S          |  | 0 0         |         |            |                 | <ul> <li>0 criteria pr criteria<br/>0 criteria pr criteria</li> </ul> |
| 19       | 1E+07 .            |          | â           | 35 PASS<br>35 PASS | LDLR           | NM_000527.4                | c.1342T>G_p.Ser648Ala                           |          | x13         | NSY        | 43.<br>21.2 D     | Ñ        | N          | 0.005 .      |              | 277228           | 1E-05           |                                   |  | õ õ         | - 2     |            | 1 0             | 0 criteria pr criteria<br>0 criteria pr criteria                      |
| 19       | 1E+07 .            | ė -      | Ť           | 35 PASS            | LOLR           | NM_000527.4                | c.1835C)T_p.Ala612Val                           | 5        | x13         | NSY        | 26.5 D            | D        | N          | 0.511        |              | 277194           | 7E-05           | . +/44.5kb/ S                     |  | ŏ ŏ         |         |            | <br>1           | 0 no asserti no asse  |
| 19       | 1E+07 .            | č ·      | Ť           | 35 PASS            | LDLR           | NM_000527.4                | c.1835C>T_p.Ala612Val                           |          | x12         | NSY        | 26.5 D            | Ď        | N          | 0.511        | 2            | 277194           | 7E-06           | . +/44.5kb/ S                     |  | õ õ         | - 2     | Ś          |                 | 0 no asserti no asse  |
| 2        | 5E+07 .            | Ğ.       | à           | 35 PASS            | MSH6           | NM_000179.2                | c.3577G>A_p.Glu1193Lys                          |          | x7          | NSY        | 33 D              | Ď        | Ď          | 0.987 .      | -            |                  |                 | . +/23.9kbi S                     |  | ò ŏ         | - i     |            |                 | 0 criteria pr criteria  |
| 1        | 6E+07 .            | G        | Α           | 35 PASS            | PCSK9          | NM_174936.3                | c.1180G>A_p.Gly394Ser                           | 2        | x7          | EE         | 27.1 D            | D        | D          | 0.824        | 26           | 236966           | 0.0001          | 0 +/25.4kb/ S                     |  | 0 0         |         |            |                 | 0 criteria pr criteria  |
| 2        | 5E+07 .            | т        | c           | 35 PASS            | MSH6           | NM_000173.2                | c.1109T>C_p.Leu370Ser                           | 2        | x4          | NSY        | 25.1 D            | D        | D          | 0.669 .      |              |                  |                 | . +/23.9kbi S                     |  | 0 0         | 2       | 2 ;        |                 | 1 criteria pr criteria  |
| 2        | 5E+07 .            | с .      | т           | 35 PASS            | MSH6           | NM_000179.2                | c.1618C>T_p.Leu540Phe                           | 2        | x4          | NSY        | 25 D              | D        | N          | 0.785 .      |              |                  |                 | <ul> <li>+/23.3kbi \$</li> </ul>  | ubstituti  | 0 0         |         | 1 1        |                 | 0 criteria pr criteria  |
| 1        | 6E+07 .            | c ·      | т           | 35 PASS            | PCSKB          | NM_174936.3                | c.1405C>T_p.Arg469Trp                           |          | хэ          | NSY        | 22.4 T            | N        | N          | 0.709        |              | 277130           | 0.0009          | . +/25.4kb/ \$                    |  | 1 3         |         | 3 (        |                 | 1 criteria pr criteria  |
| 3        | 4E+07 .            | -        | Α           | 35 PASS            | MLH1           | NM_000249.3                | c.1217G>A_p.Ser406Asn                           |          | x12         | NSY        | 13.46 T           | N        | N          | 0.31         | 249          |                  | 0.0009          | . +/57.5kb/* \$                   |  | 6 4         |         | 1 1        |                 | O reviewed no asse  |
| 3        | 4E+07 .            |          | A           | 35 PASS            | MLH1           | NM_000249.3                | c.1217G>A_p.Ser406Asn                           | 2        | x12         | NSY        | 13.46 T           | N        | N          | 0.31         | 249          |                  | 0.0009          | . +/57.5kb/* S                    |  | 6 4         |         | 1 1        |                 | O reviewed no asse  |
| 1        | 6E+06 .            | T I      | c .         | 35 PASS            | PMS2           | NM_000535.6                | c.52A>G_p.lle18Val                              | 2        | :x2         | NSY        | 24.6 D            | D        | N          | 0.656        | 2457         |                  | 0.0091          | /38.2kb/ S                        |  | 15 6        |         | 1 1        |                 | 0 reviewed criteria   |
| 17       | 4E+07 .            | G ,      | Ŷ.          | 35 PASS            | BRCA1          | NM_007294.3                | c86C>T  | 3        | UTR<br>3/4  |            |                   |          |            |              | 113<br>14.97 | 154744           | 0.0007          |                                   |  | 1 0         |         | 3 1        |                 | 0 criteria pr criteria  |
| 3<br>13  | 4E+07 .            |          | 2           | 35 PASS<br>35 PASS | MLH1<br>BRCA2  | NM_000249.3<br>NM_000059.3 | <.307-29C>A                                     | 3        | 13/4<br>x22 | INT<br>NSY | · · · ·           | 'n       |            | . 0.518      | 1437<br>2486 | 277100<br>276544 | 0.0054 0.009    | . +/57.5kb/* \$                   |  | 0 4         |         |            |                 | 0 reviewed reviewe  |
| 3        | 3E+07 .<br>4E+07 . | 4        | G           | 35 PASS            |                | NM_000249.3                | c.8851G>A_p.Als2951Thr<br>c.791-79A>G           | 2        | .x22        |            | 26.9 T            | 0        | 14         | 0.510        | 2400         |                  | 0.003           | . +/84.2kb+ \$<br>0 +/57.5kb/* \$ |  | 20 4<br>0 0 | i i     |            |                 | 0 reviewed no asse<br>0 no asserti no asse                            |
| 19       | 4E+07 .<br>1F+07   | ŝ        | Ă           | 35 PASS            |                | NM 000527.4                | c 1043G3A in Are350Gla                          |          | 13710       | NSY        | 22 n              | N        | N          | . 0.176      | .50          | 245590           | 2E-05           | 0 +/44 5kb/ 3                     |  | ň ň         |         | í          | 1 1             | 0 no asserti no asse<br>0 no asserti no asse                          |
|          |                    |          |             |                    |                | and the firm               |   |          |             |            | ee,0              |          |            |              |              | , - 16.00        |                 | Print Inter .                     | , and the second |             |         |            |                 |   |



| #VCF/CF V | CF/POS VCF/I       | ID VCF/REF | VCF/ALT V | VCF/QU/ VCF/FIL    |                | CAVA/TRANSCR               | CAVA/CSN  | IMPACT : VA      | lι         | CAVA/CI CADD_p M        | taSVN Mutatio | on PROVEA | VEST4_s BI         | OR:GN I      | BIOR::GN I       | BIOR::GN BIO   | SIGN CAVA/TF CAVA/T* B                           | IOR::CL E | BIOR::CL BIOR::C | CL BIOR:: | CL BIOR::CL BIO | R::CL BIOR::CL BIOR::C   |
|-----------|--------------------|------------|-----------|--------------------|----------------|----------------------------|---|------------------|------------|-------------------------|---------------|-----------|--------------------|--------------|------------------|----------------|--|-----------|------------------|-----------|-----------------|--|
| 1         | 6E+07 .            |            | ACTG      | 35 PASS            |                | NM_174936.3                | c.63_65dupGCT_p.Leu23dup                                | 2 .x1            |            | IF                      |               |           |                    |              |                  |                | +/25.4kb/ Insertion                              | 7         | 2                | 0         | 0 0             | 1 criteria pr no asser   |
| 2         | SE+07 .            |            | A<br>G    | 35 PASS            | MSH6<br>MSH6   | NM_000173.2<br>NM_000173.2 | c.3556+146G>A   | 3 16/7           |            | INT                     |               | <u>.</u>  | ·                  | 1556         |                  |                | +/23.9kb+ Substituti                             | 0         | *                | *         | 0 0             | 0 reviewed reviewe   |
| 13        | 5E+07 .<br>3E+07 . |            | A.TA      | 35 PASS<br>35 PASS | BRCA2          | NM_000059.3                | c.1186C>G_p.Leu396Val<br>c.68-4dupA                     | 2 x4<br>3 x2/3   |            | NSY 21.7 T<br>SS        | U             | N.        | 0.32               |              | 276930<br>274652 | 0.0056 .       | +/23.9kb+ Substituti<br>+/84.2kb+ Insertion      | 10        | 12               | 2         | 0 0             | O reviewed no asser<br>O reviewed reviewe                              |
| 13        | 3E+07 .            |            | ATA       | 35 PASS            | BRCA2          | NM_000053.3                | c.68-4dupA  | 3 2/3            |            | SS                      |               |           |                    |              | 274652           | 0.0023 .       | +/84.2kbi Insertion                              | š         | 12               | 3         | õ õ             | 0 reviewed reviewe   |
| 19        | 1E+07 .            |            | G         | 35 PASS            | LDLR           | NM_000527.4                | c.1510A>G_p.Lys504Glu                                   | 2 x10            |            | NSY 21.8 T              | Ď             | Ň         | 0.347              |              | 246252           | 2E-05          | 0 +/44.5kb/ Substituti                           | Ť         | 1                | 2         | 0 1             | 1 criteria pr criteria   |
| 2         | 2E+07 .            | с          | т         | 35 PASS            | APOB           | NM_000384.2                | c.5066G>A_p.Arg1689His                                  | 2 x26            | 5 <b>i</b> | NSY 25.9 T              | D             | D         | 0.718              | 396          | 276904           | 0.0014 .       | -/42.6kb/ Substituti                             | 0         | 3                | 5         | 0 0             | 1 criteria pr criteria   |
| 7         | 6E+06 .            |            | с         | 35 PASS            | PMS2           | NM_000535.6                | c.2324A>G_p.Asn775Ser                                   | 2 x14            |            | NSY 23.2 T              | D             | D         | 0.391              |              | 275058           | 0.0003 .       | -/38.2kb/ Substituti                             | 14        | 1                |           | 0 0             | 0 reviewed criteria  |
| 17        | 4E+07 .            |            | ç         | 35 PASS            | BRCA1          | NM_007294.3                | c.736T>G_p.Leu246Val                                    | 2 x10            |            | NSY 11.52 D             | 2             | N         | 0.358              |              | 276040           | 0.0003 .       | -/81.2kb/: Substituti                            | 9         | 6                | 5         | 0 0             | O reviewed no asser  |
| 19        | 1E+07 .<br>6E+06 . | _          | Â         | 35 PASS<br>35 PASS | LDLR<br>PMS2   | NM_000527.4<br>NM_000535.6 | c.313+1G>A<br>c.251-2A>T                                | 1 \3/4           |            | ESS 24.8.<br>ESS 27.    | B             |           |                    |              | 246208<br>233164 | 3E-05<br>4E-06 | 0 +/44.5kb/ Substituti<br>0 -/38.2kb/ Substituti | 0         | 1                | 0         | 3 16            | 1 criteria pr criteria p   |
| 3         | 4E+07 .            |            | ĉc        | 35 PASS            | MLH1           | NM_000249.3                | c.1410-2_1410-1delinsCC                                 | 1 12/            |            | ESS . 21.               | 0             | •         |                    |              | 200104           | 42-06          | +/57.5kb/* Complex                               | ň         | 0                | 0         | 2 0             | 1 criteria pr criteria (<br>1 criteria pr criteria )                   |
| 3         | 4E+07 .            |            | čč        | 35 PASS            | MLH1           | NM_000249.3                | c.1410-2_1410-1delinsCC                                 | 1 12/            |            | ESS                     |               |           |                    |              |                  |                | +/57.5kb/* Complex                               | ŏ         | ŏ                | ŏ         | i i             | 1 criteria pr criteria   |
| 2         | SE+07 .            |            | AAC       | 35 PASS            | MSH6           | NM_000179.2                | c.843_844insAC  | 1 x4             |            | FS                      |               |           |                    | 1            | 245844           | 4E-06          | 0 +/23.3kbi Insertion                            | ō         | ō                | ō         | 0 0             | 0  |
| 7         | 6E+06 .            |            | С         | 35 PASS            | PMS2           | NM_000535.6                | c.1312_1313delAA  | 1 x11            |            | FS                      |               |           |                    |              |                  |                | -/38.2kb/ Deletion                               | 0         | 0                | 0         | 0 0             | 0  |
| 13        | 3E+07 .            | AGCAAG     | ATGCTG    | 35 PASS            | BRCA2          | NM_000059.3                | c.6024_6035delinsTGCTGTT+                               | 1 x11            |            | F\$                     | •             |           |                    |              |                  |                | +/84.2kb, Complex                                | 0         | 0                | 0         | 0 0             | 0  |
| 2         | 2E+07 .<br>2E+07 . | ç          | Ŷ.        | 35 PASS<br>35 PASS | APOB<br>APOB   | NM_000384.2<br>NM_000384.2 | c.409G>T_p.Glu137X                                      | 1 .x5            |            | SG 34.<br>SG 34.        | ÷.            |           | 0.796 .<br>0.796 . |              |                  |                | -/42.6kb/ Substituti                             | 0         | 0                | ~         | 0 2             | 0 criteria pr criteria j   |
| 2         | 2E+07 .            | č          | 2         | 35 PASS<br>35 PASS | APOB           | NM_000384                  | c.409G>T_p.Glu137X                                      | 1 x5             |            | SG 34.                  | 2             |           | 0.736              |              |                  |                | -/42.6kb/ Substituti<br>-/42.6kb/ Substituti     | ő         | 0                | <u>~</u>  | 0 2             | 0 criteria pr criteria<br>0 criteria pr criteria )                     |
| 19        | 1E+07 .            | -          | â         | 35 PASS            | LDLR           | NM_000527                  |   | 7                |            | SG 42.                  | ê             |           | 0.71               |              |                  |                | +/44.5kb/ Substituti                             | ŏ         | ŏ                | ŏ         | 1 2             | 1 criteria pr criteria   |
| 1         | 6E+07 .            |            | GGAGGA    | 35 PASS            | PCSK3          | NM_174936                  |   |                  |            | IF                      | -             |           |                    | 19           | 175140           | 0.0001         | 0 +/25.4kb/ Insertion                            | õ         | 1                | 0         | 0 0             | 0 criteria pr criteria ;   |
| 2         | 2E+07 .            | CTCA       | с         | 35 PASS            | APOB           |                            |   | 6                | i 🚺        | IF                      |               |           |                    | 1266         | 245070           | 0.0052 .       | -/42.6kb/ Deletion                               | 3         | 6                | 4         | 0 0             | 1 criteria pr criteria   |
| 2         | 2E+07 .            |            | c         | 35 PASS            | APOB           | NM_000384                  | Mutation ty   | vde 🕨            | 1          | IF                      | -             |           |                    |              | 245070           | 0.0052 .       | -/42.6kb/ Deletion                               | 3         | 6                | 4         | 0 0             | 1 criteria pr criteria   |
| 1         | 6E+06 .            |            | G         | 35 PASS            | PMS2           |                            | -   |                  |            | IM 22.7 D               | D             | N         | 0.953              | 1            | 245322           | 4E-06          | 0 -/38.2kb/ Substituti                           | 0         | 0                | 0         | 2 2             | 1 criteria pr criteria j   |
| 17        | 4E+07 .<br>4E+07 . |            | GCCT      | 35 PASS<br>35 PASS | BRCA1<br>BRCA1 | NM_007294<br>NM_007294     | and locati  | on I             |            | IM                      | -             |           |                    |              |                  |                | -/81.2kb/: Insertion<br>-/81.2kb/: Deletion      | 0         | 0                | 0         | 0 0             | 0  |
| 17        | 4E+07 .            |            | G         | 35 PASS            | BRCA1          | NM_007294                  | anu iocati  | UII ,            |            | rə<br>SI 1118           | N             |           | 0.431              |              |                  |                | -/81.2kb/; Substituti                            | ő         | 0                | 0         | 0 0             | 0  |
| 13        | 3E+07 .            |            | AT        | 35 PASS            | BRCA2          | NM_000053                  |   | 7                |            | FS .                    |               |           | 0.401              |              |                  |                | +/84.2kbi Insertion                              | ŏ         | ŏ                | ŏ         | õ õ             | ŏ  |
| 7         | 6E+06 .            |            | c         | 35 PASS            | PMS2           | NM_000535                  |   | s                |            | IF                      |               |           |                    |              |                  |                | -/38.2kb/ Deletion                               | ō         | ō                | ō         | o o             | 0  |
| 2         | 5E+07 .            |            | G         | 35 PASS            | MSH6           | NM_000173.2                | c.oowonza_p.nrgiziraiy                                  | 2 .20            |            | EE 33 D                 | D             | D         | 0.937              | 1            | 30978            | 3E-05          | 0 +/23.9kb, Substituti                           | 0         | 0                | 5         | 0 0             | 0 criteria pr criteria j   |
| 2         | 5E+07 .            |            | G         | 35 PASS            | MSH6           | NM_000179.2                | :.3649A>G_p.Arg1217Gly                                  | 2 .x8            |            | EE 33 D                 | D             | D         | 0.937              | 1            | 30978            | 3E-05          | 0 +/23.9kb, Substituti                           | 0         | 0                | 5         | 0 0             | 0 criteria pr criteria j   |
| 19        | 1E+07 .            |            | T         | 35 PASS            | LDLR           | NM_000527.4                | c.2388C>T_p.  | 2 x16            |            | EE .                    |               |           |                    | 11           | 277170           | 4E-05 .        | +/44.5kb/ Substituti                             | 0         | 2                | 1         | 0 0             | 1 criteria pr criteria (   |
| 2         | 5E+07 .<br>5E+07 . |            | GGGG<br>T | 35 PASS<br>35 PASS | MSH6<br>MSH6   | NM_000179.2<br>NM_000179.2 | c.3802_3803insGGG_p.Met1;<br>c.3170_3172defTGG_p.Leu105 | 2 x9             |            | EE .<br>EE .            |               |           |                    |              |                  |                | +/23.9kbi Insertion<br>+/23.9kbi Deletion        | 0         | 0                | 0         | 0 0             | 0  |
| 19        | 1E+07 .            | C          | ÷         | 35 PASS            | LDLR           | NM_000527.4                | c.1920C)T_p.  | 2 .x4<br>3 x13   |            | SY .                    | In c          | silico    | )                  | 117.9        | 277244           | 0.0043         | +/23.5kb/ Substituti                             | š         | 2                | 2         | 0 0             | 1 criteria pr criteria j   |
| 19        | 1E+07 .            |            | ċ         | 35 PASS            | LDLR           | NM_000527.4                | c.1061-8T>C   | 3 7/8            |            | ss .                    | 111.5         |           | ,                  |              | 276844           | 0.0055         | +/44.5kb/ Substituti                             | 12        | 2                | ō         | õ õ             | 1 criteria pr criteria j   |
| 2         | 5E+07 .            | À          | AT, ATT   |                    |                | NM_000179.2                | c.3557-5_3557-4dupTT                                    | 3 6/7            |            |                         |               |           |                    |              |                  |                | +/23.3kbi Insertion                              | 3         | 1                | ō         | 0 0             | O reviewed criteria  |
| 2         | SE+07 .            |            | A .       |                    |                | NM_000179.2                | c.3557-4deff  | 3 6/7            |            | SS .                    | oredi         | CTIO      | ns                 |              |                  |                | +/23.3kb+ Deletion                               | 2         | 2                | 0         | 0 0             | 1 criteria pr criteria p   |
| 19        | 1E+07 .            |            | T         | Gen                |                | NM_000527.4                | c.2389+57C>T  | 3 16/            |            |                         |               | •         |                    | 189          | 30954            | 0.0061         | 1 +/44.5kb/ Substituti                           | 0         | 1                | 0         | 0 0             | 0 criteria pr criteria (   |
| 17        | 4E+07 .<br>5E+07 . |            | CACA      | Gen                | ਦ              | NM_007294.3                | c.5194-2337_5194-2335dupT(                              | 3 18/            |            |                         |               |           |                    |              |                  |                | -/81.2kb/: Insertion                             | 1         | 0                | 0         | 0 0             | 0 reviewed reviewed  |
| 17        | 4E+07 .            |            | C,CT      |                    |                | NM_000179.2<br>NM_007294.3 | c.4002-10delT<br>c20+11C>T                              | 3 1/2            |            | INI<br>SPU              |               |           |                    | 69           | 156718           | 0.0004         | +/23.9kb+ Deletion<br>-/81.2kb/: Substituti      | 2         | 2                |           | 0 0             | 0 criteria pr criteria j<br>1 criteria pr no assei                     |
| 17        | 4E+07 .            |            | CAT       | 35 PASS            | BRCA1          | NM_007294.3                | c19-2219-21dupAT  | 3 1/2            |            | SPU                     |               |           |                    | 00           | 150110           | 0.0004         | -/81.2kb/: Insertion                             | 1         | 1                |           | õ õ             | 0 criteria pr criteria i   |
| 17        | 4E+07 .            |            | G         | 35 PASS            | BRCA1          | NM_007294.3                | c20+52120+525del5                                       | 3 1/2            |            | SPU                     |               |           |                    | 95           | 30914            | 0.0031         | 0 -/81.2kb/: Deletion                            | ó         | i                | ō         | õ õ             | 0 no asserti no asser  |
| 17        | 4E+07 .            | с          | т         | 35 PASS            | BRCA1          | NM_007294.3                | c.+1332G>A  | 3 UTF            |            | 3PU                     |               |           |                    | 1496         | 159758           | 0.0094 .       | -/81.2kb/: Substituti                            | 1         | 0                | 0         | 0 0             | 0 reviewed reviewed  |
| 19        | 1E+07 .            | TTA        | т         | 35 PASS            | LDLR           | NM_000527.4                | c.+2210_+2211deITA                                      | 3 UT             |            | 3PU                     |               |           |                    |              |                  |                | +/44.5kb/ Deletion                               | 0         | 1                | 0         | 0 0             | 0 criteria pr criteria j   |
| 2         | 2E+07 .            | c          | Ŷ.        | 35 PASS            | APOB<br>LDLR   | NM_000384.2                | c.409G>T_p.Glu137X                                      | 1 x5             |            | SG 34.                  | â             |           | 0.796 .            |              |                  | · . ·          | -/42.6kb/ Substituti                             | 0         | 0                | *         | 0 2             | 0 criteria pr criteria (   |
| 13        | 1E+07 .<br>2E+07 . | G          | 2         | 35 PASS<br>35 PASS | APOB           | NM_000527.4<br>NM_000384.2 | c.1359-1G>A<br>c.4503T>G_p.Tyr1501X                     | 1 59/1<br>1 (x26 |            | ESS 24.8.<br>SG 27.5.   |               |           | . 0.862            |              | 245718<br>246156 | 0<br>4E-06     | 0 +/44.5kb/ Substituti<br>0 -/42.6kb/ Substituti | 0         | 0                | 0         | 3 10            | <ol> <li>criteria pr criteria (<br/>0 criteria pr criteria)</li> </ol> |
| 1         | 6E+06 .            | Ĝ          | Ă         | 35 PASS            | PMS2           | NM_000535.6                | c.730C>T_p.Gln244X                                      | 1 1/27           |            | SG 43.                  | â             |           | 0.865              | · '.         | 240100           | 46-00          | -/38.2kb/ Substituti                             | ŏ         | ŏ                | ŏ         | 0 1             | 0 criteria pr criteria  <br>0 criteria pr criteria                     |
| ŕ         | 6E+06 .            | Ğ          | A         | 35 PASS            | PMS2           | NM_000535.6                | c.730C>T_p.Gin244X                                      | 1 x7             |            | SG 43.                  | Ä             |           | 0.865 .            |              |                  |                | -/38.2kb/ Substituti                             | ŏ         | õ                | *         | õ i             | 0 criteria pr criteria j   |
| 19        | 1E+07 .            | т          | G         | 35 PASS            | LDLR           | NM_000527.4                | c.1942T>G_p.Ser648Ala                                   | 2 x13            |            | NSY 21.2 D              | N             | N         | 0.401              |              | 277228           | 1E-05 .        | +/44.5kb/ Substituti                             | 0         | 0                | 0         | 1 0             | 0 criteria pr criteria   |
| 19        | 1E+07 .            | с          | т         | 35 PASS            | LDLR           | NM_000527.4                | c.1835C>T_p.Ala612Val                                   | 2 x12            |            | NSY 26.5 D              | D             | N         | 0.511              |              | 277194           | 7E-06 .        | +/44.5kb/ Substituti                             | 0         | 0                | *         | 0 1             | 0 no asserti no asser  |
| 19        | 1E+07 .            | ç          | Ţ         | 35 PASS            | LDLR           | NM_000527.4                | c.1835C>T_p.Ala612Val                                   | 2 x12            |            | NSY 26.5 D              | 2             | N         | 0.511              | 2            | 277194           | 7E-06 .        | +/44.5kb/ Substituti                             | 0         | 0                | 0         | 0 1             | 0 no asserti no asser  |
| 2         | 5E+07 .<br>6E+07 . | G          | Ŷ.        | 35 PASS<br>35 PASS | MSH6<br>PCSK9  | NM_000179.2                | c.3577G>A_p.Glu1193Lys<br>c.1180G>A_p.Gly394Ser         | 2 .xr            |            | NSY 33 D<br>EE 27.1 D   | D             | B         | 0.987 .            |              |                  | 0.0001         | +/23.3kbi Substituti                             | 0         | 0                | 0         | 2 0             | 0 criteria pr criteria (   |
|           | 5E+07 .            | T          | ĉ         | 35 PASS<br>35 PASS | MSH6           | NM_174936.3<br>NM_000179.2 | c.1109G2A_p.Giy334Ser<br>c.1109T>C_p.Leu370Ser          | 2                |            | EE 27.1 D<br>NSY 25.1 D | Ď             | Ď         | 0.824              | 20           | 236966           | 0.0001         | 0 +/25.4kb/ Substituti<br>+/23.3kb: Substituti   | ő         | 0                | 2         | 3 1             | 0 criteria pr criteria<br>1 criteria pr criteria                       |
| 2         | 5E+07 .            | ċ          | Ť         | 35 PASS            | MSH6           | NM_000173.2                | c.1618C>T_p.Leu540Phe                                   | 2 14             |            | NSY 25 D                | Ď             | N         | 0.785              |              |                  |                | +/23.3kbi Substituti                             | ŏ         | ŏ                | 1         | õ õ             | 0 criteria pr criteria   |
| 1         | 6E+07 .            | č          | т         | 35 PASS            | PCSK9          | NM_174936.3                | c.1405C>T_p.Arg469Trp                                   | 2 x9             |            | NSY 22.4 T              | N             | N         | 0.709              | 240          | 277130           | 0.0009         | +/25.4kb/ Substituti                             | 1         | 3                | 3         | 0 1             | 1 criteria pr criteria   |
| 3         | 4E+07 .            | G          | A         | 35 PASS            | MLH1           | NM_000249.3                | c.1217G>A_p.Ser406Asn                                   | 2 x12            |            | NSY 13.46 T             | N             | N         | 0.31               | 249          | 276804           | 0.0009 .       | +/57.5kb/* Substituti                            | 6         | 4                | 1         | 0 0             | O reviewed no asser  |
| 3         | 4E+07 .            | G          | A         | 35 PASS            | MLH1           | NM_000249.3                | c.1217G>A_p.Ser406Asn                                   | 2 x12            |            | NSY 13.46 T             | N             | N         | 0.31               |              | 276804           | 0.0009 .       | +/57.5kb/* Substituti                            | 6         | 4                | 1         | 0 0             | O reviewed no asser  |
| 7         | 6E+06 .            | Ţ          | ç         | 35 PASS            | PMS2           | NM_000535.6                | c.52A>G_p.lle18Val                                      | 2 x2             |            | NSY 24.6 D              | D             | N         | 0.656              |              | 271244           | 0.0091 .       | -/38.2kb/ Substituti                             | 15        | 6                |           | 0 0             | 0 reviewed criteria  |
| 17        | 4E+07 .<br>4E+07 . | G          | <u>^</u>  | 35 PASS<br>35 PASS | BRCA1<br>MLH1  | NM_007294.3<br>NM_000249.3 | c86C>T<br>c.307-29C>A                                   | 3 UTF<br>3 \3/4  |            | SPU                     |               |           |                    | 113<br>14.97 | 154744<br>277100 | 0.0007 .       | -/81.2kb/: Substituti<br>+/57.5kb/* Substituti   | 1         | 0                |           | 0 0             | 0 criteria pr criteria j<br>0 criteria pr criteria j                   |
| 3<br>13   | 4E+07 .<br>3E+07 . | 6          | 2         | 35 PASS<br>35 PASS | MLH1<br>BRCA2  | NM_000249.3<br>NM_000059.3 | c.307-29C>A<br>c.8851G>A_p.Ala295fThr                   | 3 \3/4<br>2 \x22 |            | INT<br>NSY 26.9 T       | 'n            | N         | . 0.518            |              | 277100           | 0.0054 .       | +/57.5kb/ Substituti<br>+/84.2kb: Substituti     | 26        | 4                |           | 0 0             | O reviewed reviewe<br>O reviewed no asse                               |
| 3         | 4E+07 .            | Ă          | Ĝ         | 35 PASS            | MLHI           | NM_000249.3                | c.791-79A>G   | 3 19/1           |            | INT                     |               |           | 0.510              | 150          | 30350            | 0.0048         | 0 +/57.5kb/ Substituti                           | õ         | -                | ŏ         | ŏŏ              | 0 no asserti no asser  |
|           |                    |            |           | 35 PASS            | IDIR           | NM 000527.4                | c 1043G2A in Are350Gla                                  | 2 17             |            | NSY 22 D                | N             | N         | 0 176              |              | 245530           | 2F-05          | 0 +/44 Skb/ Substituti                           | ő         | ů.               | 0         |                 |  |
| 19        | 1E+07              | 14         | <u> </u>  | PA33               |                |                            |   |                  |            |                         |               |           |                    |              |                  |                |  |           |                  |           |                 | 0 no asserti no asser  |



| #VCF/CF \    | CF/POS VCF/ID      | VCF/REF VCF         | ALT VCF/QU/ VCF/       | FILT CAVA/ | CAVA/TRANSCR               | CAVA/CSN  | IMPACT : VA/L   | CAVA/C     | CADD_p_MetaSVI                          | Mutation PROVE | A VEST4_6 BI  | OR::GN BIOR::G                        | N BIOR::GN BIO       | R::GN CAVA/TF CAVA/T*   | SIOR::CL BIOR::CL | BIOR::CL BIOR::C | L BIOR::CL BIOR | RECL & ORECI               | CL BIOR::CI                    |
|--------------|--------------------|---------------------|------------------------|------------|----------------------------|---|-----------------|------------|---|----------------|---------------|---------------------------------------|----------------------|---|-------------------|------------------|-----------------|----------------------------|--------------------------------|
| 1            | 6E+07 .            | A ACT               |                        |            | NM_174936.3                | c.63_65dupGCT_p.Leu23dup                        | 2 (x1           | IF         |   |                |               |                                       |                      | +/25.4kb/ Insertion   | 72                | 0                | 0 0             |                            | pr no assert                   |
| 2            | 5E+07 .            | G A                 | 35 PASS                |            | NM_000179.2                | c.3556+146G>A                                   | 3 6/7           | INT        | ·                                       | 1 L            | · ·           | ·                                     |                      | +/23.9kb: Substituti  | 0 0               | 0                | 0 0             | 0 reviewed                 |                                |
| 2            | 5E+07 .            | Ç G                 | 35 PASS                |            | NM_000179.2                | c.1186C>G_p.Leu396Val                           | 2 .x4           | NSY        | 21.7 T                                  | D N            | 0.32          | 1556 27693                            |                      | +/23.9kb, Substituti  | 16 2              | 0                | 0 0             | 0 reviewed                 |                                |
| 13           | 3E+07 .<br>3E+07 . | т а,та<br>т а,та    | 35 PASS<br>35 PASS     |            | NM_000053.3<br>NM_000053.3 | c.68-4dupA<br>c.68-4dupA                        | 3 12/3          | SS<br>SS   | · ·                                     |                |               | 787 27465<br>787 27465                |                      | +/84.2kbi Insertion<br>+/84.2kbi Insertion  | 9 12              | 3                |                 | 0 reviewed<br>0 reviewed   |                                |
| 13           | 3E+07 .<br>1E+07 . | A G                 | 35 PASS<br>35 PASS     |            | NM_000527.4                | c.05-4dupA<br>c.1510A>G_p.Lvs504Glu             | 2 x10           | SS<br>NSY  | <br>21.8 T                              | <br>D N        | 0.347         | 5 24625                               |                      | •/64.2kb/ Insertion<br>0 •/44.5kb/ Substituti                                     | 3 12              | 3                | 0 0             |                            | pr criteria p                  |
| 2            | 2E+07 .            | ĉ                   | 35 PASS                |            | NM_000384.2                | c.5066G>A_p.Arg1689His                          | 2 x26           | NSY        | 25.9 T                                  |                | 0.718         | 396 27690                             |                      | -/42.6kb/ Substituti  | 0 3               | ŝ                | n n             | 1 citeria p                |                                |
| 7            | 6E+06 .            | Ť Ċ                 | 35 PASS                |            | NM_000535.6                | c.2324A>G_p.Ash775Ser                           | 2 x14           | NSY        | 23.2 T                                  | 0 0            | 0.391         | 88 27505                              |                      | -/38.2kb/ Substituti  | 14 1              | 1                | ňů              | 0 r viewed                 |                                |
| 17           | 4E+07 .            | Ă Č                 | 35 PASS                |            | NM_007294.3                | c.736T>G_p.Leu246Val                            | 2 x10           | NSY        | 11.52 D                                 | D N            | 0.358         | 82 27604                              |                      | -/81.2kb/; Substituti   | 3 6               | 5                | ō õ             | 0 reviewed                 |                                |
| 19           | 1E+07 .            | G A                 | 35 PASS                |            | NM_000527.4                | c.313+1G>A                                      | 1 \3/4          | ESS        | 24.8 .                                  | D .            |               | 7 24620                               | 8 3E-05              | 0 +/44.5kb/ Substituti  | 0 1               | 0                | 3 16            |                            | pr criteria p                  |
| 7            | 6E+06 .            | T A                 | 35 PASS                |            | NM_000535.6                | c.251-2A>T                                      | 1 \3/4          | ESS        | 27.                                     | D.             |               | 1 23316                               | 4 4E-06              | 0 -/38.2kb/ Substituti  | 0 0               | 0                | 2 8             | 1 c teria p                | pr criteria p                  |
| 3            | 4E+07 .            | AG CC               | 35 PASS                |            | NM_000249.3                | c.1410-2_1410-1delinsCC                         | 1 12/13         | ESS        |   |                |               |                                       |                      | +/57.5kb/* Complex  | 0 0               | 0                | 1 1             |                            | pr criteria p                  |
| 3            | 4E+07 .            | AG CC               | 35 PASS                |            | NM_000249.3                | c.1410-2_1410-1delinsCC                         | 1 12/13         | ESS        |   |                |               |                                       |                      | +/57.5kb/* Complex  | 0 0               | 0                | 1 1             | 1 c <mark>i</mark> teria p | pr criteria p                  |
| 2            | 5E+07 .            | A AAC               | 35 PASS                |            | NM_000173.2                | c.843_844insAC                                  | 1 .x4           | FS         | · ·                                     |                |               | 1 24584                               | 4 4E-06              | 0 +/23.3kbs Insertion   | 0 0               | 0                | 0 0             | 0.                         |                                |
| , i          | 6E+06 .<br>3E+07 . | CTT C<br>AGCAAC ATG | 35 PASS<br>CTG 35 PASS |            | NM_000535.6<br>NM_000059.3 | c.1312_1313delAA<br>c.6024_6035delinsTGCTGTT+   | 1 x11           | FS<br>FS   |   |                |               |                                       |                      | -/38.2kb/ Deletion<br>+/84.2kb, Complex   | 0 0               |                  |                 | 0.                         |                                |
| 13           | 3E+07 .            | AGUARD ATG          | 35 PASS<br>35 PASS     |            | NM_000384.2                | c.409G>T_p.Glu137X                              | 1 211           | SG         | 34                                      |                | 0.796         | •                                     |                      | +/84.2kbi Complex<br>-/42.6kb/ Substituti   | 0 0               |                  |                 | 0.                         | pr criteria p                  |
| 2            | 2E+07 .            | č î                 | 35 PASS                |            | NM_000384.2                | c.403G>T_p.Glu137X                              | 1 1 1 25        | SG         | 34 .                                    | <u>.</u>       | 0.796         |                                       |                      | -/42.6kb/ Substituti  | 0 0               |                  | , <u> </u>      |                            | pr criteria p                  |
| 2            | 2E+07 .            | čä                  | 35 PASS                |            | NM_000384                  |   | : 102           | SG         | 34                                      | Ä .            | 0.796         |                                       |                      | -/42.6kb/ Substituti  | ŏŏ                | ŏ                | 0 2             | 0 c teria p                |                                |
| 19           | 1E+07 .            | Č Á                 | 35 PASS                |            | NM_000527                  |   |                 | SG         | 42 .                                    | D .            | 0.71 .        |                                       |                      | +/44.5kb/ Substituti  | ō ō               | ō                | 1 2             |                            | pr criteria p                  |
| 1            | 6E+07 .            | G GGA               | GGA 35 PASS            | PCSK3      | NM_174936                  |   |                 | IF         |   |                |               | 19 17514                              |                      | 0 +/25.4kb/ Insertion   | <u> </u>          |                  |                 | 0 c teria p                | pr criteria p                  |
| 2            | 2E+07 .            | CTCA C              | 35 PASS                |            | NM_000384                  | Mutation +                                      | vna 6           | IF         |   |                |               | 1266 24507                            |                      | -/42.6kb/ Deletion  |                   |                  |                 |                            | pr criteria p                  |
| 2            | 2E+07 .            | CTCA C              | 35 PASS                |            | NM_000384                  | Mutation t                                      | vue 🛯           | IF         | ·                                       | L L            |               | 1266 24507                            |                      | -/42.6kb/ Deletion  |                   | Allel            | n               |                            | pr criteria p                  |
| 7            | 6E+06 .            | A G                 | 35 PASS                |            |                            |   | •••             | 104        | 22.7 D                                  | D N            | 0.953         | 1 24532                               | 2 4E-06              | 0 -/38.2kb/ Substituti  |                   | Allel            | 3               | 1 c teria p                | pr criteria p                  |
| 17           | 4E+07 .<br>4E+07 . | G GCC<br>Π T        | T 35 PASS<br>35 PASS   |            | NM_007294<br>NM_007294     | and locati                                      | on I            | IM<br>EC   | · ·                                     | · ·            |               | •                                     |                      | -/81.2kb/; Insertion<br>-/81.2kb/; Deletion                                       |                   |                  |                 | 1 ·                        |                                |
| 17           | 4E+07 .            | c G                 | 35 PASS                |            | NM_007294                  | anu iocali                                      | UII ,           | 51         | . 11 18                                 | <br>M          | 0.431         |                                       |                      | -/81.2kb/: Substituti   | fı                | requei           | ากบ             | K 1                        |                                |
| 13           | 3E+07 .            | A AT                | 35 PASS                |            | NM_000053                  |   | 7               | FS         | 1.10                                    |                | 0.401         |                                       |                      | +/84.2kbs Insertion   |                   | equei            | юу              | K 1                        |                                |
| 7            | 6E+06 .            | CTGA C              | 35 PASS                |            | NM_000535                  |   | ŝ               | IF         |   |                |               |                                       |                      | -/38.2kb/ Deletion  |                   | -                | -               | _60                        |                                |
| 2            | 5E+07 .            | A G                 | 35 PASS                |            | NM_000173.2                | coolectrica_p.argizitialy                       | 2 120           | EE         | 33 D                                    | D D            | 0.937         | 1 3097                                | 8 3E-05              | 0 +/23.3kb, Substituti  | 0 0               | 5 1              | 0 0             | 0 c teria p                | pr criteria p                  |
| 2            | 5E+07 .            | A G                 | 35 PASS                | MSH6       | NM_000179.2                | :.3649A>G_p.Arg1217Gly                          | 2 x8            | EE         | 33 D                                    | D D            | 0.937         | 1 3097                                | 8 3E-05              | 0 +/23.9kb: Substituti  | 0 0               | 5                | 0 0             | 0 c teria p                |                                |
| 19           | 1E+07 .            | с т                 | 35 PASS                |            | NM_000527.4                | c.2388C>T_p.                                    | 2 x16           | EE         | ·                                       |                |               | 11 27717                              | 0 4E-05.             | +/44.5kb/ Substituti  | 0 2               | 1 1              | 0 0             | 1 c <mark>teria</mark> p   | pr criteria p                  |
| 2            | 5E+07 .            | G GGG               |                        |            | NM_000179.2                | c.3802_3803insGGG_p.Met1;                       | 2 x9            | EE         | × .                                     |                |               |                                       |                      | +/23.9kbi Insertion   | 0 0               | 0                | 0 0             | 0.                         |                                |
| 2            | 5E+07 .            | TTGG T              | 35 PASS                |            | NM_000179.2                | c.3170_3172delTGG_p.Leu105"                     |                 | EE         | · I                                     | n silico       | <u>^</u>      | · · · · · · · · · · · · · · · · · · · |                      | +/23.3kbs Deletion  | 0 0               | 0                | 0 0             | 0.                         | ·                              |
| 19           | 1E+07 .            | с т<br>т с          | 35 PASS                |            | NM_000527.4                | c.1920C>T_p.                                    | 3 x13<br>3 x7/8 | SY         | 2 I I I I I I I I I I I I I I I I I I I | II SIIICO      | 0             | 1179 27724<br>1525 27684              |                      | +/44.5kb/ Substituti  | 5 2               | 2                | 0 0             |                            | pr criteria p                  |
| 13           | 1E+07 .<br>5E+07 . | A AT.A              | 35 PASS                | LUCK       | NM_000527.4<br>NM_000179.2 | c.1061-8T>C<br>c.3557-5_3557-4dupTT             | 3 6/7           | SS<br>SS   | 1                                       | •• •           |               | 1525 21604                            | 4 0.0055 .           | +/44.5kb/ Substituti<br>+/23.9kbi Insertion                                       | 12 2              | ŏ                |                 | 0 reviewed                 | pr criteria p                  |
| 2            | 5E+07 .            | AT A                | "                      |            | NM_000179.2                | c.3557-4deff                                    | 3 6/7           | \$\$       | ' nr                                    | edictio        | ne            |                                       |                      | +/23.3kbi Deletion  | 2 2               | ő                | n n             |                            | pr criteria p                  |
| 19           | 1E+07 .            | с т                 | <b>•</b> •••           |            | NM_000527.4                | c.2389+57C>T                                    | 3 16/17         | INT        |   | Suictio        | 113           | 189 3095                              | 4 0.0061             | 1 +/44.5kb/ Substituti  | 0 1               | ŏ                | ŏ ŏ             | 0 c teria p                |                                |
| 17           | 4E+07 .            | Č ČAC               | 🗚 Ger                  | ne         | NM_007294.3                | c.5194-2337_5194-2335dupT(                      |                 | INT        |   |                |               |                                       |                      | -/81.2kb/: Insertion  | 1 0               | ō i              | ō ō             | 0 reviewed                 |                                |
| 2            | 5E+07 .            | CTT C,CT            |                        |            | NM_000179.2                | c.4002-10delT                                   | 3 3/10          | INT        |   |                |               |                                       |                      | +/23.9kb+ Deletion  | 0 0               | 1                | 0 0             | 0 c teria p                |                                |
| 17           | 4E+07 .            | G A                 |                        |            | NM_007294.3                | c20+11C>T                                       | 3 1/2           | 5PU        |   |                |               | 69 15671                              | 8 0.0004 .           | -/81.2kb/: Substituti   | 2 2               | 1 0              | 0 0             |                            | pr no assert                   |
| 17           | 4E+07 .            | C CAT               | 35 PASS                |            | NM_007294.3                | c19-2219-21dupAT                                | 3 1/2           | 5PU        |   |                |               |                                       | · · ·                | -/81.2kb/: Insertion  | 1 1               | 0                | 0 0             | 0 c teris p                |                                |
| 17           | 4E+07 .            | GITTITI G           | 35 PASS                |            | NM_007294.3                | c20+52120+525del5                               | 3 1/2           | SPU        | · ·                                     | · ·            |               | 35 3031                               |                      | 0 -/81.2kb/; Deletion   | 0 1               | 0                | 0 0             | 0 n asser                  |                                |
| 17           | 4E+07 .<br>1E+07 . | с т<br>ПА Т         | 35 PASS<br>35 PASS     |            | NM_007294.3<br>NM_000527.4 | c.+1332G>A                                      | 3 UTR<br>3 UTR  | 3PU<br>3PU |   |                |               | 1496 15975                            | 8 0.0094 .           | -/81.2kb/: Substituti<br>+/44.5kb/ Deletion                                       | 1 0               | 0                |                 | 0 reviewed                 |                                |
| 19           | 1E+07 .<br>2E+07 . | 0 6                 | 35 PASS<br>35 PASS     |            | NM_000384.2                | c.+2210_+2211deITA<br>c.409G>T_p.Glu137X        | 3 UIR<br>1 X5   | SG         | 34                                      | A .            | 0,796         |                                       | · ·                  | +/44.5kb/ Deletion<br>-/42.6kb/ Substituti  | 0 0               | 0                | , u             | 0 citeria p<br>0 citeria p | pr criteria p<br>pr criteria p |
| 19           | 1E+07 .            | ĞÂ                  | 35 PASS                |            | NM_000527.4                | c.1359-1G>A                                     | 1 9/10          | ESS        | 24.8                                    | D .            | 0.100         | 0 24571                               | s' 0'                | 0 +/44.5kb/ Substituti  | ŏ ŏ               | ŏ                | 3 10            |                            | pr criteria p                  |
| 2            | 2E+07 .            | A C                 | 35 PASS                | APOB       | NM_000384.2                | c.4503T>G_p.Tyr1501X                            | 1 x26           | SG         | 27.5 .                                  | Α .            | 0.862         | 1 24615                               |                      | 0 -/42.6kb/ Substituti  | ō ō               | ō                | 0 1             | 0 c teris p                |                                |
| 7            | 6E+06 .            | G A                 | 35 PASS                | PMS2       | NM_000535.6                | c.730C>T_p.Gin244X                              | 1 x7            | SG         | 43 .                                    | Α.             | 0.865 .       |                                       |                      | -/38.2kb/ Substituti  | 0 0               | 0                | D 1             | 0 c teria p                | pr criteria p                  |
| 7            | 6E+06 .            | G A                 | 35 PASS                |            | NM_000535.6                | c.730C>T_p.Gln244X                              | 1 .x7           | SG         | 43 .                                    | Α.             | 0.865 .       |                                       |                      | -/38.2kb/ Substituti  | 0 0               | 0                | D 1             | 0 c <mark>teria</mark> p   |                                |
| 19           | 1E+07 .            | T G                 | 35 PASS                |            | NM_000527.4                | c.1942T>G_p.Ser648Ala                           | 2 x13           | NSY        | 21.2 D                                  | N N            | 0.401         | 4 27722                               |                      | +/44.5kb/ Substituti  | 0 0               | 0                | 1 0             | 0 c teria p                |                                |
| 19           | 1E+07 .            | C T                 | 35 PASS                |            | NM_000527.4                | c.1835C>T_p.Ala612Val                           | 2 (x12          | NSY        | 26.5 D                                  | D N            | 0.511         | 2 27719                               |                      | +/44.5kb/ Substituti  | 0 0               | 0                | 1               | 0 n asser                  |                                |
| 19           | 1E+07 .            | C T                 | 35 PASS<br>35 PASS     |            | NM_000527.4                | c.1835C>T_p.Ala612Val                           | 2 x12<br>2 x7   | NSY<br>NSY | 26.5 D<br>33 D                          |                | 0.511 0.387 . | 2 27719                               | 4 7E-06 .            | +/44.5kb/ Substituti  | 0 0               | 0                | 0 1             | 0 n asser                  |                                |
| 2            | 5E+07 .<br>6E+07 . | G A                 | 35 PASS<br>35 PASS     |            | NM_000179.2<br>NM_174936.3 | c.3577G>A_p.Glu1193Lys<br>c.1180G>A_p.Gly394Ser | 2 1 1           | EE         | 27.1 D                                  |                | 0.824         | 26 23696                              | 6 0.0001             | <ul> <li>+/23.9kbi Substituti</li> <li>0</li> <li>+/25.4kb/ Substituti</li> </ul> | 0 0               |                  | 2 0             | 0 ceteriap<br>0 ceteriap   |                                |
| 2            | 5E+07 .            | ŤĈ                  | 35 PASS                |            | NM_000179.2                | c.1109T>C_p.Leu370Ser                           | 2 14            | NSY        | 25.1 D                                  |                | 0.669 .       | 20 20000                              | 0.0001               | +/23.3kbi Substituti  | 0 0               |                  | 3 1             | 1 citeria p                |                                |
| 2            | 5E+07 .            | ć Ť                 | 35 PASS                |            | NM_000173.2                | c.1618C>T_p.Leu540Phe                           | 2 14            | NSY        | 25 D                                    | D N            | 0.785         |                                       |                      | +/23.3kbi Substituti  | õ õ               | 1                | ň n             | 0 c teria p                |                                |
| 1            | 6E+07 .            | ć Ť                 | 35 PASS                |            | NM_174936.3                | c.1405C>T_p.Arg469Trp                           | 2 x9            | NSY        | 22.4 T                                  | N N            | 0.709         | 240 27713                             | 0.0009               | +/25.4kb/ Substituti  | 1 3               | 3                | 0 1             |                            | pr criteria p                  |
| 3            | 4E+07 .            | G Á                 | 35 PASS                |            | NM_000249.3                | c.1217G>A_p.Ser406Asn                           | 2 x12           | NSY        | 13.46 T                                 | N N            | 0.31          | 249 27680                             |                      | +/57.5kb/* Substituti   | 6 4               | 1                | o o             | 0 r viewed                 |                                |
| 3            | 4E+07 .            | G A                 | 35 PASS                | MLH1       | NM_000249.3                | c.1217G>A_p.Ser406Asn                           | 2 (x12          | NSY        | 13.46 T                                 | N N            | 0.31          | 249 27680                             | 4 0.0003 .           | +/57.5kb/* Substituti   | 6 4               | 1                | 0 0             | 0 reviewed                 | d no assert                    |
| 7            | 6E+06 .            | т с                 | 35 PASS                |            | NM_000535.6                | c.52A>G_p.llc18Val                              | 2 X2            | NSY        | 24.6 D                                  | D N            | 0.656         | 2457 27124                            |                      | -/38.2kb/ Substituti  | 15 6              | 1                | 0 0             | 0 reviewed                 |                                |
| 17           | 4E+07 .            | G A                 | 35 PASS                |            | NM_007294.3                | c86C>T  | 3 UTR           | SPU        |   |                |               | 113 15474                             |                      | -/81.2kb/: Substituti   | 1 0               | 3                | 0 0             | 0 c teria p                |                                |
|              |                    |                     | 35 PASS                | MLH1       | NM_000249.3                | <.307-29C>A                                     | 3 3/4           | INT        |   |                |               | 1497 27710                            | 0 0.0054 .           | +/57.5kb/* Substituti   | 0 4               | 0 1              | 1 0             | 0 reviewed                 | d reviewed                     |
| 3            | 4E+07 .            | C A                 |                        |            |                            |   |                 |            | ·                                       |                |               |                                       |                      |   |                   | ě.               | Š               | 0 1                        | 4                              |
| 3            | 3E+07 .            |                     | 35 PASS                | BRCA2      | NM_000059.3                | c.8851G>A_p.Ala2951Thr                          | 2 x22           | NSY        | 26.9 T                                  | D N            | 0.518         | 2486 27654                            | 4 0.009 .            | +/84.2kb: Substituti  | 26 4              | ŏ                | ŏŏ              | 0 reviewed                 |                                |
| 3<br>13<br>3 |                    |                     |                        | BRCA2      |                            |   |                 |            | 26.9 T                                  |                | 0.518         |                                       | 4 0.009.<br>0 0.0048 |   | 26 4<br>0 0       | 0                |                 | 0 n <mark>o</mark> assert  |                                |

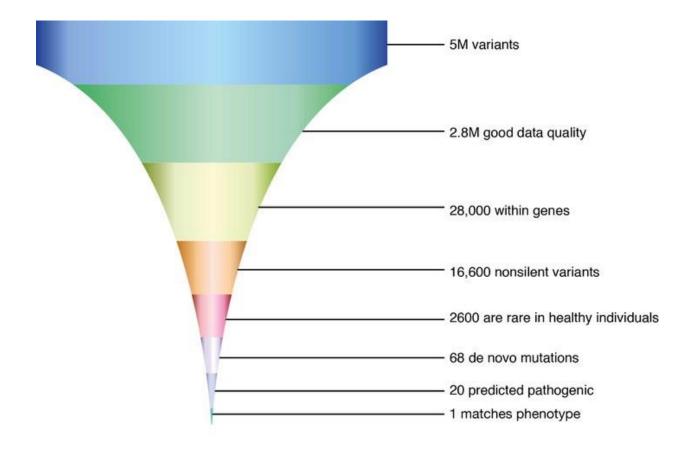


## Variant filtering: criteria

- Genes list
- Sequencing parameters (filter artifactuals)
- Variant types (exonic intronic;
- Variant class (frameshift synonymous)
- Population frequency
- Pathogenicity prediction score
- Conservation score
- Clinical interpretation of genetic variants by the ACMG/AMP 2015 guideline
- Presence in mutation databases

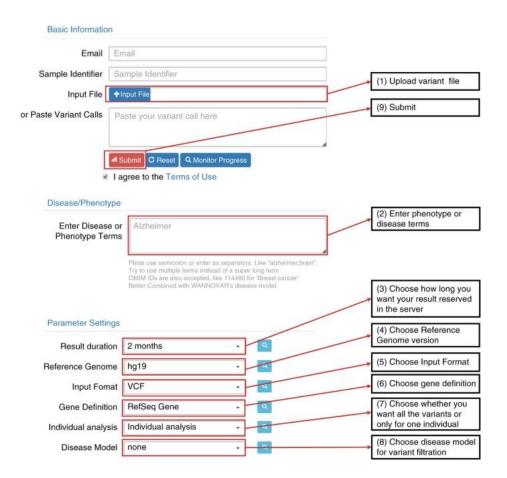


#### Variant filtering: criteria





#### wANNOVAR (http://wannovar.usc.edu)



wANNOVAR implemented a:

## Variant-reduction pipeline based on commonly used filters and disease models such as selecting:

- only the nonsynonymous variants and splicing variants
- rare or novel variants in the 1000 Genomes Project database
- predicted deleterious variants.

#### Phenotype-based variant prioritization

sample's specific phenotype or disease information is available and may help identify causal variants.



#### **wANNOVAR** limitations

- Default 'variant-reduction' schemes (disease models) may not be optimal for the **specific use case** (e.g. eliminate true causal variants in some scenarios during the filtering procedure)
- **complex diseases**: rule-based 'hard' filtering versus a probabilistic prioritization approach
- Originally developed as annotation tools for genetic variants from a single genome, with limited functionality on analyzing multiple genomes (not support case-control association analysis, or family-based association analysis)
- ANNOVAR provides several different types of **deleteriousness-prediction scores**, and it leaves the choice of selecting annotations to users



## Variant filtering: criteria

- Genes list
- Sequencing parameters (filter artifactuals)
- Variant types (exonic intronic;
- Variant class (frameshift synonymous)
- Population frequency
- Pathogenicity prediction score
- Conservation score
- Clinical interpretation of genetic variants by the ACMG/AMP 2015 guideline
- Presence in mutation databases

(PMID: 28118812; https://www.nature.com/articles/s41525-021-00227-3)



#### Variant filtering: identify variants in specific genomic regions

- Gene panel disease-oriented
- Identity-by-descent (IBD), the detection of shared segments inherited from a common ancestor
- Linkage analysis
- Exome capture regions, capture array manufacturers will provide the regions in BED file



## Browser Extensible Data (BED) format

- Provides a flexible way to define the data lines that are displayed in an annotation track. These are generally used for user defined sequence features as well as **graphical representations of features**.
- BED lines have three required fields and nine additional optional fields.
- The first three required **BED fields** are:
  - 1. chrom The name of the chromosome
  - 2. chromStart The starting position of the feature in the chromosome or scaffold. The first base in a chromosome is numbered 0.
  - **3. chromEnd** The ending position of the feature in the chromosome

| chr1 | 213941196 | 213942363 |
|------|-----------|-----------|
| chr1 | 213942363 | 213943530 |
| chr1 | 213943530 | 213944697 |
| chr2 | 158364697 | 158365864 |
| chr2 | 158365864 | 158367031 |
| chr3 | 127477031 | 127478198 |
| chr3 | 127478198 | 127479365 |
| chr3 | 127479365 | 127480532 |
| chr3 | 127480532 | 127481699 |
|      |           |           |



## Browser Extensible Data (BED) format

#### • What software uses bed files?

- Alignment viewers can use these data to graphically display certain features.
- bedtools uses this format to query for nearby features.
- Some annotation files are in this format.
- Feature detection packages use this as output.
- How are these files generated?
- Feature detection algorithms.
- Lots of databases that hold certain genomic features report their data in this format.
- Sometimes manually curated from alignments (via bedtools, bamtools, etc.).



#### Variant filtering: identify variants in specific genomic regions

- Users can supply your own region annotation databases in generic, BED or GFF formats.
- Region-based annotation looks for overlap of a query variant with a region (this region could be a single position) in a database, and it does not care about exact match of positions, and it does not care about nucleotide identity at all.
- Users can select annotation tracks that are already provided by the UCSC Genome Browser annotation databases
- Annotate variants against GFF3-formatted annotation databases, using the regionbased annotation procedure



#### General Feature Format (GFF) / General Transfer Format (GTF)

- GFF or GTF are a tab-delimited text file that holds information any and every feature that can be applied to a nucleic acid or protein sequence.
- Everything from CDS, microRNAs, binding domains, ORFs, and more can be handled by this format.
- many variations of the original GFF format, latest accepted format (GFF3)

Sample GTF output from Ensembl data dump:

| 1 transcribed_unprocessed_pseudogene<br>1 processed_transcript |  | "ENSG00000223972";<br>"ENSG00000223972"; |  |  |   |
|--|--|--|--|--|---|
| 4  |  |  |  |  | Þ |

Sample GFF output from Ensembl export:

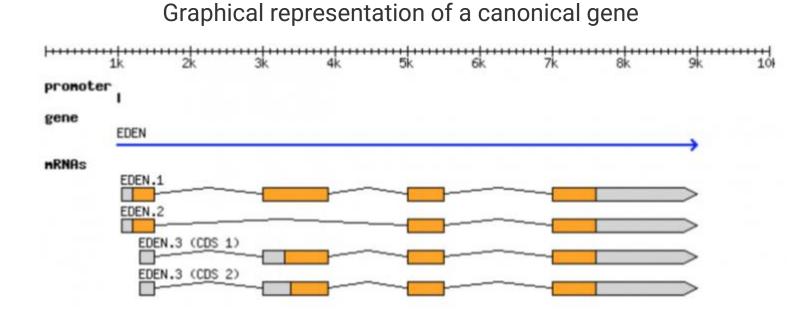
| х | Ensembl Repea | t 2419108 | 2419128 | 42      |        |   | hid=trf; hstart=1; hend=21             |
|---|---------------|-----------|---------|---------|--------|---|--|
| Х | Ensembl Repea | t 2419108 | 2419410 | 2502    | -      |   | hid=AluSx; hstart=1; hend=303          |
| Х | Ensembl Repea | t 2419108 | 2419128 | 0       |        |   | hid=dust; hstart=2419108; hend=2419128 |
| Х | Ensembl Pred. | trans.    | 2416676 | 2418760 | 450.19 | - | 2 genscan=GENSCAN0000019335            |
| Х | Ensembl Varia | tion      | 2413425 | 2413425 |        | + |  |
| Х | Ensembl Varia | tion      | 2413805 | 2413805 |        | + |  |

#### GFF3 has 9 required fields

- 1. Sequence ID
- 2. **Source** (Describes the algorithm that generated this feature, e.g Genescane or Genebank).
- 3. Feature Type (Describes what the feature is: mRNA, domain, exon, etc.)
- 4. Feature Start
- 5. Feature End
- 6. **Score** (*Typically E-values for sequence similarity and P-values for predictions*).
- 7. Strand
- 8. Phase (Indicates where the feature begins with reference to the reading frame).
- 9. Attributes (A semicolon-separated list of tag-value pairs, providing additional information about each feature)



#### GFF3 file



The same information can be represented in GFF3 format



#### GFF3 file

| 0  | ##gff-version 3.2.1      |       |      |        |   |   |   |
|----|--------------------------|-------|------|--------|---|---|---|
| 1  | ##sequence-region ctg123 | 1 149 | 7228 |        |   |   |   |
| 2  | ctg123 . gene            | 1000  | 9000 |        | + |   | ID=gene00001;Name=EDEN                            |
| 3  | ctg123 . TF_binding_site | 1000  | 1012 |        | + |   | ID=tfbs00001;Parent=gene00001                     |
| 4  | ctg123 . mRNA            | 1050  | 9000 |        | + |   | ID=mRNA00001;Parent=gene00001;Name=EDEN.1         |
| 5  | ctg123 . mRNA            | 1050  | 9000 |        | + |   | ID=mRNA00002;Parent=gene00001;Name=EDEN.2         |
| 6  | ctg123 . mRNA            | 1300  | 9000 |        | + |   | ID=mRNA00003;Parent=gene00001;Name=EDEN.3         |
| 7  | ctg123 . exon            | 1300  | 1500 |        | + |   | ID=exon00001;Parent=mRNA00003                     |
| 8  | ctg123 . exon            | 1050  | 1500 |        | + |   | ID=exon00002;Parent=mRNA00001,mRNA00002           |
| 9  | ctg123 . exon            | 3000  | 3902 |        | + | • | ID=exon00003;Parent=mRNA00001,mRNA00003           |
| 10 | ctg123 . exon            | 5000  | 5500 |        | + |   | ID=exon00004;Parent=mRNA00001,mRNA00002,mRNA00003 |
| 11 | ctg123 . exon            | 7000  | 9000 |        | + |   | ID=exon00005;Parent=mRNA00001,mRNA00002,mRNA00003 |
| 12 | ctg123 . CDS             | 1201  | 1500 |        | + | 0 | ID=cds00001;Parent=mRNA00001;Name=edenprotein.1   |
| 13 | ctg123 . CDS             | 3000  | 3902 |        | + | 0 | ID=cds00001;Parent=mRNA00001;Name=edenprotein.1   |
| 14 | ctg123 . CDS             | 5000  | 5500 |        | + | 0 | ID=cds00001;Parent=mRNA00001;Name=edenprotein.1   |
| 15 | ctg123 . CDS             | 7000  | 7600 |        | + | 0 | ID=cds00001;Parent=mRNA00001;Name=edenprotein.1   |
| 16 | ctg123 . CDS             | 1201  | 1500 |        | + | 0 | ID=cds00002;Parent=mRNA00002;Name=edenprotein.2   |
| 17 | ctg123 . CDS             | 5000  | 5500 |        | + | 0 | ID=cds00002;Parent=mRNA00002;Name=edenprotein.2   |
| 18 | ctg123 . CDS             | 7000  | 7600 |        | + | 0 | ID=cds00002;Parent=mRNA00002;Name=edenprotein.2   |
| 19 | ctg123 . CDS             | 3301  | 3902 |        | + | 0 | ID=cds00003;Parent=mRNA00003;Name=edenprotein.3   |
| 20 | ctg123 . CDS             | 5000  | 5500 | 1      | + | 1 | ID=cds00003;Parent=mRNA00003;Name=edenprotein.3   |
| 21 | ctg123 . CDS             | 7000  | 7600 | $\sim$ | + | 1 | ID=cds00003;Parent=mRNA00003;Name=edenprotein.3   |
| 22 | ctg123 . CDS             | 3391  | 3902 |        | + | 0 | ID=cds00004;Parent=mRNA00003;Name=edenprotein.4   |
| 23 | ctg123 . CDS             | 5000  | 5500 |        | + | 1 | ID=cds00004;Parent=mRNA00003;Name=edenprotein.4   |
| 24 | ctg123 . CDS             | 7000  | 7600 |        | + | 1 | ID=cds00004;Parent=mRNA00003;Name=edenprotein.4   |
|    |                          |       |      |        |   |   |   |



#### GFF3 file

- What Software uses GFF3?
- Any tool that requires information about gene position for analysis such as:
  - Mapping RNA-seq such as Tophat, HTSeq, Genome Browsers like IGV
- How is this file generated?
- Feature identification software report motifs/features in this format.
- Almost all sequence annotation databases report in this format.



## Variant filtering: criteria

- Genes list
- Sequencing parameters (filter artifactuals)
- Variant types (exonic intronic;
- Variant class (frameshift synonymous)
- Population frequency
- Pathogenicity prediction score
- Conservation score
- Clinical interpretation of genetic variants by the ACMG/AMP 2015 guideline
- Presence in mutation databases



#### Variant filtering: sequencing parameters

NGS data are prone to artifactual variant calls due to e.g. short-read alignment

- Allele balance (the ratio of reads aligned at a variant locus that support the alternate allele) 0.2 0.8. More stringent threshold of 0.3–0.7 has a very high transmission rate, and an estimated false negative rate of ~1.41%
- Genotype quality (GQ) threshold: >20
- Read **Depth of Coverage** (DP): >10
- Presence of repetitive or polymeric sequences



## Variant filtering: criteria

- Genes list
- Sequencing parameters (filter artifactuals)
- Variant types (exonic intronic)
- Variant class (frameshift synonymous)
- Population frequency
- Pathogenicity prediction score
- Conservation score
- Clinical interpretation of genetic variants by the ACMG/AMP 2015 guideline
- Presence in mutation databases



## Types of mutations

- Point (missense, same sense, stop gain, ...)
- Frameshift
- Splicing
- Regulatory
- Insertion/deletion (small)
- Insertion/deletion (large)
- Repeats



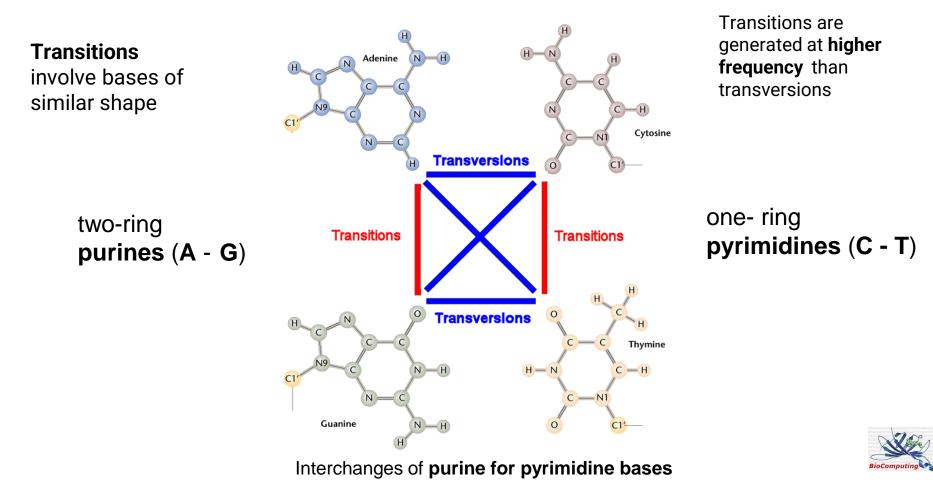


#### **Point mutations**

|              |   |                                 | Secon                    | d letter                               |                                 |                  |
|--------------|---|---------------------------------|--------------------------|--|---------------------------------|------------------|
|              |   | U                               | С                        | А                                      | G                               |                  |
|              | U | UUU<br>UUC<br>UUA<br>UUG<br>Leu | UCU<br>UCC<br>UCA<br>UCG | UAU<br>UAC Tyr<br>UAA Stop<br>UAG Stop | UGU<br>UGC<br>UGA<br>UGG<br>Trp | U<br>C<br>A<br>G |
| First letter | с | CUU<br>CUC<br>CUA<br>CUG        | CCU<br>CCC<br>CCA<br>CCG | CAU<br>CAC<br>CAA<br>CAA<br>CAG<br>Gin | CGU<br>CGC<br>CGA<br>CGG        |                  |
| First        | A | AUU<br>AUC<br>AUA<br>AUG Met    | ACU<br>ACC<br>ACA<br>ACG | AAU<br>AAC<br>AAA<br>AAG               | AGU<br>AGC<br>AGA<br>AGG<br>Arg | U C A G          |
|              | G | GUU<br>GUC<br>GUA<br>GUG        | GCU<br>GCC<br>GCA<br>GCG | GAU<br>GAC<br>Asp<br>GAA<br>GAG<br>Glu | GGU<br>GGC<br>GGA<br>GGG        | U<br>C<br>A<br>G |

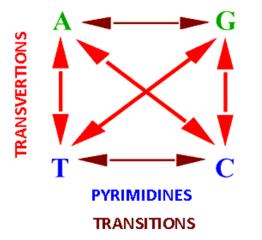


#### **Point mutations**



#### **Point mutations**

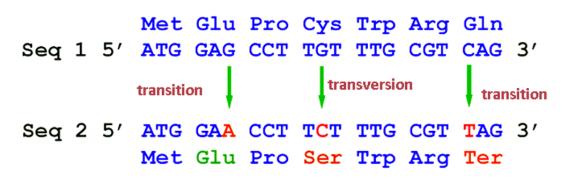
**PURINES** 



Point mutations are allowed due to the physicochemical properties of nucleobases. They can take the shape of several conformational states, facilitating alternative coupling to those discovered by Watson & Crick

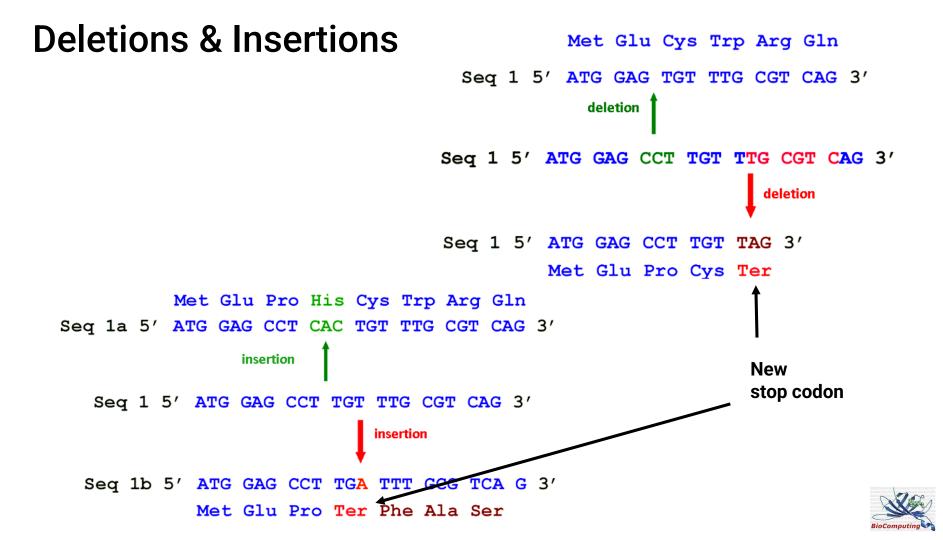


Dna nucleobases substitution events

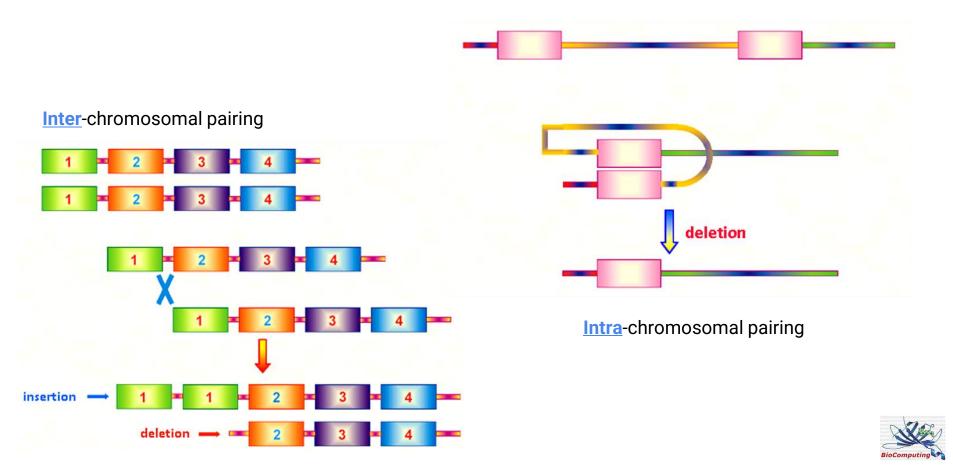


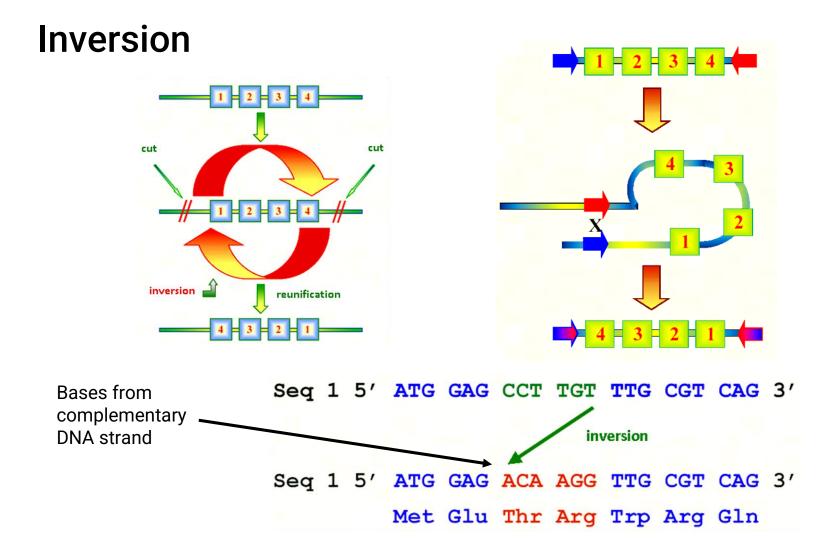
Transitions are less likely to result in amino acid substitutions, and are therefore more likely to persist as "*silent substitutions*" in populations as single nucleotide polymorphisms (SNPs).





### **Unequal Crossing over**







## **Types of mutations**

- 1. MSH2 NM\_000251.2 c.1590A>G\_p.Glu530=
- 2. BRCA1 NM\_007294.3 c.736T>G\_p.Leu246Val
- 3. BRCA2 NM\_000059.3 c.6474delT\_p.Gln2159AsnfsTer9
- 4. PMS2 NM\_000535.6 c.730C>T\_p.Gln244X
- 5. PCSK9 NM\_174936.3 c.524-1G>A
- 6. MLH1 NM\_000249.3 c.307-29C>A



#### Sequence variant nomenclature

#### https://varnomen.hgvs.org/



Human Mutation

www.hovs.org

Special Article

#### HGVS Recommendations for the Description of Sequence Variants: 2016 Update Hum Mutat (2016) 37:564-569

Johan T. den Dunnen,<sup>1</sup>\* Raymond Dalgleish,<sup>2</sup> Donna R. Maglott,<sup>3</sup> Reece K. Hart,<sup>4</sup> Marc S. Greenblatt,<sup>5</sup> Jean McGowan-Jordan,<sup>6</sup> Anne-Francoise Roux,<sup>7</sup> Timothy Smith,<sup>8</sup> Stylianos E. Antonarakis,<sup>9</sup> and Peter E.M. Taschner<sup>10</sup> on behalf of the Human Genome Variation Society (HGVS), the Human Variome Project (HVP), and the Human Genome Organisation (HUGO) "mutation" > disease-associated variant "polymorphism" > not disease-associated "pathogenic" > disease-associated

#### http://www.hgvs.org/varnomen/HGVS-basics2017.pdf



### Sequence variant nomenclature

- DNA A, G, C, T g.957A>T, c.63-3T>C
- RNA a, g, c, u r.957a>u, r.(?), r.spl?
- protein (mostly deduced) three / one letter amino acid code \* = stop codon p.(His78Gln)
- use official HGNC gene symbols
- provide reference sequence covering complete sequence largest transcript preferably a LRG e.g. LRG-123 give accession.version number e.g. NM 012654.3
- indicate type of Reference Sequence:
  - coding DNA c.
  - genomic g.
  - o mitochondrial m.
  - non-coding RNA n.
  - $\circ \quad \mathsf{RNA} \ \mathsf{r}.$
  - $\circ$  protein p.



## Mutalyzer: https://mutalyzer.nl/

Bioinformatics, 37(18), 2021, 2811–2817 doi: 10.1093/bioinformatics/btab051 Advance Access Publication Date: 4 February 2021 Original Paper Mutalyzer 2 tool suite is designed to automatically apply the HGVS guidelines

Sequence analysis

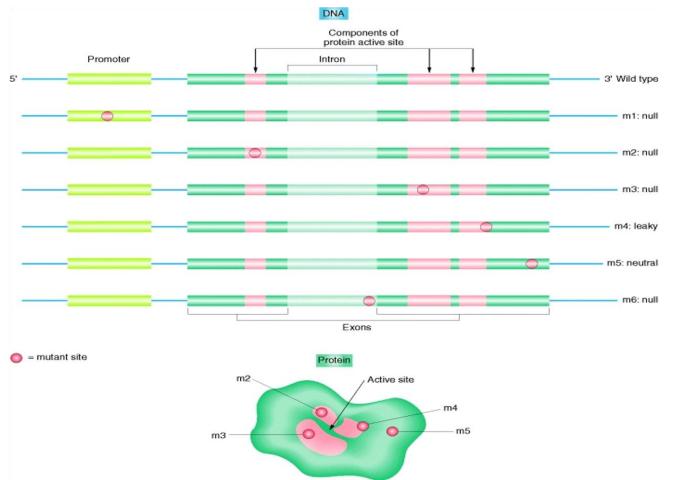
## Mutalyzer 2: next generation HGVS nomenclature checker

```
Mihai Lefter ()<sup>1,*</sup>, Jonathan K. Vis<sup>1,2</sup>, Martijn Vermaat<sup>1</sup>, Johan T. den Dunnen<sup>1,2</sup>, Peter E. M. Taschner<sup>1,3</sup> and Jeroen F. J. Laros ()<sup>1,2,4</sup>
```

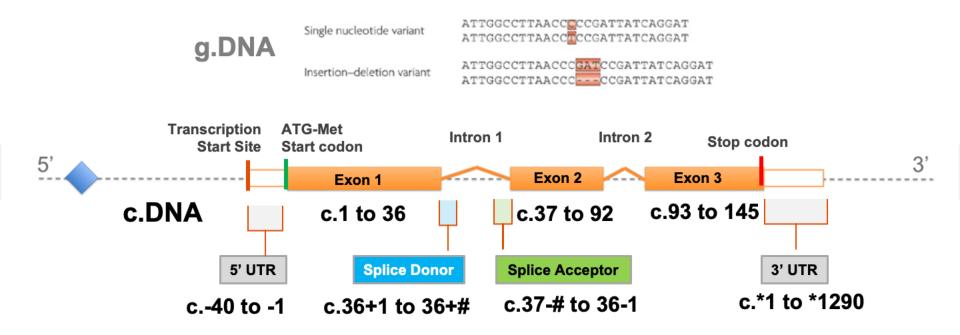
The source code is available on GitHub (<u>https://github.com/mutalyzer/mutalyzer</u>)

- Name Checker / Normalizer, provides checking and disambiguation of variant descriptions
- Position Converter, which converts descriptions between chromosomal and transcript references
- Description Extractor, generates HGVS variant descriptions given a reference sequence and an observed sequence
- *Mapper*, maps a description to another reference.
- Batch Processor, can be used to process up to 50 descriptions with the Normalizer



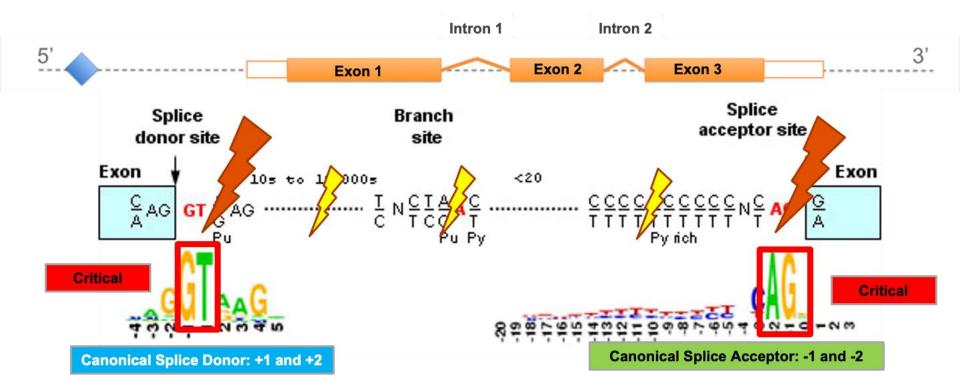




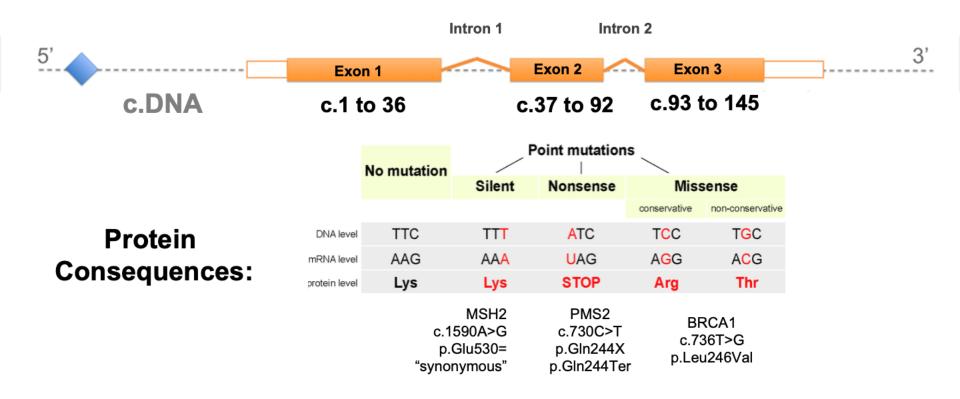


MLH1 NM\_000249.3 c.307-29C>A



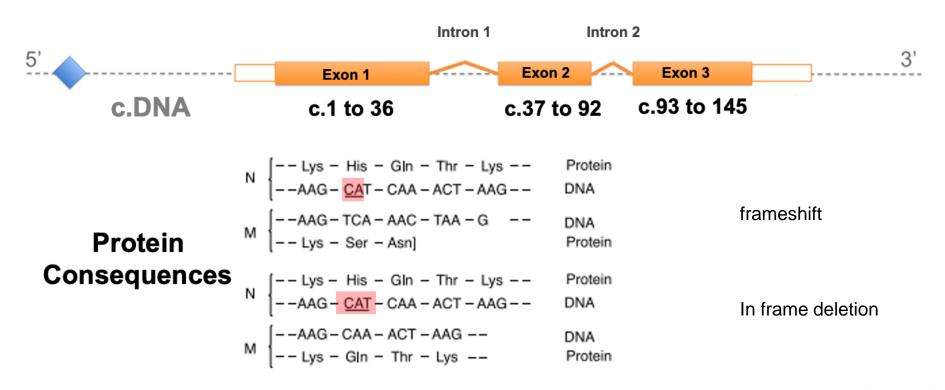






**The functional impact** of an SNP is depends on its **location** (i.e., coding or non-coding region) and **effects** to the related **protein sequence** (i.e., synonymous or nonsynonymous).

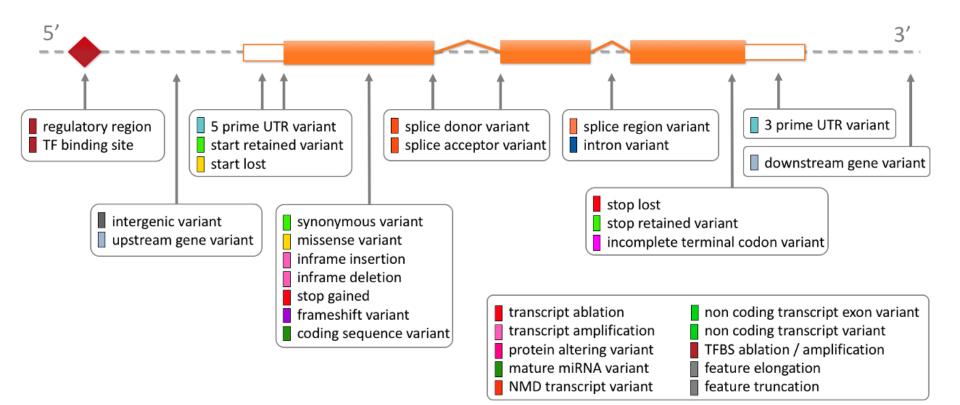




SNPs leading to the truncation of a protein sequence are mostly considered to inhibit protein function



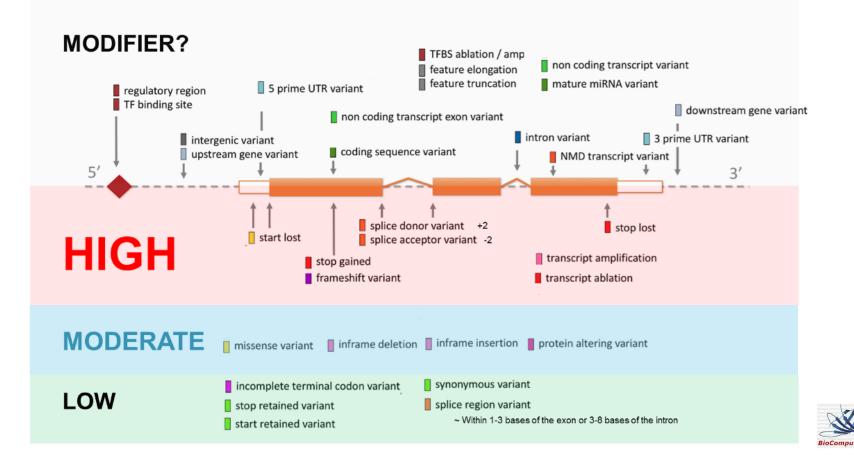
### Variant Severity: Variable definitions but helps prioritize



https://m.ensembl.org/info/genome/variation/prediction/predicted\_data.html



#### Variant Severity: Variable definitions but helps prioritize



# Variant filtering: criteria

- Genes list
- Sequencing parameters (filter artifactuals)
- Variant types (exonic intronic)
- Variant class (frameshift synonymous)
- Population frequency
- Pathogenicity prediction score
- Conservation score
- Clinical interpretation of genetic variants by the ACMG/AMP 2015 guideline
- Presence in mutation databases



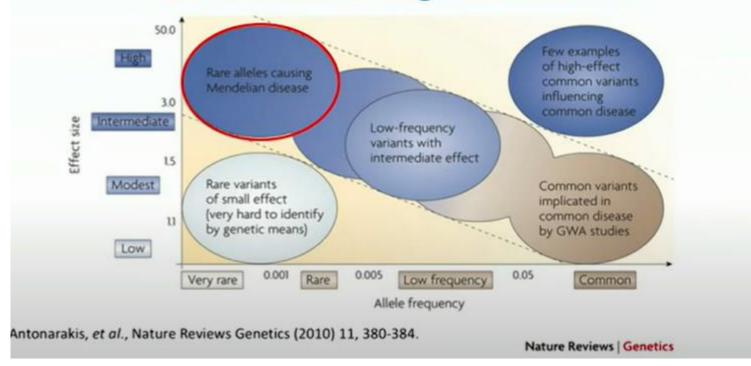
## Variant filtering: Variant frequency

## What's in an exome

- Every genome contains many rare, potentially functional variants
  - ~500 rare missense variants (1/3 of which are predicted damaging by *in silico* predictors)
  - ~100 LoF variants: ~20 homozygous, ~20 rare
  - ~100 rare variants in known disease genes
  - ~50 reported disease-causing mutations (!)
  - o 1-2 de novo coding mutations
  - Unknown number of sequencing errors



## Mendelian disease: Mainly looking for rare variants with large effect size





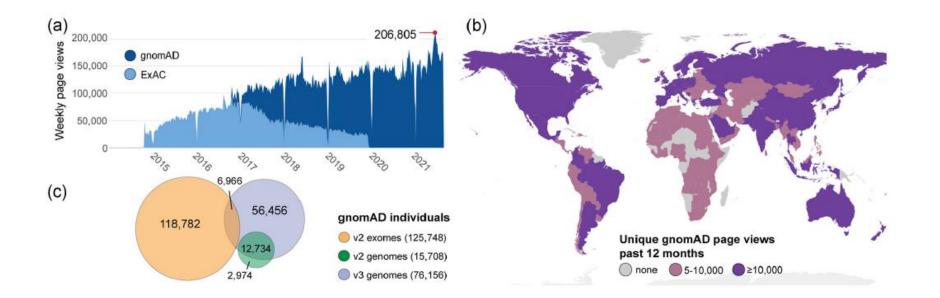
# Variant filtering: Variant frequency

- Population databases
- GnomAD
- ExAC
- Trans-Omics for PrecisionMedicine (TOPMed)-BRAVO
- Geisinger Healthcare System DiscovEHR dataset
- Internal laboratory panel of normal



### Variant filtering: GnomAD

https://doi.org/10.1002/humu.24309





#### Variant filtering: Genome Aggregation Database (GnomAD)

#### • 195,000 individuals

- 85% of all possible synonymous CpG-to TpG transitions observed; across non-CpG trinucleotide contexts, less than 12% of possible synonymous variants; 4% of nonsense variants
- With the existing sample size of gnomAD, each individual has
  - **200 very rare coding variants** (gnomAD allele frequency <0.1%).
  - tens of variants that are absent from gnomAD
  - **27 ± 13 novel coding variants** that are absent in all other gnomAD individuals (variants unique to that individual
- Case-control from common adult-onset disease studies (type 2 diabetes, psychiatric disorders, and cardiovascular disease (60 studies)
- Database of Genotypes and Phenotypes (dbGaP)
- Report the population with highest Allele frequency for each variant (Popmax AF)



# Variant filtering: GnomAD

- Uniform joint variant calling
- variant quality control (QC)
- individuals known to be affected with severe pediatric disease excluded
- Quality Score Recalibration (VQSR) have been applied to distinguish true genetic variants from artifacts
- depth  $\geq$  10, genotype quality  $\geq$  20, minor allele fraction  $\geq$  0.2 for nonreference heterozygous variants

•Using gnomAD - tips and tricks (video)

•gnomAD: Using large genomic data sets to interpret human genetic variation (video)



# Variant filtering: Variant frequency

Disease model

- De novo or Dominant Absent in Public database or MAF < 0.001 in each of the eight gnomAD populations (e.g., African, Latino, East Asian, etc.)
- For **dominant**, the number of homozygous alternate alleles in gnomAD to be <10
- Recessive MAF < 0.01
- Complex MAF < 0.0001
- Somatic: high confidence: absent in Public database or MAF < 0.001



# Identifying de novo variants

**De novo definition:** A variant that has arisen in an individual for the first time and is not inherited from a parent.

- can be caused by chance, impaired DNA repair, or increased mutation of the genome due to mutagens such as radiation and particular chemicals
- The human de novo mutation rate 1.29 x 10<sup>-8</sup> per base pair per generation
- 70 de novo against 4-5 million inherited variants, for each proband
- In protein coding exome we expect 1 de novo mutation against 50.000 inherited variants
- For 99.9% variant calling precision, **50 false-positive** calls for each de novo mutations

Filtering for artifactual and population frequency



#### Variant filtering: de novo variants

