VariantFiltering: filtering of coding and non-coding genetic variants

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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 or 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.

VariantFiltering exploits the R/Bioconductor infrastructure to read and stream over input files and to annotate the input variants with diverse functional information. A core set of functional annotations are generated by *VariantFiltering* but this set can be modified and extended using Bioconductor annotation packages such as *MafDb.ALL.wgs.phase3.release.v5a.20130502*, which stores and exposes to the user minimum allele frequency (MAF) values frozen from the latest realease of the 1000 Genomes project.

The main input is a multisample Variant Call Format (VCF) file which is parsed using the functionality from the *VariantAnnotation* package for that purpose. This functionality allows also *VariantFiltering* to stream over large VCF files to reduce the memory footprint.

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:

```
> library(VariantFiltering)
> CEUvcf <- file.path(system.file("extdata", package="VariantFiltering"), "CEUtrio.vcf.bgz")
> vfpar <- VariantFilteringParam(vcfFilenames=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.UCSC.hg19 (UCSC hg19 Genome Reference Consortium GR
  Variant-centric annotation package: SNPlocs.Hsapiens.dbSNP.20120608 (dbSNP Build 137)
  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
  Other annotation pkg/obj: MafDb.ESP6500SI.V2.SSA137,
                            MafDb.ALL.wgs.phase1.release.v3.20101123,
                            MafDb.ExAC.r0.3.sites,
                            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 overriden when calling the construction function. To quickly see all the available arguments and their default values we should type:

```
> args(VariantFilteringParam)
```

```
function (vcfFilenames, pedFilename = NA_character_, bsgenome = "BSgenome.Hsapiens.UCSC.hg19",
    orgdb = "org.Hs.eg.db", txdb = "TxDb.Hsapiens.UCSC.hg19.knownGene",
    snpdb = "SNPlocs.Hsapiens.dbSNP.20120608", spliceSiteMatricesFilenames = NA,
    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, otherAnnotations = c("MafDb.ESP6500SI.V2.SSA137",
        "MafDb.ALL.wgs.phase1.release.v3.20101123", "MafDb.ExAC.r0.3.sites",
        "PolyPhen.Hsapiens.dbSNP131", "SIFT.Hsapiens.dbSNP137",
        "phastCons100way.UCSC.hg19", "humanGenesPhylostrata"),
    geneKeytype = NA_character_, yieldSize = NA_integer_)
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
                     INDELQual                          LowQual
                          TRUE                             TRUE
                       SNPQual    VQSRTrancheINDEL99.00to99.90
                          TRUE                             TRUE
VQSRTrancheINDEL99.90to100.00+  VQSRTrancheINDEL99.90to100.00
                          TRUE                             TRUE
    VQSRTrancheSNP99.00to99.90   VQSRTrancheSNP99.90to100.00+
                          TRUE                             TRUE
   VQSRTrancheSNP99.90to100.00
                          TRUE
  Functional annotation filters
       dbSNP         OMIM  variantType aaChangeType      SOterms
       FALSE        FALSE        FALSE        FALSE        FALSE
    Populations used for MAF filtering: AFESP, EA_AFESP, AA_AFESP, AFKG, AMR_AFKG, ASN_AFKG, AFR_AFKG, EUR_
    Include MAF NA values: yes
    Maximum MAF: 1.00
```

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  SO:0001629   splice_site_variant            2       0.33
2  SO:0001583      missense_variant           15       2.45
3  SO:0002012            start_lost            1       0.16
4  SO:0001587           stop_gained            1       0.16
5  SO:0001624   3_prime_UTR_variant           16       2.62
6  SO:0001623   5_prime_UTR_variant            7       1.15
7  SO:0001631 upstream_gene_variant           43       7.04
8  SO:0001627        intron_variant          308      50.41
9  SO:0001819    synonymous_variant           24       3.93
10 SO:0001628    intergenic_variant          268      43.86
11 SO:0001589    frameshift_variant            1       0.16
```

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
1     spliceSite            2       0.33
2         intron          308      50.41
3        fiveUTR            7       1.15
4       threeUTR           16       2.62
5         coding           41       6.71
6     intergenic          268      43.86
7       promoter           43       7.04
8     frameshift            1       0.16
9       nonsense            1       0.16
10 nonsynonymous           15       2.45
11    synonymous           24       3.93
```

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:0001629     6       splice_site_variant            2       0.33
2  SO:0001568     5          splicing_variant            2       0.33
3  SO:0001624     7       3_prime_UTR_variant           16       2.62
4  SO:0001627     5            intron_variant          308      50.41
5  SO:0001623     7       5_prime_UTR_variant            7       1.15
6  SO:0001622     6               UTR_variant           23       3.76
7  SO:0001583    10          missense_variant           15       2.45
8  SO:0002012     8                start_lost            1       0.16
9  SO:0001582     7   initiator_codon_variant            1       0.16
10 SO:0001589     8        frameshift_variant            1       0.16
11 SO:0001587     4               stop_gained            1       0.16
12 SO:0001906     3        feature_truncation            1       0.16
13 SO:0001992     9     nonsynonymous_variant           16       2.62
14 SO:0001650     8           inframe_variant           16       2.62
15 SO:0001818     7  protein_altering_variant           17       2.78
16 SO:0001631     4     upstream_gene_variant           43       7.04
17 SO:0001628     3        intergenic_variant          299      48.94
18 SO:0001819     7        synonymous_variant           24       3.93
19 SO:0001580     6   coding_sequence_variant           41       6.71
20 SO:0001968     5 coding_transcript_variant           59       9.66
21 SO:0001791     5              exon_variant           59       9.66
22 SO:0001576     4        transcript_variant          353      57.77
23 SO:0001564     3              gene_variant          353      57.77
24 SO:0001878     2           feature_variant          611     100.00
25 SO:0001537     1        structural_variant          611     100.00
26 SO:0001060     0          sequence_variant          611     100.00
```

Here the `Level` column refers to the shortest-path distance to the most general SO term `sequence_variant` within the SO ayclic 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
1  SO:0001629     6     splice_site_variant            2       0.33
6  SO:0001622     6             UTR_variant           23       3.76
19 SO:0001580     6 coding_sequence_variant           41       6.71
```

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
> bamFiles(uind)
```

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

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

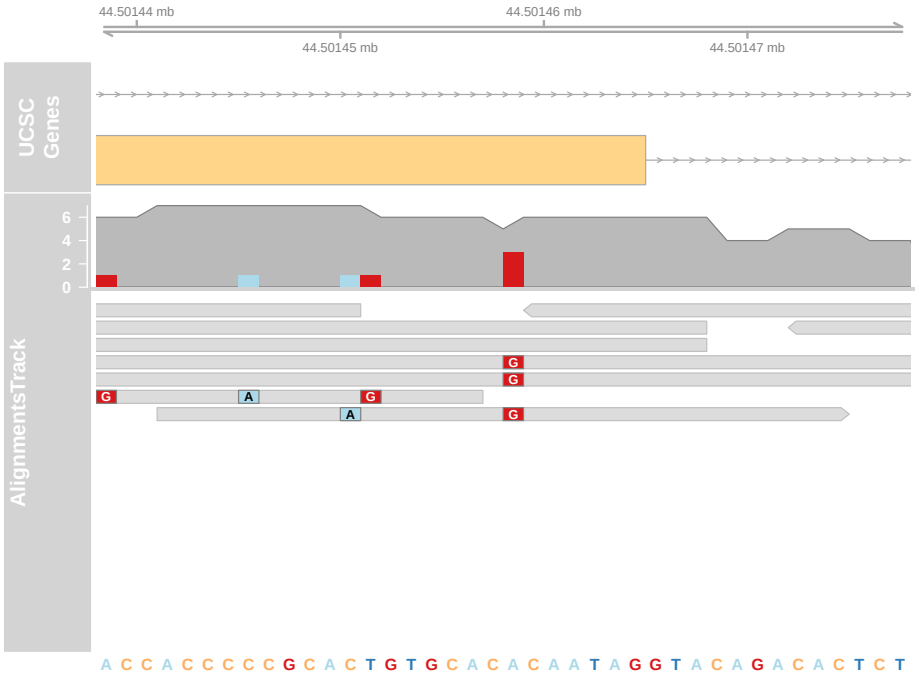


Figure 1: Browser-like display of a variant.

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
                     INDELQual                          LowQual
                          TRUE                             TRUE
                       SNPQual   VQSRTrancheINDEL99.00to99.90
                          TRUE                             TRUE
VQSRTrancheINDEL99.90to100.00+  VQSRTrancheINDEL99.90to100.00
                          TRUE                             TRUE
    VQSRTrancheSNP99.00to99.90   VQSRTrancheSNP99.90to100.00+
                          TRUE                             TRUE
   VQSRTrancheSNP99.90to100.00
                          TRUE
  Functional annotation filters
       dbSNP         OMIM  variantType aaChangeType      SOterms
       FALSE        FALSE        FALSE        FALSE        FALSE
    Populations used for MAF filtering: AFESP, EA_AFESP, AA_AFESP, AFKG, AMR_AFKG, ASN_AFKG, AFR_AFKG, EUR_
    Include MAF NA values: yes
    Maximum MAF: 1.00
```

```
> uindSO2sam <- summary(uind, method="SOfull")
> uindSO2sam[uindSO2sam$Level == 6, ]
```

```
         SOID Level             Description Nr. Variants % Variants
1  SO:0001629     6     splice_site_variant            1       0.19
6  SO:0001622     6             UTR_variant           20       3.75
19 SO:0001580     6 coding_sequence_variant           41       7.69
```

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
                     INDELQual                          LowQual
                          TRUE                             TRUE
                       SNPQual   VQSRTrancheINDEL99.00to99.90
```

```
                          TRUE                                TRUE
VQSRTrancheINDEL99.90to100.00+  VQSRTrancheINDEL99.90to100.00
                          TRUE                                TRUE
    VQSRTrancheSNP99.00to99.90   VQSRTrancheSNP99.90to100.00+
                          TRUE                                TRUE
   VQSRTrancheSNP99.90to100.00
                          TRUE
  Functional annotation filters
       dbSNP         OMIM  variantType aaChangeType      SOterms
       FALSE        FALSE        FALSE        FALSE        FALSE
    Populations used for MAF filtering: AFESP, EA_AFESP, AA_AFESP, AFKG, AMR_AFKG, ASN_AFKG, AFR_AFKG, EUR_
    Include MAF NA values: yes
    Maximum MAF: 1.00
```

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 14
names(14): INDELQual LowQual SNPQual ... variantType aaChangeType SOterms
```

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))
```

```
                     INDELQual                       LowQual
                          TRUE                          TRUE
                       SNPQual  VQSRTrancheINDEL99.00to99.90
                          TRUE                          TRUE
VQSRTrancheINDEL99.90to100.00+  VQSRTrancheINDEL99.90to100.00
                          TRUE                          TRUE
    VQSRTrancheSNP99.00to99.90   VQSRTrancheSNP99.90to100.00+
                          TRUE                          TRUE
   VQSRTrancheSNP99.90to100.00                         dbSNP
                          TRUE                         FALSE
                          OMIM                   variantType
                         FALSE                         FALSE
                  aaChangeType                       SOterms
                         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))
```

```
                     INDELQual                       LowQual
                          TRUE                          TRUE
                       SNPQual  VQSRTrancheINDEL99.00to99.90
                          TRUE                          TRUE
VQSRTrancheINDEL99.90to100.00+  VQSRTrancheINDEL99.90to100.00
                          TRUE                          TRUE
    VQSRTrancheSNP99.00to99.90   VQSRTrancheSNP99.90to100.00+
                          TRUE                          TRUE
```

```
   VQSRTrancheSNP99.90to100.00                        dbSNP
                          TRUE                         TRUE
                          OMIM                  variantType
                          TRUE                         TRUE
                 aaChangeType                      SOterms
                          TRUE                         TRUE
```

```
> summary(uind)
```

```
        SOID           Description Nr. Variants % Variants
1 SO:0001583      missense_variant            7       3.07
2 SO:0002012            start_lost            1       0.44
3 SO:0001624   3_prime_UTR_variant            7       3.07
4 SO:0001623   5_prime_UTR_variant            5       2.19
5 SO:0001631 upstream_gene_variant           29      12.72
6 SO:0001627        intron_variant          194      85.09
7 SO:0001819    synonymous_variant           20       8.77
8 SO:0001628    intergenic_variant            6       2.63
```

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)
```

```
         SOID           Description Nr. Variants % Variants
1  SO:0001629   splice_site_variant            1       0.12
2  SO:0001583      missense_variant           18       2.25
3  SO:0002012            start_lost            1       0.12
4  SO:0001587           stop_gained            1       0.12
5  SO:0001624   3_prime_UTR_variant           21       2.62
6  SO:0001623   5_prime_UTR_variant            7       0.88
7  SO:0001631 upstream_gene_variant           49       6.12
8  SO:0001627        intron_variant          361      45.12
9  SO:0001819    synonymous_variant           27       3.38
10 SO:0001628    intergenic_variant          406      50.75
```

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 `SOterms` 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:

```
> cutoffs(uind)$SOterms <- "UTR_variant"
> active(filters(uind))["SOterms"] <- TRUE
> summary(uind)
```

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

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

```
         SOID Level               Description Nr. Variants % Variants
1  SO:0001624     7       3_prime_UTR_variant           21         75
2  SO:0001623     7       5_prime_UTR_variant            7         25
3  SO:0001622     6               UTR_variant           28        100
4  SO:0001968     5 coding_transcript_variant           28        100
5  SO:0001791     5              exon_variant           28        100
6  SO:0001576     4        transcript_variant           28        100
7  SO:0001564     3              gene_variant           28        100
```

```
8  SO:0001878     2           feature_variant           28        100
9  SO:0001537     1        structural_variant           28        100
10 SO:0001060     0          sequence_variant           28        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="SOfull"`, 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))
```

```
                     INDELQual                         LowQual
                         FALSE                           FALSE
                       SNPQual   VQSRTrancheINDEL99.00to99.90
                         FALSE                           FALSE
VQSRTrancheINDEL99.90to100.00.  VQSRTrancheINDEL99.90to100.00
                         FALSE                           FALSE
    VQSRTrancheSNP99.00to99.90   VQSRTrancheSNP99.90to100.00.
                         FALSE                           FALSE
   VQSRTrancheSNP99.90to100.00                           dbSNP
                         FALSE                           FALSE
                          OMIM                     variantType
                         FALSE                           FALSE
                  aaChangeType                         SOterms
                         FALSE                           FALSE
                     startLost
                          TRUE
```

```
> summary(uind)
```

```
        SOID           Description Nr. Variants % Variants
1 SO:0001583      missense_variant            1        100
2 SO:0002012           start_lost             1        100
3 SO:0001623   5_prime_UTR_variant            1        100
4 SO:0001631 upstream_gene_variant            1        100
5 SO: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 the help page of the *VariantFilteringResults* class contains a list of available accessor methods to update them. Here we illustrate the use of one of them, the one controlling minimum allele frequency (MAF) values:

```
> active(filters(uind)) <- FALSE
> MAFmask <- MAFpop(uind)
> MAFmask
```

```
     AFESP   EA_AFESP   AA_AFESP       AFKG   AMR_AFKG   ASN_AFKG   AFR_AFKG
      TRUE       TRUE       TRUE       TRUE       TRUE       TRUE       TRUE
  EUR_AFKG     AFExAC AFR_AFExAC AMR_AFExAC Adj_AFExAC EAS_AFExAC FIN_AFExAC
      TRUE       TRUE       TRUE       TRUE       TRUE       TRUE       TRUE
NFE_AFExAC OTH_AFExAC SAS_AFExAC
      TRUE       TRUE       TRUE
```

```
> MAFpop(uind) <- !MAFmask
> MAFpop(uind, "ASN_AFKG") <- TRUE
> MAFpop(uind)
```

```
     AFESP   EA_AFESP   AA_AFESP       AFKG   AMR_AFKG   ASN_AFKG   AFR_AFKG
     FALSE      FALSE      FALSE      FALSE      FALSE       TRUE      FALSE
  EUR_AFKG     AFExAC AFR_AFExAC AMR_AFExAC Adj_AFExAC EAS_AFExAC FIN_AFExAC
     FALSE      FALSE      FALSE      FALSE      FALSE      FALSE      FALSE
NFE_AFExAC OTH_AFExAC SAS_AFExAC
     FALSE      FALSE      FALSE
```

```
> maxMAF(uind) <- 0.01
> summary(uind)
```

```
        SOID           Description Nr. Variants % Variants
1 SO:0001583      missense_variant            7       2.55
2 SO:0001624   3_prime_UTR_variant            7       2.55
3 SO:0001631 upstream_gene_variant           11       4.00
4 SO:0001627        intron_variant          130      47.27
5 SO:0001819    synonymous_variant            5       1.82
6 SO:0001628    intergenic_variant          138      50.18
7 SO:0001589    frameshift_variant            1       0.36
```

In this case we selected variants with MAF< 0.01 in the asian 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 int his vignette.

To use an inherintance 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.
- **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")
```

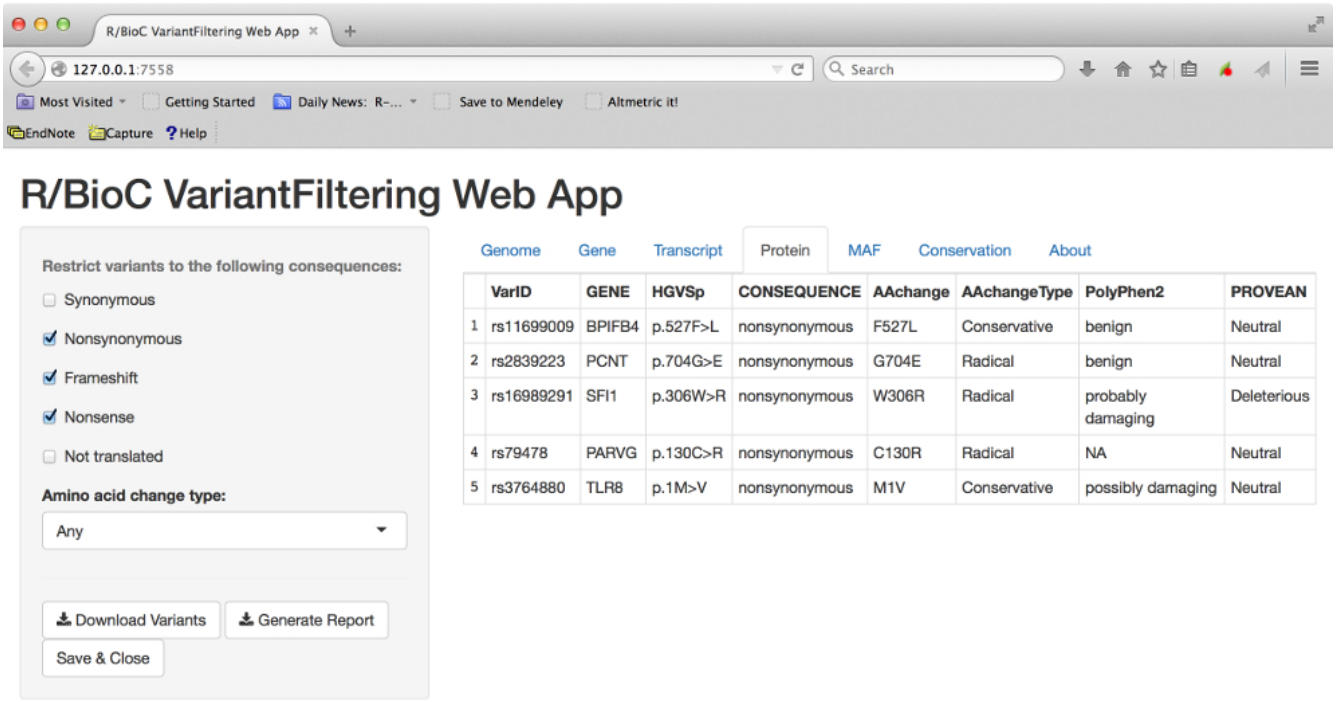
The default value on the `type` argument (`"shiny"`) starts a shiny web app which allows one to interactively filter the variants, obtaining an udpated *VariantFilteringResults* object and downloading the filtered variants and the corresponding full reproducible R code, if necessary.

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 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())
```



	VarID	GENE	HGVSp	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	SFI1	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.

- R version 3.2.0 Patched (2015-04-23 r68254), `x86_64-apple-darwin13.4.0`
- Locale: `C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8`
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: AnnotationDbi 1.30.1, BSgenome 1.36.0, BSgenome.Hsapiens.UCSC.hg19 1.4.0, Biobase 2.28.0, BiocGenerics 0.14.0, Biostrings 2.36.1, DBI 0.3.1, GenomeInfoDb 1.4.0, GenomicFeatures 1.20.1, GenomicRanges 1.20.3, IRanges 2.2.1, MafDb.ALL.wgs.phase1.release.v3.20101123 3.1.0, MafDb.ESP6500SI.V2.SSA137 3.1.0, MafDb.ExAC.r0.3.sites 3.1.0, PolyPhen.Hsapiens.dbSNP131 1.0.2, RSQLite 1.0.0, Rsamtools 1.20.2, S4Vectors 0.6.0, SIFT.Hsapiens.dbSNP137 1.0.0, SNPlocs.Hsapiens.dbSNP.20120608 0.99.9, TxDb.Hsapiens.UCSC.hg19.knownGene 3.1.2, VariantAnnotation 1.14.1, VariantFiltering 1.4.3, XVector 0.8.0, org.Hs.eg.db 3.1.2, phastCons100way.UCSC.hg19 3.1.0, rtracklayer 1.28.3
- Loaded via a namespace (and not attached): BiocParallel 1.2.2, BiocStyle 1.6.0, Formula 1.2-1, GenomicAlignments 1.4.1, Gviz 1.12.0, Hmisc 3.16-0, MASS 7.3-40, R6 2.0.1, RBGL 1.44.0, RColorBrewer 1.1-2, RCurl 1.95-4.6, Rcpp 0.11.6, XML 3.98-1.1, acepack 1.3-3.3, biomaRt 2.24.0, biovizBase 1.16.0, bitops 1.0-6, cluster 2.0.1, colorspace 1.2-6, dichromat 2.0-0, digest 0.6.8, foreign 0.8-63, futile.logger 1.4.1, futile.options 1.0.0, ggplot2 1.0.1, graph 1.46.0, grid 3.2.0, gridExtra 0.9.1, gtable 0.1.2, htmltools 0.2.6, httpuv 1.3.2, lambda.r 1.1.7, lattice 0.20-31, latticeExtra 0.6-26, magrittr 1.5, matrixStats 0.14.0, mime 0.3, munsell 0.4.2, nnet 7.3-9, plyr 1.8.2, proto 0.3-10, reshape2 1.4.1, rpart 4.1-9, scales 0.2.4, shiny 0.12.0, splines 3.2.0, stringi 0.4-1, stringr 1.0.0, survival 2.38-1, tools 3.2.0, xtable 1.7-4, zlibbioc 1.14.0