Supplementary Material

SeqTailor: a user-friendly webserver for the extraction of DNA or protein sequences from next-generation sequencing data

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CHR_HSCHR19LRC_PGF2_CTG3_1	CHR_HSCHR3_4_CTG3	CHR_HSCHR6_MHC_COX_CTG1
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CHR_HSCHR21_5_CTG2	CHR_HSCHR5_1_CTG1	CHR_HSCHR8_5_CTG7
CHR_HSCHR21_6_CTG1_1	CHR_HSCHR5_1_CTG1_1	CHR_HSCHR8_6_CTG1
CHR_HSCHR21_8_CTG1_1	CHR_HSCHR5_1_CTG5	CHR_HSCHR8_6_CTG7
CHR_HSCHR22_1_CTG1	CHR_HSCHR5_2_CTG1	CHR_HSCHR8_7_CTG1
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CHR_HSCHR22_1_CTG3	CHR_HSCHR5_2_CTG5	CHR_HSCHR8_8_CTG1
CHR_HSCHR22_1_CTG4	CHR_HSCHR5_3_CTG1	CHR_HSCHR8_9_CTG1
CHR_HSCHR22_1_CTG5	CHR_HSCHR5_3_CTG1_1	CHR_HSCHR9_1_CTG1
CHR_HSCHR22_1_CTG6	CHR_HSCHR5_3_CTG5	CHR_HSCHR9_1_CTG2
CHR_HSCHR22_1_CTG7	CHR_HSCHR5_4_CTG1	CHR_HSCHR9_1_CTG3
CHR_HSCHR22_2_CTG1	CHR_HSCHR5_4_CTG1_1	CHR_HSCHR9_1_CTG4
CHR_HSCHR22_3_CTG1	CHR_HSCHR5_5_CTG1	CHR_HSCHR9_1_CTG5
CHR_HSCHR22_4_CTG1	CHR_HSCHR5_6_CTG1	CHR_HSCHR9_1_CTG6
CHR_HSCHR22_5_CTG1	CHR_HSCHR5_7_CTG1	CHR_HSCHR9_1_CTG7
CHR_HSCHR22_6_CTG1	CHR_HSCHR5_8_CTG1	CHR_HSCHRX_1_CTG3
CHR_HSCHR22_7_CTG1	CHR_HSCHR5_9_CTG1	CHR_HSCHRX_2_CTG12
CHR_HSCHR22_8_CTG1	CHR_HSCHR6_1_CTG10	CHR_HSCHRX_2_CTG3
CHR_HSCHR3_1_CTG1	CHR_HSCHR6_1_CTG2	CHR_HSCHRX_3_CTG7
CHR_HSCHR3_1_CTG2_1	CHR_HSCHR6_1_CTG3	

Table S1: The 329 alternate loci and scaffolds in Human GRCh38 assembly supported in the SeqTailor webserver.

protein-coding
IG genes
TR genes
non-stop decay
nonsense mediated decay
polymorphic pseudogene
processed pseudogene
processed transcript
pseudogene
transcribed processed pseudogene
transcribed unitary pseudogene
transcribed unprocessed pseudogene
translated processed pseudogene
unitary pseudogene
unprocessed pseudogene

Table S2: The 15 transcript biotypes that belonging to the categories of protein coding and pseudogenes, that have been adopted in the data collection in the SeqTailor webserver.

Comments	Actions
Genetic Variants in VCF Files (CHROM, POS, ID, REF, ALT)	
Normal	Normal
Duplicated	Skipped
Field mistake	Skipped
Empty REF allele	Skipped
Unmatched REF allele to the reference genome	Skipped
Identical REF and ALT allele	Skipped
Negative window size	Skipped
Too long window size	Reduced window size to 5,000bp
Genetic Ranges in BED Files (CHROM, START, END)	
Normal	Normal
Duplicated	Skipped
Field mistake	Skipped
Negative START	Set Start to 0
Negative END	Skipped
Negative START and END	Skipped
START is greater than END	Swap START with END
Too long genomic range	Trim at 10,000bp from START

Table S3: The exception handling in the SeqTailor webserver for VCF files and BED files.

Organism	Standard Genetic Codes	Mitochondrial Genetic Codes
Human	Table 1	Table 2
Chimpanzee	Table 1	Table 2
Mouse	Table 1	Table 2
Rat	Table 1	Table 2
Cow	Table 1	Table 2
Chicken	Table 1	Table 2
Lizard	Table 1	Table 2
Zebrafish	Table 1	Table 2
Fruitfly	Table 1	Table 5
Arabidopsis	Table 1	Table 1
Rice	Table 1	Table 1

Table S4: The standard and mitochondrial genetic codes tables for the supported 11 organisms in the SeqTailor webserver, according to the NCBI Taxonomy Database.

Genetic Code Table 1						
Codon	**Amino Acid**			**Codon**	**Amino Acid**	
TTT	Phe	F		ATT	Ile	I
TCT	Ser	S		ACT	Thr	T
TAT	Tyr	Y		AAT	Asn	N
TGT	Cys	C		AGT	Ser	S
TTC	Phe	F		ATC	Ile	I
TCC	Ser	S		ACC	Thr	T
TAC	Tyr	Y		AAC	Asn	N
TGC	Cys	C		AGC	Ser	S
TTA	Leu	L		ATA	Ile	I
TCA	Ser	S		ACA	Thr	T
TAA	Ter	*		AAA	Lys	K
TGA	Ter	*		AGA	Arg	R
TTG	Leu	L		ATG	Met	M
TCG	Ser	S		ACG	Thr	T
TAG	Ter	*		AAG	Lys	K
TGG	Trp	W		AGG	Arg	R
CTT	Leu	L		GTT	Val	V
CCT	Pro	P		GCT	Ala	A
CAT	His	H		GAT	Asp	D
CGT	Arg	R		GGT	Gly	G
CTC	Leu	L		GTC	Val	V
CCC	Pro	P		GCC	Ala	A
CAC	His	H		GAC	Asp	D
CGC	Arg	R		GGC	Gly	G
CTA	Leu	L		GTA	Val	V
CCA	Pro	P		GCA	Ala	A
CAA	Gln	Q		GAA	Glu	E
CGA	Arg	R		GGA	Gly	G
CTG	Leu	L		GTG	Val	V
CCG	Pro	P		GCG	Ala	A
CAG	Gln	Q		GAG	Glu	E
CGG	Arg	R		GGG	Gly	G

Table S5: The genetic codes table 1.

Genetic Code Table 2						
Codon	**Amino Acid**			**Codon**	**Amino Acid**	
TTT	Phe	F		ATT	Ile	I
TCT	Ser	S		ACT	Thr	T
TAT	Tyr	Y		AAT	Asn	N
TGT	Cys	C		AGT	Ser	S
TTC	Phe	F		ATC	Ile	I
TCC	Ser	S		ACC	Thr	T
TAC	Tyr	Y		AAC	Asn	N
TGC	Cys	C		AGC	Ser	S
TTA	Leu	L		ATA	Met	M
TCA	Ser	S		ACA	Thr	T
TAA	Ter	*		AAA	Lys	K
TGA	Trp	W		AGA	Ter	*
TTG	Leu	L		ATG	Met	M
TCG	Ser	S		ACG	Thr	T
TAG	Ter	*		AAG	Lys	K
TGG	Trp	W		AGG	Ter	*
CTT	Leu	L		GTT	Val	V
CCT	Pro	P		GCT	Ala	A
CAT	His	H		GAT	Asp	D
CGT	Arg	R		GGT	Gly	G
CTC	Leu	L		GTC	Val	V
CCC	Pro	P		GCC	Ala	A
CAC	His	H		GAC	Asp	D
CGC	Arg	R		GGC	Gly	G
CTA	Leu	L		GTA	Val	V
CCA	Pro	P		GCA	Ala	A
CAA	Gln	Q		GAA	Glu	E
CGA	Arg	R		GGA	Gly	G
CTG	Leu	L		GTG	Val	V
CCG	Pro	P		GCG	Ala	A
CAG	Gln	Q		GAG	Glu	E
CGG	Arg	R		GGG	Gly	G

Table S6: The genetic codes table 2.

Genetic Code Table 5						
Codon	**Amino Acid**			**Codon**	**Amino Acid**	
TTT	Phe	F		ATT	Ile	I
TCT	Ser	S		ACT	Thr	T
TAT	Tyr	Y		AAT	Asn	N
TGT	Cys	C		AGT	Ser	S
TTC	Phe	F		ATC	Ile	I
TCC	Ser	S		ACC	Thr	T
TAC	Tyr	Y		AAC	Asn	N
TGC	Cys	C		AGC	Ser	S
TTA	Leu	L		ATA	Met	M
TCA	Ser	S		ACA	Thr	T
TAA	Ter	*		AAA	Lys	K
TGA	Trp	W		AGA	Ser	S
TTG	Leu	L		ATG	Met	M
TCG	Ser	S		ACG	Thr	T
TAG	Ter	*		AAG	Lys	K
TGG	Trp	W		AGG	Ser	S
CTT	Leu	L		GTT	Val	V
CCT	Pro	P		GCT	Ala	A
CAT	His	H		GAT	Asp	D
CGT	Arg	R		GGT	Gly	G
CTC	Leu	L		GTC	Val	V
CCC	Pro	P		GCC	Ala	A
CAC	His	H		GAC	Asp	D
CGC	Arg	R		GGC	Gly	G
CTA	Leu	L		GTA	Val	V
CCA	Pro	P		GCA	Ala	A
CAA	Gln	Q		GAA	Glu	E
CGA	Arg	R		GGA	Gly	G
CTG	Leu	L		GTG	Val	V
CCG	Pro	P		GCG	Ala	A
CAG	Gln	Q		GAG	Glu	E
CGG	Arg	R		GGG	Gly	G

Table S7: The genetic codes table 5.

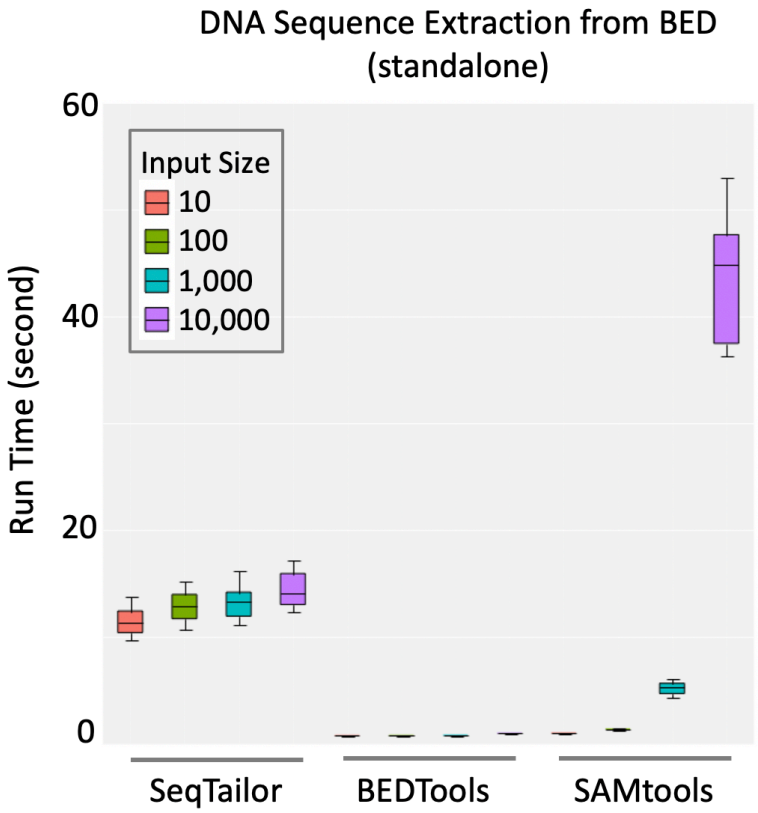


Figure S1: Runtime performance in extracting DNA reference sequences from varying sizes of input BED data, by SeqTailor-standalone, BEDTools, and SAMtools, in a script-based manner.

Case Study

SeqTailor aims to makes it efficient to further investigate the genomic variant data and renders sequence-based software more accessible. To demonstrate the practical power of SeqTailor to bridge the gap between genomic variations and sequence-based tools for analyses and predictions, we exhibited a case study on pathogenic genetic variants with different effects and different clinical consequences identified in five human genes (*MSH2*, *BRAF*, *GJB2*, *BRCA2*, and *IL2RG*) by the HGMD professional database (1) and the ClinVar database (2).

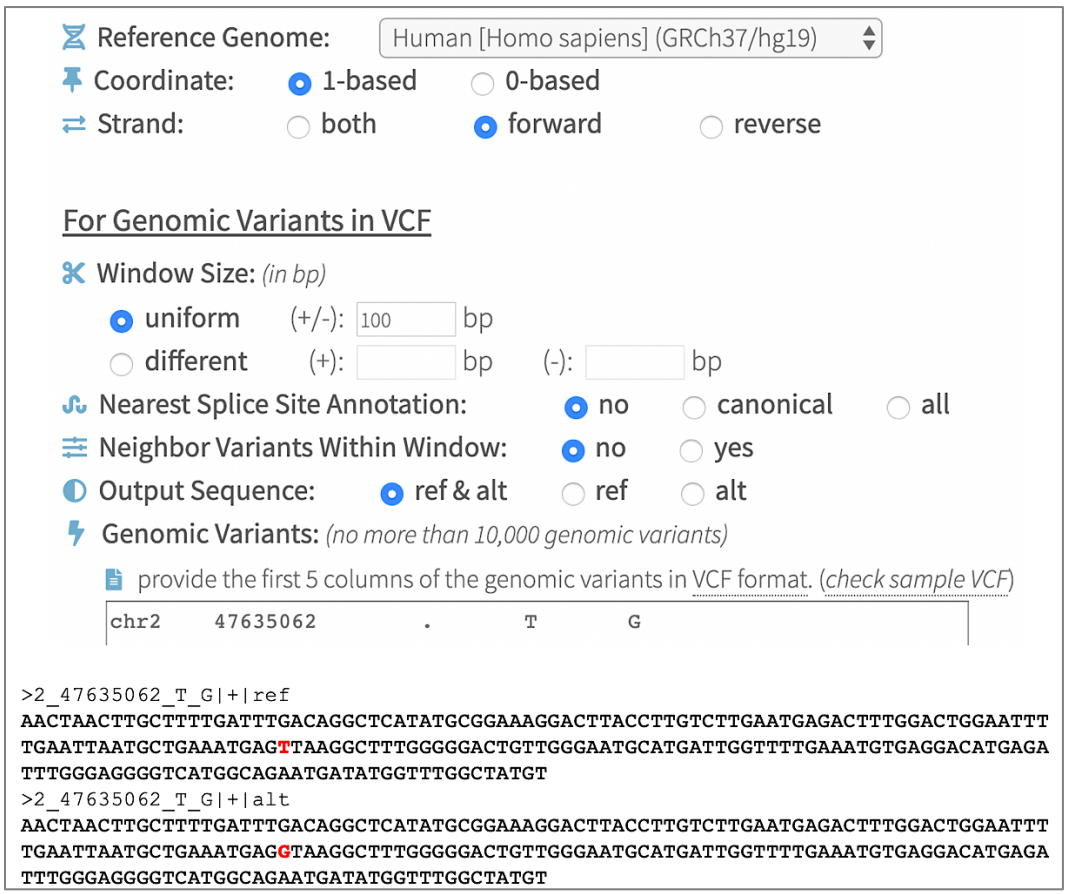
Case Study 1: DNA sequence extraction for a variant in *MSH2*

A single nucleotide variant (ClinVar: SCV000107433.2, dbSNP: rs587779138), changes T to G on Chr2:47635062 forward strand of GRCh37 assembly, is a deep intronic variant of *MSH2* gene that has been shown to cause Lynch syndrome through the creation of a new splice donor site with pseudoexon activation (3).

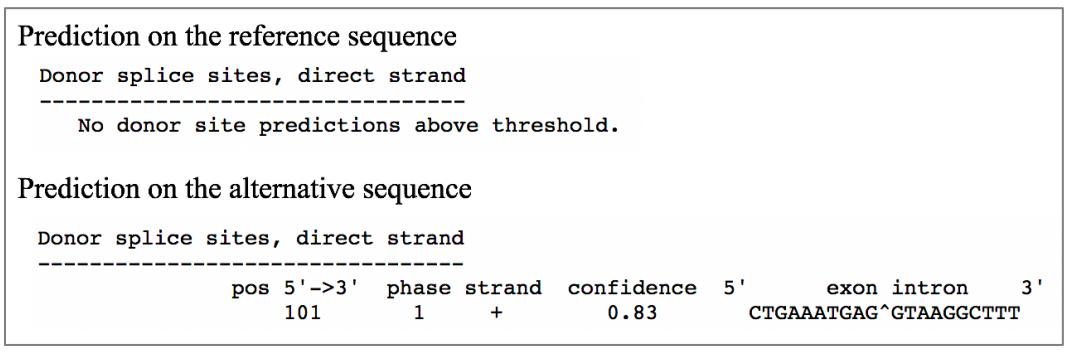
NM_000251.2(MSH2):c.212-478T>G

Allele ID:	96369
Variant type:	single nucleotide variant
Cytogenetic location:	2p21
Genomic location:	• Chr2: 47407923 (on Assembly GRCh38) • Chr2: 47635062 (on Assembly GRCh37)
HGVS:	• NG_007110.2:g.9800T>G • NM_000251.2:c.212-478T>G • NC_000002.12:g.47407923T>G (GRCh38) • LRG_218t1:c.212-478T>G • NC_000002.11:g.47635062T>G (GRCh37) • NM_000251.1:c.212-478T>G • LRG_218:g.9800T>G

With SeqTailor, the DNA sequence (+/-100 bp) around this variant (chr2-47635062-T-G) was rapidly extracted. In the output sequence, this variant is located at position 101.



The output sequences can be directly used as the input for sequence-based splicing prediction tools (e.g. NetGene2 (4)), to evaluate the impact of this variant on splicing. A number of tools are available for this purpose, and NetGene2 was selected here for demonstration purposes only. When provided with the reference sequence and alternative sequence, NetGene2 gave the splicing predictions for both sequences, as shown below. In this example, NetGene2 identified donor splice site at position 101 on the alternative sequence, but no donor splice site in the reference sequence.

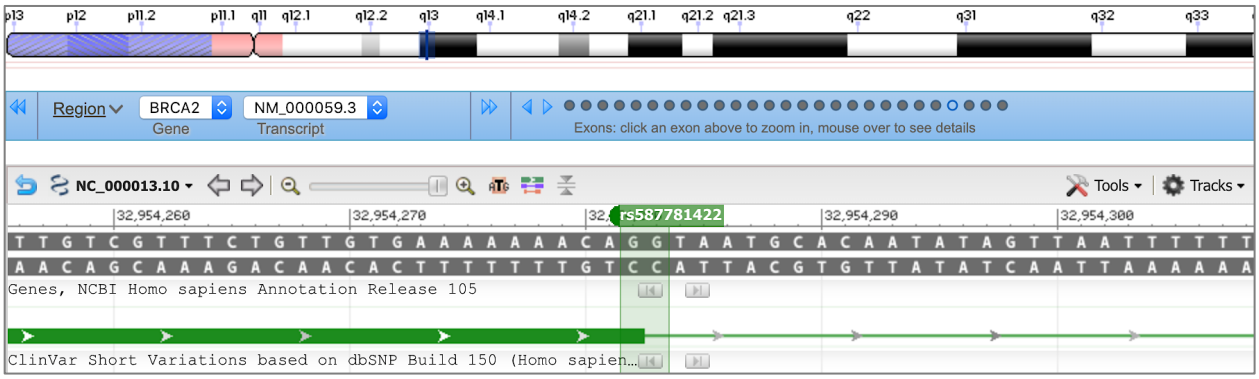


Case Study 2: DNA sequence extraction for a variant in *BRCA2*

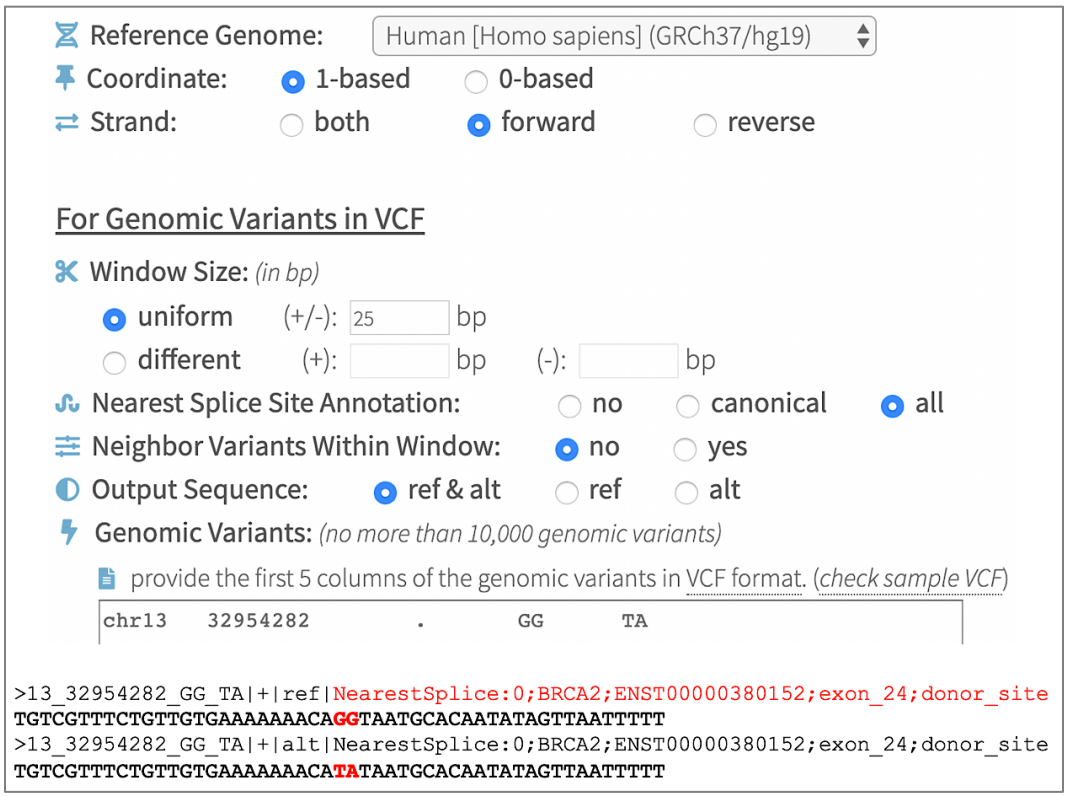
An indel (ClinVar: SCV000637244.1, dbSNP: rs587781422), deletes GG and inserts TA on Chr13:32954282–32954283 forward strand of the GRCh37 assembly, is a splicing variant of the *BRCA2* gene that has been associated with hereditary breast-ovarian cancer (5). This 2-bp variant sits exactly at the splicing site, spanning from the last nucleotide of exon 24 of *BRCA2* gene to the first nucleotide of the following intron.

NM_000059.3(BRCA2):c.9256_9256+1delGGinsTA

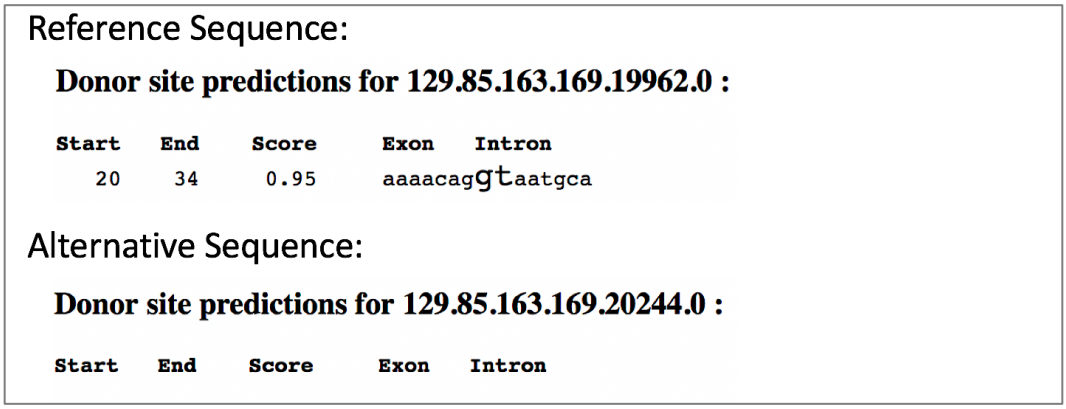
Allele ID:	150706
Variant type:	Indel
Cytogenetic location:	13q13.1
Genomic location:	• Chr13: 32380145 - 32380146 (on Assembly GRCh38) • Chr13: 32954282 - 32954283 (on Assembly GRCh37)
HGVS:	• NG_012772.3:g.69666_69667delGGinsTA • NM_000059.3:c.9256_9256+1delGGinsTA • NC_000013.11:g.32380145_32380146delGGinsTA (GRCh38) • LRG_293t1:c.9256_9256+1delGGinsTA • NC_000013.10:g.32954282_32954283delGGinsTA (GRCh37) • LRG_293:g.69666_69667delGGinsTA



By submitting this variant (chr13-32954282-GG-TA) and choosing to annotate the nearest splice site, SeqTailor extracted the ref./alt. DNA sequences, and provided the distance from the variant to the nearest splice site (+ve distance: downstream, -ve distance: upstream, and 0: exactly at the splice site), as well as the belonging gene symbol, transcript ID, exon number, and donor/acceptor site information. Please note that, in SeqTailor, the nearest splice site refers to the first nucleotide of the nearest exon (as acceptor site) or the last nucleotide of the nearest exon (as donor site).



The output sequences can be rapidly used for splicing prediction (e.g. NNSPLICE (6)). Again, the tool used here were selected for demonstration purposes only. NNSPLICE identified a donor splice site in the reference sequence, but not in the alternative sequence.

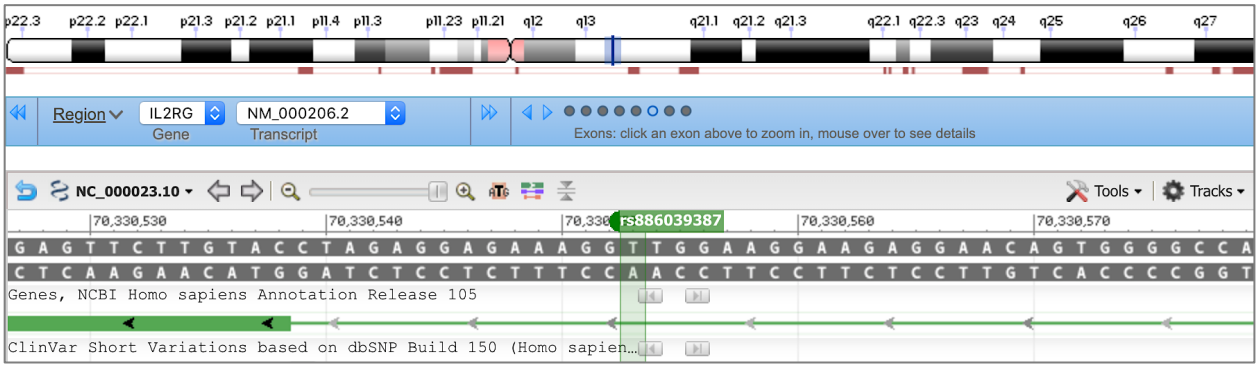


Case Study 3: DNA sequence extraction for a variant in IL2RG

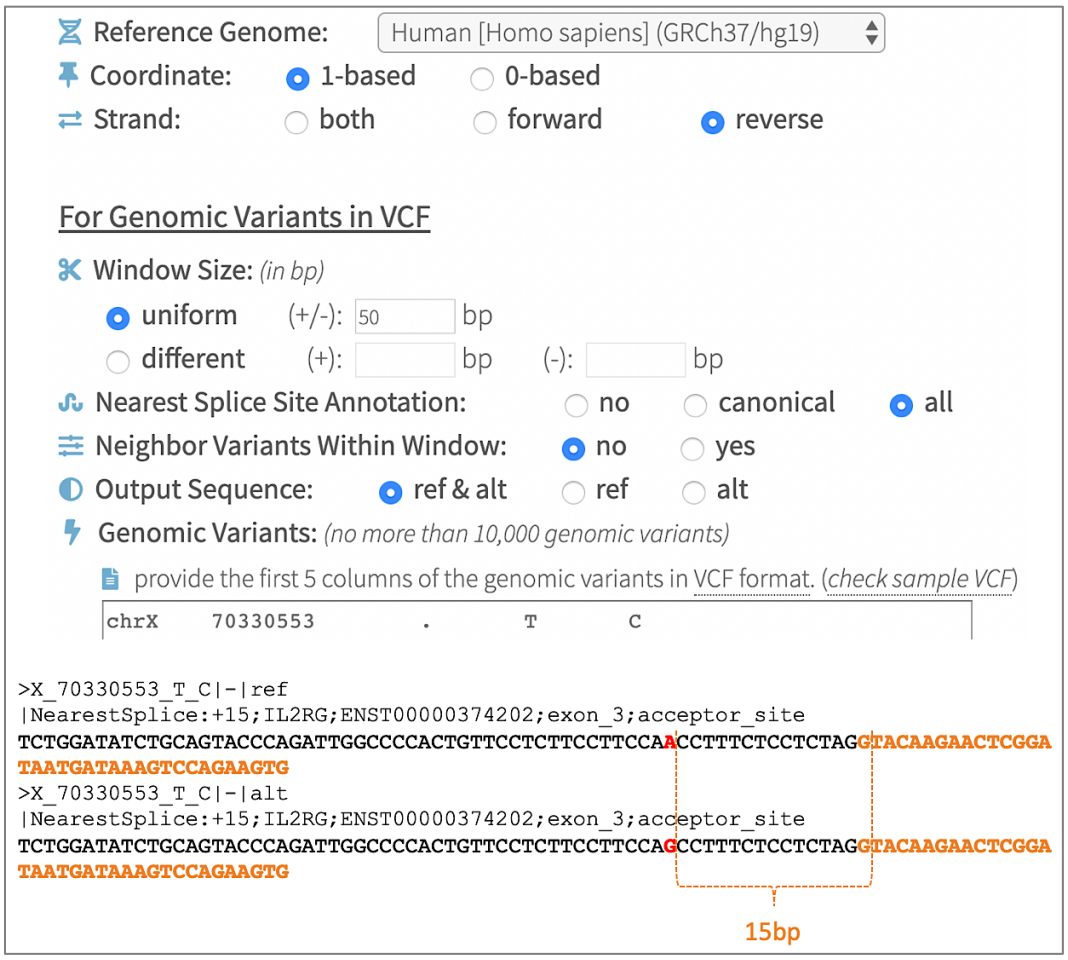
A single nucleotide variant (ClinVar: SCV000637244.1, dbSNP: rs886039387), changes A to G on ChrX:70330553 reverse strand of the GRCh37 assembly, is an intronic variant of the *IL2RG* gene that has been reported in association with X-linked severe combined immunodeficiency (7). This variant does not directly change the encoded amino acid sequence, but the experimental studies have shown that this intronic mutation causes aberrant splicing in the mRNA as shown by RT-PCR on B-cell line of an individual with this variant (7).

NM_000206.2(IL2RG):c.270-15A>G

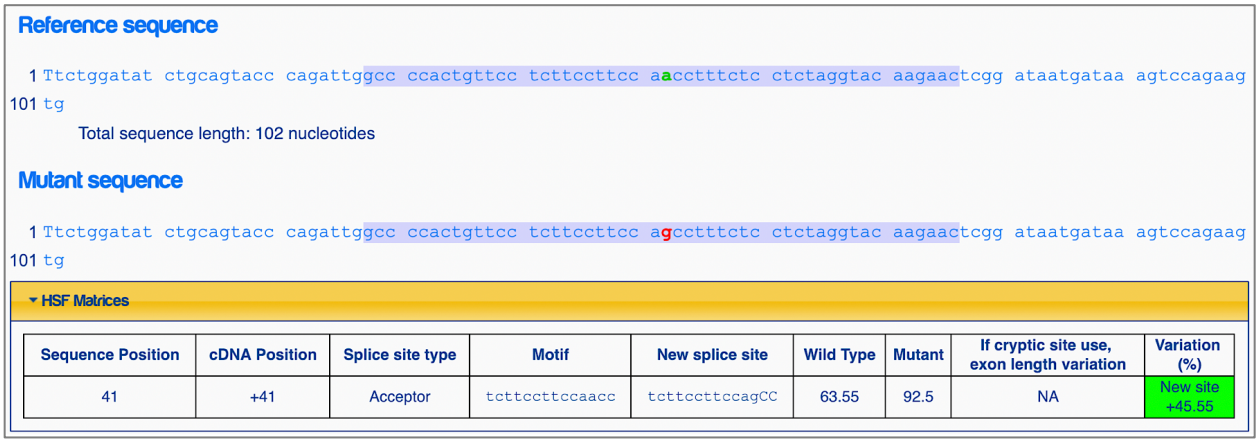
Allele ID:	260341
Variant type:	single nucleotide variant
Cytogenetic location:	Xq13.1
Genomic location:	• ChrX: 71110703 (on Assembly GRCh38) • ChrX: 70330553 (on Assembly GRCh37)
HGVS:	• NG_009088.1:g.5851A>G • NM_000206.2:c.270-15A>G • NC_000023.11:g.71110703T>C (GRCh38) • LRG_150t1:c.270-15A>G • NC_000023.10:g.70330553T>C (GRCh37) • LRG_150:g.5851A>G



As the *IL2RG* gene is on the reverse strand, its VCF format is converted to (chrX-70330553-T-C). SeqTailor extracted its ref./alt. DNA sequences, and informed its nearest splice site (acceptor site) is located at 15bp downstream from the position of the variant. The sequence colored in orange represent the exons.



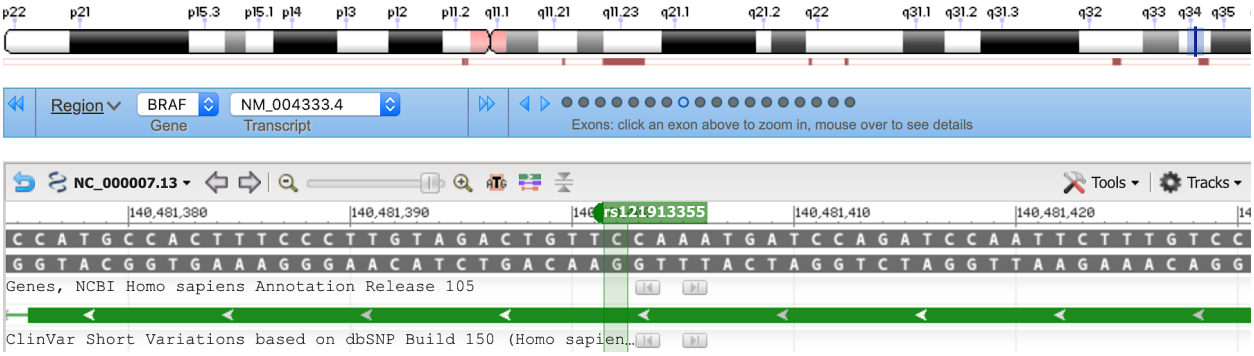
The output ref./alt. DNA sequences were directly applied to Human Splicing Finder (8) for splicing analysis. As shown below, Human Splicing Finder predicted a new acceptor site might be created at the position of this variant, by giving splicing score 92.5 for the mutant versus the splicing score 63.55 for the wild-type.



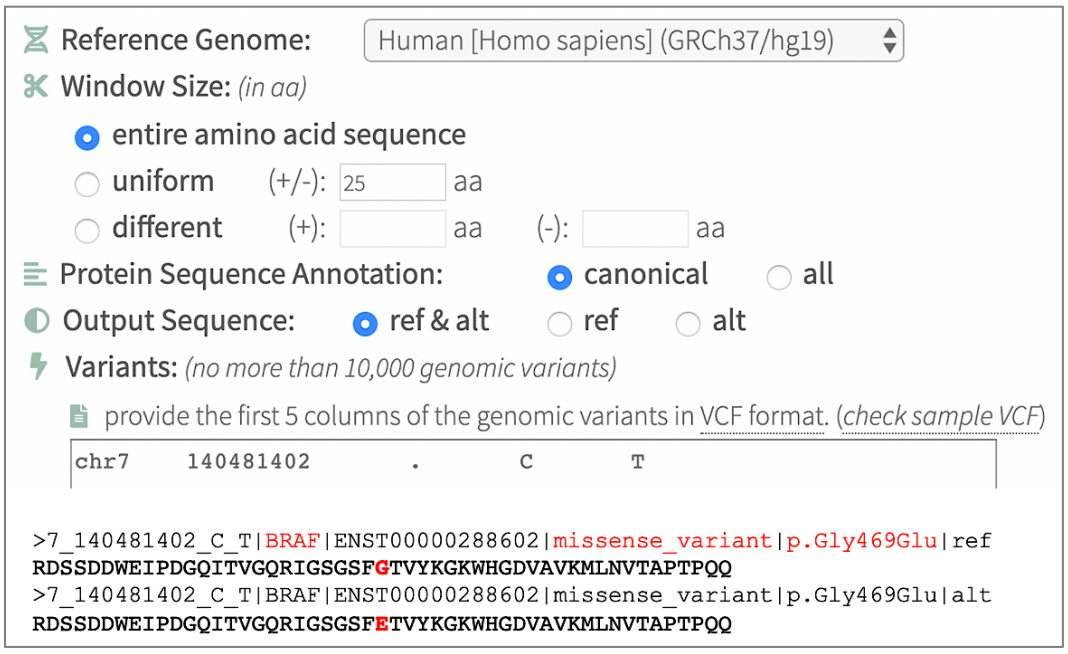
Case Study 4: Protein sequence extraction for a variant in *BRAF*

A single nucleotide variant (ClinVar: SCV000616361.3, dbSNP: rs121913355), changes G to A on Chr7: 140481402 reverse strand of the GRCh37 assembly, is a missense variant of *BRAF* that replaces the glycine (G) residue in position 469 with a glutamic acid (E), and has been associated with cardio-facio-cutaneous syndrome (9).

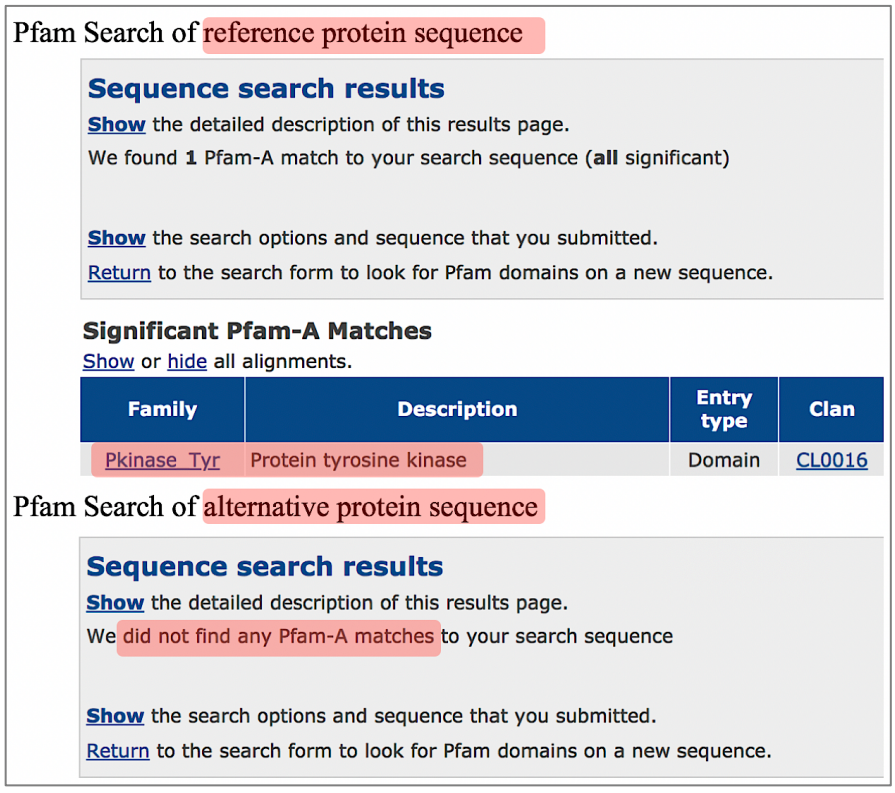
Allele ID:	29013
Variant type:	single nucleotide variant
Cytogenetic location:	7q34
Genomic location:	• Chr7: 140781602 (on Assembly GRCh38) • Chr7: 140481402 (on Assembly GRCh37)
Other names:	• p.G469E:GGA>GAA
Protein change:	G469E
HGVS:	• NG_007873.3:g.148163G>A • NM_004333.5:c.1406G>A • NP_004324.2:p.Gly469Glu • NC_000007.14:g.140781602C>T (GRCh38) • LRG_299t1:c.1406G>A • NC_000007.13:g.140481402C>T (GRCh37) • NG_007873.2:g.148163G>A • NM_004333.4:c.1406G>A • P15056:p.Gly469Glu • LRG_299p1:p.Gly469Glu • LRG_299:g.148163G>A



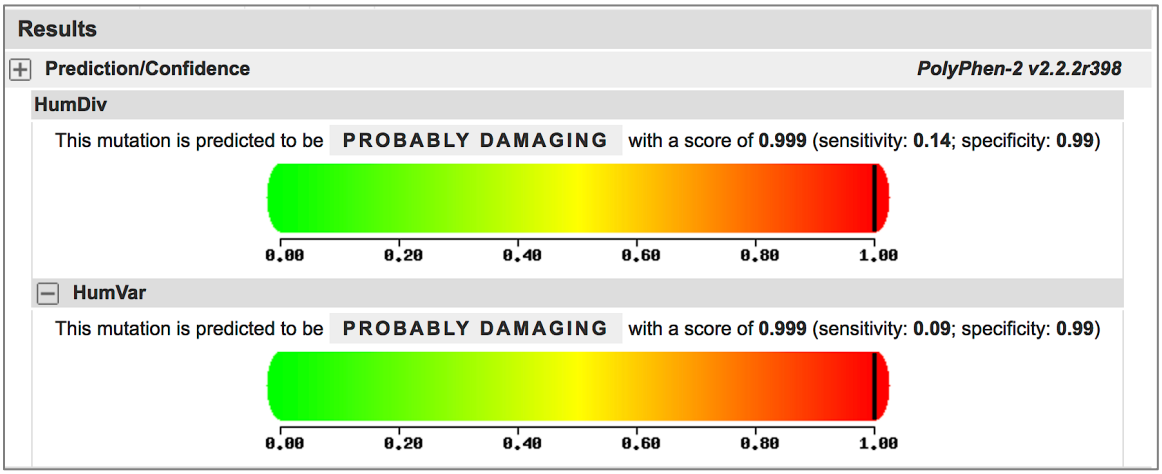
As the *BRAF* gene is located on the reverse strand, the HGVS nomenclature of this variant becomes (g.140481402C>T), thus its VCF format becomes (chr7-140481402-C-T). SeqTailor was then used to annotate the variant by the built-in SnpEff (10), followed by extracting the protein sequence (+/- 25 aa) around this missense variant of *BRAF*.



Using the ref./alt. protein sequences, protein family or domain prediction tools (e.g. Pfam (11)) can be used to determine if the variant will lead to a loss of functionally important protein domains. In this case, *BRAF* is a protein kinase transducing mitogenic signals from cell membrane to nucleus, and its kinase domain plays a key role in its function. A Pfam search identified the protein kinase domain in the ref. protein sequence, but not in the alt. protein sequence, suggesting the missense variant may damages the conserved kinase domain, thereby impairing *BRAF* protein function.



Furthermore, the protein sequence and the altered amino acid can be submitted to PolyPhen-2 (12), to predict the functional effect of the missense variant. In this case, PolyPhen-2 assigned a score of 0.999 to this variant, implying a 'probably damaging' effect.

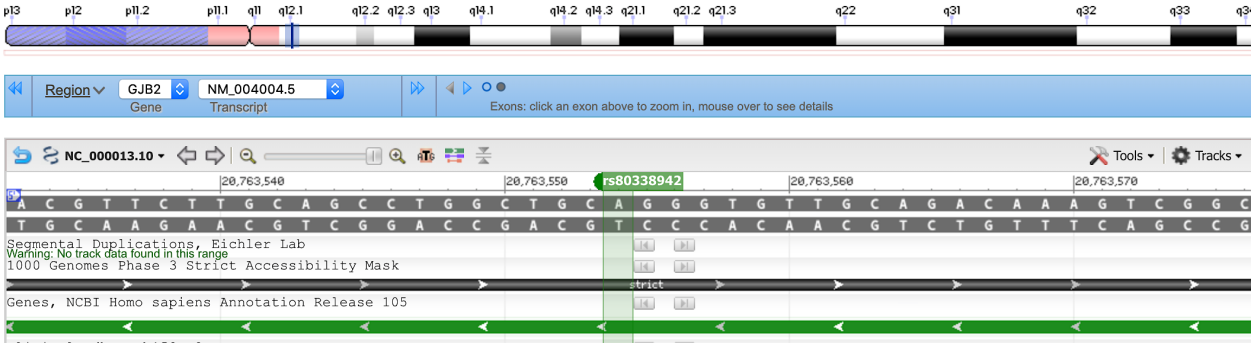


Case Study 5: Protein sequence extraction for a variant in *GJB2*

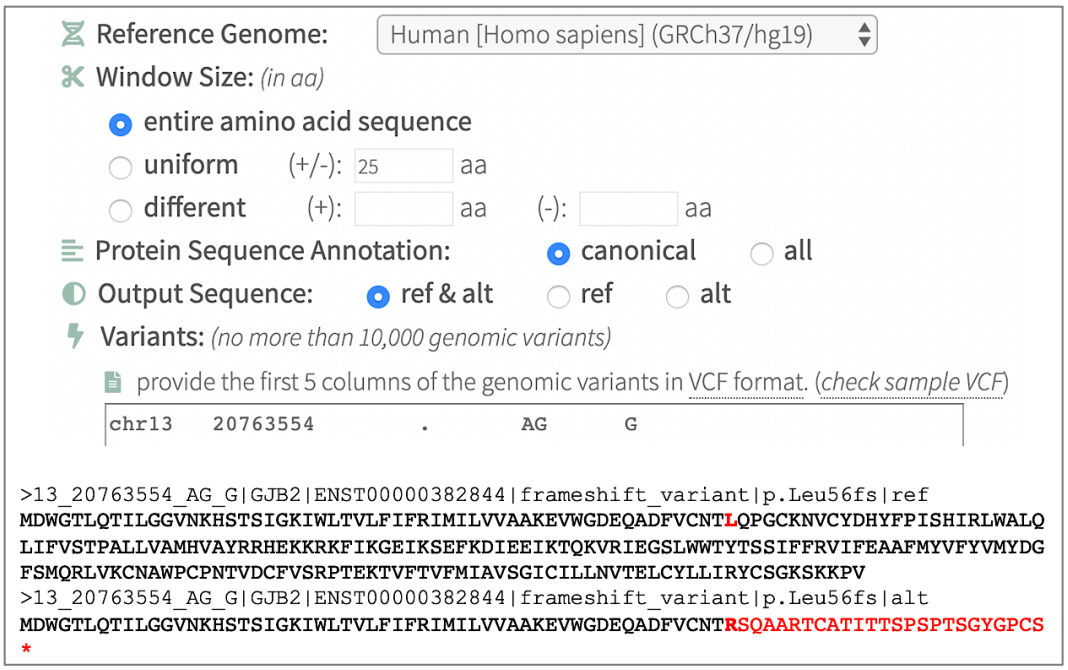
A single nucleotide variant (ClinVar: SCV000840535.3, dbSNP: rs80338942), deletes T on Chr13:20763554 revsere strand of the GRCh37 assembly, is a frameshift variant of the *GJB2* gene that changes the amino acid from Leu to Arg at position 56, and has been found to cause Nonsyndromic hearing loss and deafness (13).

NM_004004.5(GJB2):c.167delT (p.Leu56Argfs)

Allele ID:	32049
Variant type:	Deletion
Cytogenetic location:	13q12.1
Genomic location:	• Chr13: 20189415 (on Assembly GRCh38) • Chr13: 20763554 (on Assembly GRCh37)
Other names:	• NM_004004.5(GJB2):c.167delT(p.Leu56Argfs) • NM_004004.5(GJB2):c.167delT
HGVS:	• NG_008358.1:g.8561delT • NM_004004.5:c.167delT • NP_003995.2:p.Leu56Argfs • NC_000013.11:g.20189415delA (GRCh38) • NC_000013.10:g.20763554delA (GRCh37) • NM_004004.5:c.167del • NC_000013.10:g.20763554del (GRCh37)



In this example, the reference protein sequence was the entire protein sequence of transcript ENST00000382844 of gene *GJB2*. The variant led to a frameshift occurring at position 56 by changing L to R, thus the following amino acids in the protein sequence will be translated differently. SeqTailor extracts and alters the reference CDS sequence, followed by re-translating the altered CDS sequence to the alternative protein sequence. In this example, the new protein sequence will terminate at 24 amino acids downstream from the frameshifted amino acid. Once the stop codon is encountered, SeqTailor gives a '*' symbol to inform the sequence termination.


Reference Genome:
Human [Homo sapiens] (GRCh37/hg19)
Window Size: (in aa)
entire amino acid sequence
uniform (+/-): 25 aa
different (+): aa (-): aa
Protein Sequence Annotation:
canonical
all
Output Sequence:
ref & alt
ref
alt
Variants: (no more than 10,000 genomic variants)
provide the first 5 columns of the genomic variants in VCF format. (check sample VCF)
chr13 20763554 . AG G
>13_20763554_AG_G|GJB2|ENST00000382844|frameshift_variant|p.Leu56fs|ref
MDWGTLQTILGGVNKHSTSIGKIWLTVLFIFRIMILVVAAKEVWGDEQADFVCNTLQPGCKNVCYDHYFPISHIRLWALQ
LIFVSTPALLVAMHVAYRRHEKKRKFIKGEIKSEFKDIEEIKTQKVRIEGSLWWTYTSSIFFRVIFEAAFMYVFYVMYDG
FSMQRLVKCNAWPCPNTVDCFVSRPTEKTVFTVFMIAVSGICILLNVTELCYLLIRYCSGKSKKPV
>13_20763554_AG_G|GJB2|ENST00000382844|frameshift_variant|p.Leu56fs|alt
MDWGTLQTILGGVNKHSTSIGKIWLTVLFIFRIMILVVAAKEVWGDEQADFVCNTRSQAARTCATITTSPSPTSGYGPCS
*

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