

# VariantFiltering: filtering of coding and non-coding genetic variants

*Dei M. Elurbe*<sup>1,2</sup> and *Robert Castelo*<sup>3,4</sup>

October 26, 2021

<sup>1</sup> CIBER de Enfermedades Raras (CIBERER), Barcelona, Spain

<sup>2</sup> Present address: CMBI, Radboud University Medical Centre, Nijmegen, The Netherlands.

<sup>2</sup> Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

<sup>3</sup> Research Program on Biomedical Informatics (GRIB), Hospital del Mar Medical Research Institute, Barcelona, Spain.

## 1 Overview

---

The aim of this software package is to facilitate the filtering and annotation of coding and non-coding genetic variants from a group of unrelated individuals, or a family of related ones among which at least one of them is affected by a genetic disorder. When working with related individuals, [VariantFiltering](#) can search for variants from the affected individuals that segregate according to a particular inheritance model acting on autosomes (dominant, recessive homozygous or recessive heterozygous -also known as compound heterozygous) or on allosomes (X-linked), or that occur *de novo*. When working with unrelated individuals, no mode of inheritance is used for filtering but it can be used to search for variants shared among individuals affected by a common genetic disorder.

The main input is a multisample Variant Call Format (VCF) file which is parsed using the R/Bioconductor infrastructure, and particularly the functionality from the [VariantAnnotation](#) package for that purpose. This infrastructure also allows [VariantFiltering](#) to stream over large VCF files to reduce the memory footprint and to annotate the input variants with diverse functional information.

A core set of functional data are annotated by [VariantFiltering](#) but this set can be modified and extended using Bioconductor annotation packages such as [MafDb.1Kgenomes.phase3.hs37d5](#), which stores and exposes to the user minor allele frequency (MAF) values frozen from the latest release of the 1000 Genomes project.

The package contains a toy data set to illustrate how it works through this vignette, and it consists of a multisample VCF file with variants from chromosomes 20, 21, 22 and allosomes X, Y from a trio of CEU individuals of the 1000 Genomes project. To further reduce the execution time of this vignette, only the code for the first analysis is actually evaluated and its results reported.

## 2 Setting up the analysis

---

To start using [VariantFiltering](#) the user should consider installing the packages listed in the `Suggests:` field from its `DESCRIPTION` file. After loading [VariantFiltering](#) the first step is to build a parameter object, of class `VariantFilteringParam` which requires at least a character string with the input VCF filename, as follows:

## VariantFiltering: filtering of coding and non-coding genetic variants

```
> library(VariantFiltering)
> CEUvcf <- file.path(system.file("extdata", package="VariantFiltering"),
+                       "CEUtrio.vcf.bgz")
> vfpar <- VariantFilteringParam(CEUvcf)
> class(vfpar)

[1] "VariantFilteringParam"
attr("package")
[1] "VariantFiltering"

> vfpar

VariantFiltering parameter object

VCF file(s): CEUtrio.vcf.bgz
Genome version(s): hg19(NCBI)
Number of individuals: 3 (NA12878, NA12891, NA12892)
Genome-centric annotation package: BSgenome.Hsapiens.1000genomes.hs37d5 (1000genomes hs37d5)
Variant-centric annotation package: SNPlocs.Hsapiens.dbSNP144.GRCh37 (dbSNP dbSNP Human BUILD 144)
Transcript-centric annotation package: TxDb.Hsapiens.UCSC.hg19.knownGene
Gene-centric annotation package: org.Hs.eg.db
Radical/Conservative AA changes: AA_chemical_properties_HanadaGojoboriLi2006.tsv
Codon usage table: humanCodonUsage.txt
Regions to annotate: CodingVariants, IntronVariants, FiveSpliceSiteVariants, ThreeSpliceSiteVariants, PromoterVariants,
Other annotation pkg/obj: MafDb.1Kgenomes.phase1.hs37d5,
                        PolyPhen.Hsapiens.dbSNP131,
                        SIFT.Hsapiens.dbSNP137,
                        phastCons100way.UCSC.hg19,
                        humanGenesPhylostrata

All transcripts: FALSE
```

The display of the *VariantFilteringParam* object indicates a number of default values which can be overridden when calling the construction function. To quickly see all the available arguments and their default values we should type:

```
> args(VariantFilteringParam)

function (vcfFilename, pedFilename = NA_character_, bsgenome = "BSgenome.Hsapiens.1000genomes.hs37d5",
  orgdb = "org.Hs.eg.db", txdb = "TxDb.Hsapiens.UCSC.hg19.knownGene",
  snpdb = "SNPlocs.Hsapiens.dbSNP144.GRCh37", weightMatricesFileNames = NA,
  weightMatricesLocations = rep(list(variantLocations()), length(weightMatricesFileNames)),
  weightMatricesStrictLocations = rep(list(FALSE), length(weightMatricesFileNames)),
  radicalAAchangeFilename = file.path(system.file("extdata",
  package = "VariantFiltering"), "AA_chemical_properties_HanadaGojoboriLi2006.tsv"),
  codonusageFilename = file.path(system.file("extdata", package = "VariantFiltering"),
  "humanCodonUsage.txt"), geneticCode = getGeneticCode("SGC0"),
  allTranscripts = FALSE, regionAnnotations = list(CodingVariants(),
  IntronVariants(), FiveSpliceSiteVariants(), ThreeSpliceSiteVariants(),
  PromoterVariants(), FiveUTRVariants(), ThreeUTRVariants()),
  intergenic = FALSE, otherAnnotations = c("MafDb.1Kgenomes.phase1.hs37d5",
  "PolyPhen.Hsapiens.dbSNP131", "SIFT.Hsapiens.dbSNP137",
  "phastCons100way.UCSC.hg19", "humanGenesPhylostrata"),
  geneKeytype = NA_character_, yieldSize = NA_integer_)
```

## VariantFiltering: filtering of coding and non-coding genetic variants

NULL

The manual page of `VariantFilteringParam` contains more information about these arguments and their default values.

### 3 Annotating variants

After setting up the parameters object, the next step is to annotate variants. This can be done using upfront an inheritance model that will substantially filter and reduce the number of variants and annotations or, as we illustrate here below, calling the function `unrelatedIndividuals()` that just annotates the variants without filtering out any of them:

```
> uind <- unrelatedIndividuals(vfpar)
```

```
> class(uind)
```

```
[1] "VariantFilteringResults"  
attr(,"package")  
[1] "VariantFiltering"
```

```
> uind
```

VariantFiltering results object

```
Genome version(s): "hg19"(NCBI)  
Number of individuals: 3 (NA12878, NA12891, NA12892)  
Variants segregate according to a(n) unrelated individuals inheritance model  
Quality filters  
  PASS INDELQual  LowQual  SNPQual  
  TRUE      TRUE    TRUE     TRUE  
Functional annotation filters  
  variantType      dbSNP      location      consequence  
  FALSE           FALSE      FALSE         FALSE  
  codonusageFC      OMIM      aaChangeType  maxMAF  
  FALSE           FALSE      FALSE         FALSE  
genePhyloStratum    S0terms  
  FALSE           FALSE
```

The resulting object belongs to the *VariantFilteringResults* class of objects, defined within *VariantFiltering*, whose purpose is to ease the task of filtering and prioritizing the annotated variants. The display of the object just tells us the genome version of the input VCF file, the number of individuals, the inheritance model and what variant filters are activated.

To get a summary of the number of variants annotated to a particular feature we should use the function `summary()`:

```
> summary(uind)
```

	SOID	Description	Nr. Variants	% Variants
1	S0:0002012	start_lost	1	0.31
2	S0:0001631	upstream_gene_variant	52	16.10
3	S0:0001624	3_prime_UTR_variant	16	4.95
4	S0:0001623	5_prime_UTR_variant	13	4.02

## VariantFiltering: filtering of coding and non-coding genetic variants

5	SO:0001589	frameshift_variant	1	0.31
6	SO:0001587	stop_gained	2	0.62
7	SO:0001583	missense_variant	16	4.95
8	SO:0001575	splice_donor_variant	3	0.93
9	SO:0001574	splice_acceptor_variant	6	1.86
10	SO:0001627	intron_variant	259	80.19
11	SO:0001819	synonymous_variant	16	4.95

The default setting of the `summary()` function is to provide feature annotations in terms of Sequence Ontology (SO) terms. The reported number of variants refer to the number of different variants in the input VCF file annotated to the feature while the percentage of variants refers to the fraction of this number over the total number of different variants in the input VCF file.

We can also obtain a summary based on the Bioconductor feature annotations provided by the functions `locateVariants()` and `predictCoding()` from the *VariantAnnotation* package, as follows:

```
> summary(uind, method="bioc")
```

	BIOCID	Nr. Variants	% Variants
2	intron	259	80.19
3	fiveUTR	13	4.02
4	threeUTR	16	4.95
5	coding	35	10.84
7	promoter	52	16.10
8	fiveSpliceSite	3	0.93
9	threeSpliceSite	6	1.86
10	frameshift	1	0.31
11	nonsense	2	0.62
12	nonsynonymous	16	4.95
13	synonymous	16	4.95

Since SO terms are organized hierarchically, we can use this structure to aggregate feature annotations into more coarse-grained SO terms using the argument `method="SOfull"`:

```
> uindSO <- summary(uind, method="SOfull")
> uindSO
```

	SOID	Level	Description	Nr. Variants	% Variants
1	SO:0002012	9	start_lost	1	0.31
2	SO:0001582	8	initiator_codon_variant	1	0.31
3	SO:0001819	8	synonymous_variant	16	4.95
4	SO:0001631	5	upstream_gene_variant	52	16.10
5	SO:0001628	4	intergenic_variant	52	16.10
6	SO:0001624	8	3_prime_UTR_variant	16	4.95
7	SO:0001623	8	5_prime_UTR_variant	13	4.02
8	SO:0001622	7	UTR_variant	29	8.98
9	SO:0001589	9	frameshift_variant	1	0.31
10	SO:0001587	5	stop_gained	2	0.62
11	SO:0001906	4	feature_truncation	2	0.62
12	SO:0001583	11	missense_variant	16	4.95
13	SO:0001992	10	nonsynonymous_variant	18	5.57

## VariantFiltering: filtering of coding and non-coding genetic variants

14	SO:0001650	9	inframe_variant	18	5.57
15	SO:0001818	8	protein_altering_variant	19	5.88
16	SO:0001580	7	coding_sequence_variant	35	10.84
17	SO:0001968	6	coding_transcript_variant	55	17.03
18	SO:0001791	6	exon_variant	55	17.03
19	SO:0001575	8	splice_donor_variant	3	0.93
20	SO:0001574	8	splice_acceptor_variant	6	1.86
21	SO:0001629	7	splice_site_variant	9	2.79
22	SO:0001627	6	intron_variant	261	80.80
23	SO:0001568	6	splicing_variant	9	2.79
24	SO:0001576	5	transcript_variant	303	93.81
25	SO:0001564	4	gene_variant	303	93.81
26	SO:0001878	3	feature_variant	323	100.00
27	SO:0001537	2	structural_variant	323	100.00
28	SO:0001060	1	sequence_variant	323	100.00
29	SO:0002072	0	sequence_comparison	323	100.00

Here the `Level` column refers to the shortest-path distance to the most general SO term `sequence_variant` within the SO acyclic digraph. We can use this level value to interrogate the annotations on a specific granularity:

```
> uindSO[uindSO$Level == 6, ]
```

	SOID	Level	Description	Nr. Variants	% Variants
17	SO:0001968	6	coding_transcript_variant	55	17.03
18	SO:0001791	6	exon_variant	55	17.03
22	SO:0001627	6	intron_variant	261	80.80
23	SO:0001568	6	splicing_variant	9	2.79

Variants are stored internally in a `VRanges` object. We can retrieve the variants as a `Vranges` object with the function `allVariants()`:

```
> allVariants(uind)
```

```
VRangesList of length 3  
names(3): NA12878 NA12891 NA12892
```

This function in fact returns a `VRangesList` object with one element per sample by default. We can change the grouping of variants with the argument `groupBy` specifying the annotation column we want to use to group variants. If the specified column does not exist, then it will return a single `VRanges` object with all annotated variants.

Using the following code we can obtain a graphical display of a variant, including the aligned reads and the running coverage, to have a visual representation of its support. For this purpose we need to have the BAM files used to perform the variant calling. In this case we are using toy BAM files stored along with the `VariantFiltering` package, which for practical reasons only include a tiny subset of the aligned reads.

```
> path2bams <- file.path(system.file("extdata", package="VariantFiltering"),  
+                         paste0(samples(uind), ".subset.bam"))  
> bv <- BamViews(bamPaths=path2bams,  
+               bamSamples=DataFrame(row.names=samples(uind)))  
> bamFiles(uind) <- bv
```

## VariantFiltering: filtering of coding and non-coding genetic variants

```
> bamFiles(uind)

BamViews dim: 0 ranges x 3 samples
names: NA12878 NA12891 NA12892
detail: use bamPaths(), bamSamples(), bamRanges(), ...

> plot(uind, what="rs6130959", sampleName="NA12892")
```

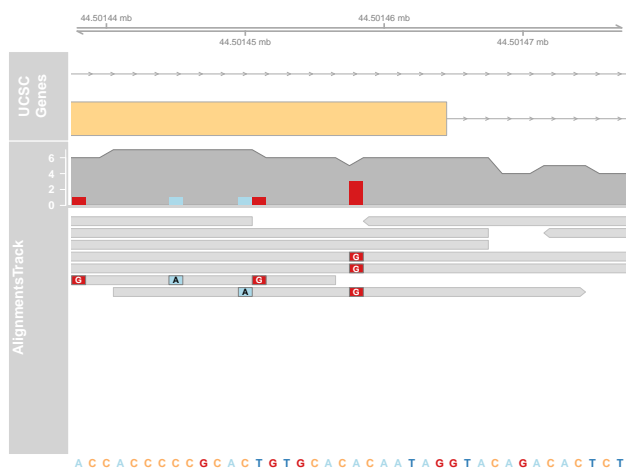


Figure 1: Browser-like display of a variant

## 4 Filters and cutoffs

In the case of having run the `unrelatedIndividuals()` annotation function we can filter variants by restricting the samples involved in the analysis, as follows:

```
> samples(uind)

[1] "NA12878" "NA12891" "NA12892"

> samples(uind) <- c("NA12891", "NA12892")
> uind

VariantFiltering results object

Genome version(s): "hg19"(NCBI)
Number of individuals: 2 (NA12891, NA12892)
Variants segregate according to a(n) unrelated individuals inheritance model
Quality filters
  PASS INDELQual  LowQual  SNPQual
  TRUE      TRUE    TRUE    TRUE
Functional annotation filters
  variantType      dbSNP      location      consequence
  FALSE           FALSE      FALSE         FALSE
  codonusageFC      OMIM      aaChangeType  maxMAF
  FALSE           FALSE      FALSE         FALSE
```

## VariantFiltering: filtering of coding and non-coding genetic variants

```
genePhyloStratum      S0terms
                   FALSE      FALSE

> uindS02sam <- summary(uind, method="S0full")
> uindS02sam[uindS02sam$Level == 6, ]
```

	S0ID	Level	Description	Nr. Variants	% Variants
17	S0:0001968	6	coding_transcript_variant	50	17.18
18	S0:0001791	6	exon_variant	50	17.18
22	S0:0001627	6	intron_variant	233	80.07
23	S0:0001568	6	splicing_variant	7	2.41

As we can see, restricting the samples for filtering variants results in fewer variants. We can set the original samples back with the function `resetSamples()`:

```
> uind <- resetSamples(uind)
> uind
```

VariantFiltering results object

Genome version(s): "hg19" (NCBI)  
Number of individuals: 3 (NA12878, NA12891, NA12892)  
Variants segregate according to a(n) unrelated individuals inheritance model  
Quality filters

PASS	INDELQual	LowQual	SNPQual
TRUE	TRUE	TRUE	TRUE

Functional annotation filters

variantType	dbSNP	location	consequence
FALSE	FALSE	FALSE	FALSE
codonusageFC	OMIM	aaChangeType	maxMAF
FALSE	FALSE	FALSE	FALSE

genePhyloStratum S0terms  
FALSE FALSE

The rest of the filtering operations we can perform on a *VariantFilteringResults* objects are implemented through the *FilterRules* class which implements a general mechanism for generating logical masks to filter vector-like objects; consult its manual page at the [IRanges](#) package for full technical details.

The *Variantfiltering* package provides a number of default filters, which can be extended by the user. To see which are these filters we just have to use the `filters()` function:

```
> filters(uind)
```

FilterRules of length 15  
names(15): PASS INDELQual LowQual SNPQual ... maxMAF genePhyloStratum S0terms

Filters may be active or inactive. Only active filters will participate in the filtering process when we interrogate for variants. To know what filters are active we should use the `active()` function as follows:

```
> active(filters(uind))
```

PASS	INDELQual	LowQual	SNPQual
TRUE	TRUE	TRUE	TRUE

## VariantFiltering: filtering of coding and non-coding genetic variants

variantType	dbSNP	location	consequence
FALSE	FALSE	FALSE	FALSE
codonusageFC	OMIM	aaChangeType	maxMAF
FALSE	FALSE	FALSE	FALSE
maxMAF	genePhyloStratum	S0terms	
FALSE	FALSE	FALSE	

By default, all filters are always inactive. To activate all of them, we can simply type:

```
> active(filters(uind)) <- TRUE
> active(filters(uind))
```

PASS	INDELQual	LowQual	SNPQual
TRUE	TRUE	TRUE	TRUE
variantType	dbSNP	location	consequence
TRUE	TRUE	TRUE	TRUE
codonusageFC	OMIM	aaChangeType	maxMAF
TRUE	TRUE	TRUE	TRUE
maxMAF	genePhyloStratum	S0terms	
TRUE	TRUE	TRUE	

```
> summary(uind)
```

	S0ID	Description	Nr. Variants	% Variants
1	S0:0001631	upstream_gene_variant	1	7.1
2	S0:0001624	3_prime_UTR_variant	1	7.1
3	S0:0001623	5_prime_UTR_variant	3	21.4
4	S0:0001575	splice_donor_variant	1	7.1
5	S0:0001627	intron_variant	2	14.3
6	S0:0001819	synonymous_variant	14	100.0

To deactivate all of them back and selectively activate one of them, we should use the bracket `[]` notation, as follows:

```
> active(filters(uind)) <- FALSE
> active(filters(uind))["dbSNP"] <- TRUE
> summary(uind)
```

	S0ID	Description	Nr. Variants	% Variants
1	S0:0002012	start_lost	1	0.28
2	S0:0001631	upstream_gene_variant	55	15.15
3	S0:0001624	3_prime_UTR_variant	16	4.41
4	S0:0001623	5_prime_UTR_variant	11	3.03
5	S0:0001587	stop_gained	2	0.55
6	S0:0001583	missense_variant	18	4.96
7	S0:0001575	splice_donor_variant	3	0.83
8	S0:0001574	splice_acceptor_variant	6	1.65
9	S0:0001627	intron_variant	296	81.54
10	S0:0001819	synonymous_variant	16	4.41



## VariantFiltering: filtering of coding and non-coding genetic variants

The previous filter just selects variants with an annotated dbSNP identifier. However, other filters may require cutoff values to decide what variants pass the filter. To set those values we can use the function `cutoffs()`. For instance, in the case of the `S0terms` filter, we should use set the cutoff values to select variants annotated to specific SO terms. Here we select, for instance, those annotated in UTR regions:

```
> change(cutoffs(uind), "S0terms") <- "UTR_variant"
> active(filters(uind))["S0terms"] <- TRUE
> summary(uind)

[1] S0ID          Description  Nr. Variants % Variants
<0 rows> (or 0-length row.names)

> summary(uind, method="S0full")

      S0ID Level          Description Nr. Variants % Variants
1  SO:0001624    8      3_prime_UTR_variant          16         59
2  SO:0001623    8      5_prime_UTR_variant          11         41
3  SO:0001622    7              UTR_variant           27        100
4  SO:0001968    6 coding_transcript_variant           27        100
5  SO:0001791    6              exon_variant           27        100
6  SO:0001576    5      transcript_variant           27        100
7  SO:0001564    4              gene_variant           27        100
8  SO:0001878    3      feature_variant           27        100
9  SO:0001537    2      structural_variant           27        100
10 SO:0001060    1      sequence_variant           27        100
11 SO:0002072    0      sequence_comparison           27        100
```

Note that the first call to `summary()` did not report any variant since there are no variants annotated to the SO term `UTR_variant`. However, when using the argument `method="S0full"`, all variants annotated to more specific SO terms in the hierarchy will be reported.

The methods `filters()` and `cutoffs()` can be employed to extend the filtering functionality. Here we show a simple example in which we add a filter to detect the loss of the codon that initiates translation. This constitutes already a feature annotated by [VariantFiltering](#) so that we can verify that it works:

```
> startLost <- function(x) {
+   mask <- start(allVariants(x, groupBy="nothing")$CDSLOC) == 1 &
+     as.character(allVariants(x, groupBy="nothing")$REFCODON) == "ATG" &
+     as.character(allVariants(x, groupBy="nothing")$VARCODON) != "ATG"
+   mask
+ }
> filters(uind)$startLost <- startLost
> active(filters(uind)) <- FALSE
> active(filters(uind))["startLost"] <- TRUE
> active(filters(uind))

      PASS      INDELQual      LowQual      SNPQual
FALSE      FALSE      FALSE      FALSE
variantType  dbSNP      location  consequence
FALSE      FALSE      FALSE      FALSE
codonusageFC OMIM      aaChangeType  maxMAF
FALSE      FALSE      FALSE      FALSE
maxMAF genePhyloStratum S0terms      startLost
```

## VariantFiltering: filtering of coding and non-coding genetic variants

```
FALSE          FALSE          FALSE          TRUE
> summary(uind)
      SOID      Description Nr. Variants % Variants
1 S0:0002012      start_lost          1         100
2 S0:0001631 upstream_gene_variant          1         100
3 S0:0001623  5_prime_UTR_variant          1         100
4 S0:0001583      missense_variant          1         100
5 S0:0001575 splice_donor_variant          1         100
6 S0:0001627      intron_variant          1         100
```

As we can see, our filter works as expected and selects the only variant which was annotated with the SO term `start_lost`. Note that there is also an additional annotation indicating this variant belongs to an UTR region resulting from an alternative CDS.

Properly updating cutoff values may be problematic if we do not know how exactly are they employed by the corresponding filters. To facilitate setting the right cutoff values we use the `change()` method and we illustrate it on the filter of minor allele frequency (MAF) values:

```
> active(filters(uind)) <- FALSE
> active(filters(uind))["maxMAF"] <- TRUE
> cutoffs(uind)$maxMAF
CutoffsList of length 2
names(2): popmask maxvalue
> cutoffs(uind)$maxMAF$popmask
      AFKGp1 AFR_AFKGp1 AMR_AFKGp1 ASN_AFKGp1 EUR_AFKGp1
      TRUE      TRUE      TRUE      TRUE      TRUE
> cutoffs(uind)$maxMAF$maxvalue
[1] 0.5
> change(cutoffs(uind)$maxMAF, "popmask") <- FALSE
> cutoffs(uind)$maxMAF$popmask
      AFKGp1 AFR_AFKGp1 AMR_AFKGp1 ASN_AFKGp1 EUR_AFKGp1
      FALSE      FALSE      FALSE      FALSE      FALSE
> change(cutoffs(uind)$maxMAF, "popmask") <- c(AFKGp1=TRUE)
> cutoffs(uind)$maxMAF$popmask
      AFKGp1 AFR_AFKGp1 AMR_AFKGp1 ASN_AFKGp1 EUR_AFKGp1
      TRUE      FALSE      FALSE      FALSE      FALSE
> change(cutoffs(uind)$maxMAF, "maxvalue") <- 0.01
> summary(uind)
      SOID      Description Nr. Variants % Variants
1 S0:0001631 upstream_gene_variant          23         18.4
2 S0:0001624  3_prime_UTR_variant           3           2.4
3 S0:0001623  5_prime_UTR_variant           5           4.0
4 S0:0001587      stop_gained              1           0.8
5 S0:0001583      missense_variant          6           4.8
6 S0:0001575 splice_donor_variant          1           0.8
```

## VariantFiltering: filtering of coding and non-coding genetic variants

7	S0:0001574	splice_acceptor_variant	3	2.4
8	S0:0001627	intron_variant	102	81.6
9	S0:0001819	synonymous_variant	4	3.2

In this case we selected variants with  $MAF < 0.01$  in the global population of the 1000 Genomes project. If we are interested in retrieving the actual set of filtered variants, we can do it using the function `filteredVariants()`:

```
> filteredVariants(uind)
VRangesList of length 3
names(3): NA12878 NA12891 NA12892
```

To further understand how to manipulate *Vranges* and *VRangesList* objects, please consult the package [VariantAnnotation](#).

## 5 Inheritance models

We can filter upfront variants that do not segregate according to a given inheritance model. In such a case, we also need to provide a PED file at the time we build the parameter object, as follows:

```
> CEUped <- file.path(system.file("extdata", package="VariantFiltering"),
+                     "CEUtrio.ped")
> param <- VariantFilteringParam(vcfFileNames=CEUvcf, pedFilename=CEUped)
```

Here we are using a PED file included in the [VariantFiltering](#) package and specifying information about the CEU trio employed in this vignette.

To use an inheritance model we need to replace the previous call to `unrelatedIndividuals()` by one specific to the inheritance model. The [VariantFiltering](#) package offers 5 possible ones:

- **Autosomal recessive inheritance analysis: Homozygous.**

Homozygous variants responsible for a recessive trait in the affected individuals can be identified calling the `autosomalRecessiveHomozygous()` function. This function selects homozygous variants that are present in all the affected individuals and occur in heterozygosity in the unaffected ones.

- **Autosomal recessive inheritance analysis: Heterozygous.**

To filter by this mode of inheritance, also known as compound heterozygous, we need two unaffected parents/ancestors and at least one affected descendant. Variants are filtered in five steps: 1. select heterozygous variants in one of the parents and homozygous in the other; 2. discard previously selected variants that are common between the two parents; 3. group variants by gene; 4. select those genes, and the variants that occur within them, which have two or more variants and there is at least one from each parent; 5. from the previously selected variants, discard those that do not occur in the affected descendants. This is implemented in the function `autosomalRecessiveHeterozygous()`.

- **Autosomal dominant inheritance analysis.**

The function `autosomalDominant()` identifies variants present in all the affected individual(s) discarding the ones that also occur in at least one of the unaffected subjects.

## VariantFiltering: filtering of coding and non-coding genetic variants

- **X-Linked inheritance analysis.**

The function `xLinked()` identifies variants that appear only in the X chromosome of the unaffected females as heterozygous, don't appear in the unaffected males analyzed and finally are present (as homozygous) in the affected male(s). This function is currently restricted to affected males, and therefore, it cannot search for X-linked segregating variants affecting daughters.

- **De Novo variants analysis**

The function `deNovo()` searches for *de novo* variants which are present in one descendant and present in both parents/ancestors. It is currently restricted to a trio of individuals.

## 6 Create a report from the filtered variants

The function `reportVariants()` allows us to easily create a report from the filtered variants into a CSV or a TSV file as follows:

```
> reportVariants(uind, type="csv", file="uind.csv")
```

The default value on the `type` argument ("`shiny`") starts a shiny web app which allows one to interactively filter the variants, obtaining an updated `VariantFilteringResults` object and downloading the filtered variants and the corresponding full reproducible R code, if necessary.

VarID	GENE	HGVS	CONSEQUENCE	AAchange	AAchangeType	PolyPhen2	PROVEAN	
1	rs11699009	BPIFB4	p.527F>L	nonsynonymous	F527L	Conservative	benign	Neutral
2	rs2839223	PCNT	p.704G>E	nonsynonymous	G704E	Radical	benign	Neutral
3	rs16989291	SF11	p.306W>R	nonsynonymous	W306R	Radical	probably damaging	Deleterious
4	rs79478	PARVG	p.130C>R	nonsynonymous	C130R	Radical	NA	Neutral
5	rs3764880	TLR8	p.1M>V	nonsynonymous	M1V	Conservative	possibly damaging	Neutral

**Figure 2: Snapshot of the shiny web app run from VariantFiltering with the function `reportVariants()`** Some of the parameters has been filled for illustrative purposes.

## 7 Using the package with parallel execution

Functions in `VariantFiltering` to annotate and filter variants leverage the functionality of the Bioconductor package `BiocParallel` to perform in parallel some of the tasks and calculations and reduce the overall execution time. These functions have an argument called `BPPARAM` that allows the user to control how this parallelism is exploited. In particular the user must give

## VariantFiltering: filtering of coding and non-coding genetic variants

as value to this argument the result from a call to the function `bpparam()`, which actually is its default behavior. Here below we modify that behavior to force a call being executed without parallelism. The interested reader should consult the help page of `bpparam()` and the vignette of the *BiocParallel* for further information.

## 8 Session information

```
> toLatex(sessionInfo())
```

- R version 4.1.1 (2021-08-10), x86\_64-pc-linux-gnu
- Locale: LC\_CTYPE=en\_US.UTF-8, LC\_NUMERIC=C, LC\_TIME=en\_GB, LC\_COLLATE=C, LC\_MONETARY=en\_US.UTF-8, LC\_MESSAGES=en\_US.UTF-8, LC\_PAPER=en\_US.UTF-8, LC\_NAME=C, LC\_ADDRESS=C, LC\_TELEPHONE=C, LC\_MEASUREMENT=en\_US.UTF-8, LC\_IDENTIFICATION=C
- Running under: Ubuntu 20.04.3 LTS
- Matrix products: default
- BLAS: /home/biocbuild/bbs-3.14-bioc/R/lib/libRblas.so
- LAPACK: /home/biocbuild/bbs-3.14-bioc/R/lib/libRlapack.so
- Base packages: base, datasets, grDevices, graphics, methods, stats, stats4, utils
- Other packages: AnnotationDbi 1.56.0, BSgenome 1.62.0, BSgenome.Hsapiens.1000genomes.hs37d5 0.99.1, Biobase 2.54.0, BiocGenerics 0.40.0, Biostrings 2.62.0, GenomInfoDb 1.30.0, GenomicFeatures 1.46.0, GenomicRanges 1.46.0, GenomicScores 2.6.0, IRanges 2.28.0, MafDb.1Kgenomes.phase1.hs37d5 3.10.0, MatrixGenerics 1.6.0, PolyPhen.Hsapiens.dbSNP131 1.0.2, RSQLite 2.2.8, Rsamtools 2.10.0, S4Vectors 0.32.0, SIFT.Hsapiens.dbSNP137 1.0.0, SNPlocs.Hsapiens.dbSNP144.GRCh37 0.99.20, SummarizedExperiment 1.24.0, TxDb.Hsapiens.UCSC.hg19.knownGene 3.2.2, VariantAnnotation 1.40.0, VariantFiltering 1.30.0, XVector 0.34.0, matrixStats 0.61.0, org.Hs.eg.db 3.14.0, phastCons100way.UCSC.hg19 3.7.2, rtracklayer 1.54.0
- Loaded via a namespace (and not attached): AnnotationFilter 1.18.0, AnnotationHub 3.2.0, BiocFileCache 2.2.0, BiocIO 1.4.0, BiocManager 1.30.16, BiocParallel 1.28.0, BiocStyle 2.22.0, BiocVersion 3.14.0, DBI 1.1.1, DT 0.19, DelayedArray 0.20.0, Formula 1.2-4, GenomInfoDbData 1.2.7, GenomicAlignments 1.30.0, Gviz 1.38.0, HDF5Array 1.22.0, Hmisc 4.6-0, KEGGREST 1.34.0, Matrix 1.3-4, ProtGenerics 1.26.0, R6 2.5.1, RBGL 1.70.0, RColorBrewer 1.1-2, RCurl 1.98-1.5, Rcpp 1.0.7, Rhdf5lib 1.16.0, XML 3.99-0.8, assertthat 0.2.1, backports 1.2.1, base64enc 0.1-3, biomaRt 2.50.0, biovizBase 1.42.0, bit 4.0.4, bit64 4.0.5, bitops 1.0-7, blob 1.2.2, cachem 1.0.6, checkmate 2.0.0, cluster 2.1.2, colorspace 2.0-2, compiler 4.1.1, crayon 1.4.1, curl 4.3.2, data.table 1.14.2, dbplyr 2.1.1, dichromat 2.0-0, digest 0.6.28, dplyr 1.0.7, ellipsis 0.3.2, ensemblDb 2.18.0, evaluate 0.14, fansi 0.5.0, fastmap 1.1.0, filelock 1.0.2, foreign 0.8-81, generics 0.1.1, ggplot2 3.3.5, glue 1.4.2, graph 1.72.0, grid 4.1.1, gridExtra 2.3, gtable 0.3.0, hms 1.1.1, htmlTable 2.3.0, htmltools 0.5.2, htmlwidgets 1.5.4, httpuv 1.6.3, httr 1.4.2, interactiveDisplayBase 1.32.0, jpeg 0.1-9, jsonlite 1.7.2, knitr 1.36, later 1.3.0, lattice 0.20-45, latticeExtra 0.6-29,

## VariantFiltering: filtering of coding and non-coding genetic variants

lazyeval 0.2.2, lifecycle 1.0.1, magrittr 2.0.1, memoise 2.0.0, mime 0.12, munsell 0.5.0, nnet 7.3-16, parallel 4.1.1, pillar 1.6.4, pkgconfig 2.0.3, png 0.1-7, prettyunits 1.1.1, progress 1.2.2, promises 1.2.0.1, purrr 0.3.4, rappdirs 0.3.3, restfulr 0.0.13, rhdf5 2.38.0, rhdf5filters 1.6.0, rjson 0.2.20, rlang 0.4.12, rmarkdown 2.11, rpart 4.1-15, rstudioapi 0.13, scales 1.1.1, shiny 1.7.1, shinyTree 0.2.7, shinyjs 2.0.0, shinythemes 1.2.0, splines 4.1.1, stringi 1.7.5, stringr 1.4.0, survival 3.2-13, tibble 3.1.5, tidyselect 1.1.1, tools 4.1.1, utf8 1.2.2, vctrs 0.3.8, xfun 0.27, xml2 1.3.2, xtable 1.8-4, yaml 2.2.1, zlibbioc 1.40.0