

# Introduction to *VariantAnnotation*

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November 2, 2015

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## 1 Introduction

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This vignette outlines a work flow for annotating and filtering genetic variants using the *VariantAnnotation* package. Sample data are in VariantCall Format (VCF) and are a subset of chromosome 22 from [1000 Genomes](#). VCF text files contain meta-information lines, a header line with column names, data lines with information about a position in the genome, and optional genotype information on samples for each position. The 1000 Genomes page describes the [VCF format](#) in detail.

Data are read in from a VCF file and variants identified according to region such as coding, intron, intergenic, spliceSite etc. Amino acid coding changes are computed for the non-synonymous variants and SIFT and PolyPhen databases provide predictions of how severely the coding changes affect protein function.

## 2 Variant Call Format (VCF) files

---

### 2.1 Data import and exploration

Data are parsed into a VCF object with `readVcf`.

```
> library(VariantAnnotation)
> fl <- system.file("extdata", "chr22.vcf.gz", package="VariantAnnotation")
> vcf <- readVcf(fl, "hg19")
> vcf
```

```
class: CollapsedVCF
```

```
dim: 10376 5
```

```
rowRanges(vcf):
```

```
GRanges with 5 metadata columns: paramRangeID, REF, ALT, QUAL, FILTER
```

```
info(vcf):
```

```
DataFrame with 22 columns: LDAF, AVGPOST, RSQ, ERATE, THETA, CIEND...
```

```
info(header(vcf)):
```

|           | Number | Type    | Description                                 |
|-----------|--------|---------|---|
| LDAF      | 1      | Float   | MLE Allele Frequency Accounting for LD      |
| AVGPOST   | 1      | Float   | Average posterior probability from MaCH/... |
| RSQ       | 1      | Float   | Genotype imputation quality from MaCH/Th... |
| ERATE     | 1      | Float   | Per-marker Mutation rate from MaCH/Thunder  |
| THETA     | 1      | Float   | Per-marker Transition rate from MaCH/Thu... |
| CIEND     | 2      | Integer | Confidence interval around END for impre... |
| CIPOS     | 2      | Integer | Confidence interval around POS for impre... |
| END       | 1      | Integer | End position of the variant described in... |
| HOMLEN    | .      | Integer | Length of base pair identical micro-homo... |
| HOMSEQ    | .      | String  | Sequence of base pair identical micro-ho... |
| SVLEN     | 1      | Integer | Difference in length between REF and ALT... |
| SVTYPE    | 1      | String  | Type of structural variant                  |
| AC        | .      | Integer | Alternate Allele Count                      |
| AN        | 1      | Integer | Total Allele Count                          |
| AA        | 1      | String  | Ancestral Allele, ftp://ftp.1000genomes.... |
| AF        | 1      | Float   | Global Allele Frequency based on AC/AN      |
| AMR_AF    | 1      | Float   | Allele Frequency for samples from AMR ba... |
| ASN_AF    | 1      | Float   | Allele Frequency for samples from ASN ba... |
| AFR_AF    | 1      | Float   | Allele Frequency for samples from AFR ba... |
| EUR_AF    | 1      | Float   | Allele Frequency for samples from EUR ba... |
| VT        | 1      | String  | indicates what type of variant the line ... |
| SNPSOURCE | .      | String  | indicates if a snp was called when analy... |

```
geno(vcf):
```

```
SimpleList of length 3: GT, DS, GL
```

```
geno(header(vcf)):
```

|    | Number | Type   | Description                       |
|----|--------|--------|-----------------------------------|
| GT | 1      | String | Genotype                          |
| DS | 1      | Float  | Genotype dosage from MaCH/Thunder |
| GL | .      | Float  | Genotype Likelihoods              |

#### 2.1.1 Header information

Header information can be extracted from the VCF with `header()`. We see there are 5 samples, 1 piece of meta information, 22 info fields and 3 geno fields.

```
> header(vcf)

class: VCFHeader
samples(5): HG00096 HG00097 HG00099 HG00100 HG00101
meta(1): META
fixed(1): ALT
info(22): LDAF AVGPST ... VT SNPSOURCE
geno(3): GT DS GL
```

Data can be further extracted using the named accessors.

```
> samples(header(vcf))

[1] "HG00096" "HG00097" "HG00099" "HG00100" "HG00101"

> geno(header(vcf))
```

DataFrame with 3 rows and 3 columns

|    | Number      | Type        | Description                       |
|----|-------------|-------------|-----------------------------------|
|    | <character> | <character> | <character>                       |
| GT | 1           | String      | Genotype                          |
| DS | 1           | Float       | Genotype dosage from MaCH/Thunder |
| GL | .           | Float       | Genotype Likelihoods              |

## 2.1.2 Genomic positions

`rowRanges` contains information from the CHROM, POS, and ID fields of the VCF file, represented as a `GRanges`. The `paramRangeID` column is meaningful when reading subsets of data and is discussed further below.

```
> head(rowRanges(vcf), 3)

GRanges object with 3 ranges and 5 metadata columns:
      seqnames      ranges strand | paramRangeID
      <Rle>      <IRanges> <Rle> | <factor>
rs7410291      22 [50300078, 50300078] * | <NA>
rs147922003    22 [50300086, 50300086] * | <NA>
rs114143073    22 [50300101, 50300101] * | <NA>
      REF          ALT          QUAL          FILTER
      <DNAStringSet> <DNAStringSetList> <numeric> <character>
rs7410291          A          G          100          PASS
rs147922003        C          T          100          PASS
rs114143073        G          A          100          PASS
-----
seqinfo: 1 sequence from hg19 genome; no seqlengths
```

Individual fields can be pulled out with named accessors. Here we see `REF` is stored as a `DNAStringSet` and `qual` is a numeric vector.

```
> ref(vcf)[1:5]

A DNAStringSet instance of length 5
width seq
[1] 1 A
[2] 1 C
[3] 1 G
[4] 1 C
[5] 1 C

> qual(vcf)[1:5]
```

```
[1] 100 100 100 100 100
```

ALT is a DNASTringSetList (allows for multiple alternate alleles per variant) or a DNASTringSet. When structural variants are present it will be a CharacterList.

```
> alt(vcf)[1:5]
```

DNASTringSetList of length 5

```
[[1]] G
[[2]] T
[[3]] A
[[4]] T
[[5]] T
```

### 2.1.3 Genotype data

Genotype data described in the FORMAT fields are parsed into the geno slot. The data are unique to each sample and each sample may have multiple values variable. Because of this, the data are parsed into matrices or arrays where the rows represent the variants and the columns the samples. Multidimensional arrays indicate multiple values per sample. In this file all variables are matrices.

```
> geno(vcf)
```

List of length 3

```
names(3): GT DS GL
```

```
> sapply(geno(vcf), class)
```

```
      GT      DS      GL
"matrix" "matrix" "matrix"
```

Let's take a closer look at the genotype dosage (DS) variable. The header provides the variable definition and type.

```
> geno(header(vcf))["DS",]
```

DataFrame with 1 row and 3 columns

|    | Number      | Type        | Description                       |
|----|-------------|-------------|-----------------------------------|
|    | <character> | <character> | <character>                       |
| DS | 1           | Float       | Genotype dosage from MaCH/Thunder |

These data are stored as a 10376 × 5 matrix. Each of the five samples (columns) has a single value per variant location (row).

```
> DS <- geno(vcf)$DS
```

```
> dim(DS)
```

```
[1] 10376      5
```

```
> DS[1:3,]
```

|             | HG00096 | HG00097 | HG00099 | HG00100 | HG00101 |
|-------------|---------|---------|---------|---------|---------|
| rs7410291   | 0       | 0       | 1       | 0       | 0       |
| rs147922003 | 0       | 0       | 0       | 0       | 0       |
| rs114143073 | 0       | 0       | 0       | 0       | 0       |

DS is also known as 'posterior mean genotypes' and range in value from [0, 2]. To get a sense of variable distribution, we compute a five number summary of the minimum, lower-hinge (first quartile), median, upper-hinge (third quartile) and maximum.

```
> fivenum(DS)
```

```
[1] 0 0 0 0 2
```

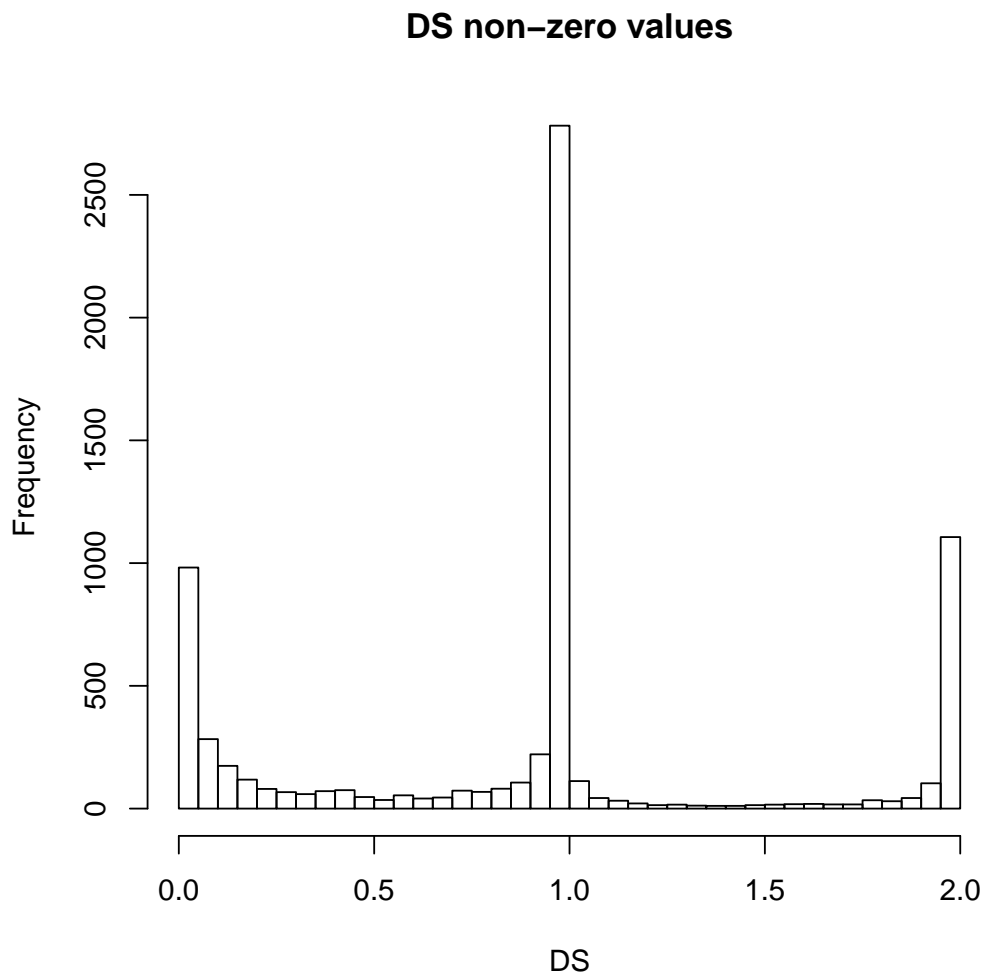
The majority of these values (86%) are zero.

```
> length(which(DS==0))/length(DS)
```

```
[1] 0.8621627
```

View the distribution of the non-zero values.

```
> hist(DS[DS != 0], breaks=seq(0, 2, by=0.05),
+      main="DS non-zero values", xlab="DS")
```



#### 2.1.4 Info data

In contrast to the genotype data, the info data are unique to the variant and the same across samples. All info variables are represented in a single DataFrame.

```
> info(vcf)[1:4, 1:5]
```

DataFrame with 4 rows and 5 columns

|           | LDAF      | AVGPOST   | RSQ       | ERATE     | THETA     |
|-----------|-----------|-----------|-----------|-----------|-----------|
|           | <numeric> | <numeric> | <numeric> | <numeric> | <numeric> |
| rs7410291 | 0.3431    | 0.9890    | 0.9856    | 2e-03     | 0.0005    |

```
rs147922003    0.0091    0.9963    0.8398    5e-04    0.0011
rs114143073    0.0098    0.9891    0.5919    7e-04    0.0008
rs141778433    0.0062    0.9950    0.6756    9e-04    0.0003
```

We will use the info data to compare quality measures between novel (i.e., not in dbSNP) and known (i.e., in dbSNP) variants and the variant type present in the file. Variants with membership in dbSNP can be identified by using the appropriate SNPlocs package for hg19.

```
> library(SNPlocs.Hsapiens.dbSNP.20101109)
> rd <- rowRanges(vcf)
> seqlevels(rd) <- "ch22"
> ch22snps <- getSNPlocs("ch22")
> dbsnpchr22 <- sub("rs", "", names(rd)) %in% ch22snps$RefSNP_id
> table(dbsnpchr22)
```

```
dbsnpchr22
FALSE TRUE
 6259 4117
```

Info variables of interest are 'VT', 'LDAF' and 'RSQ'. The header offers more details on these variables.

```
> info(header(vcf))[c("VT", "LDAF", "RSQ"),]
```

DataFrame with 3 rows and 3 columns

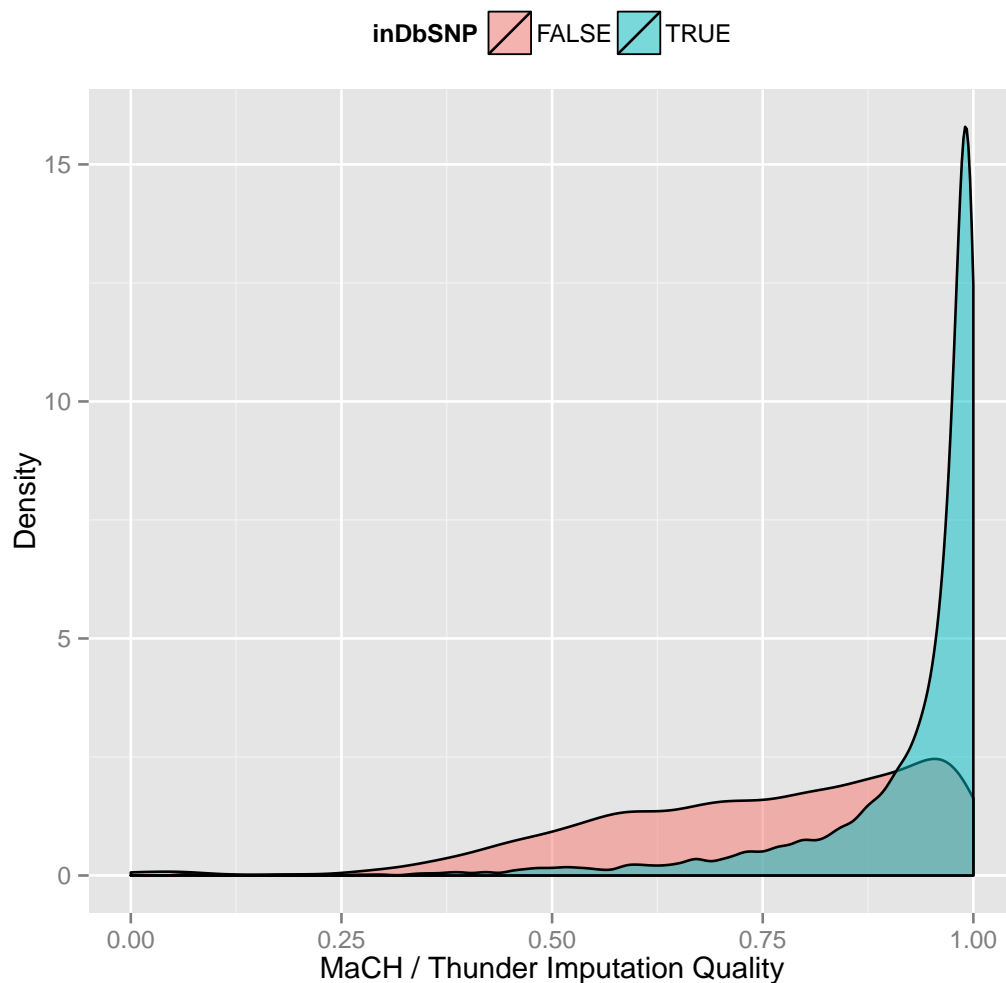
|      | Number      | Type        |  |
|------|-------------|-------------|--|
|      | <character> | <character> |  |
| VT   | 1           | String      |  |
| LDAF | 1           | Float       |  |
| RSQ  | 1           | Float       |  |
|      |             |             | Description  |
|      |             |             | <character>  |
| VT   |             |             | indicates what type of variant the line represents |
| LDAF |             |             | MLE Allele Frequency Accounting for LD             |
| RSQ  |             |             | Genotype imputation quality from MaCH/Thunder      |

Create a data frame of quality measures of interest ...

```
> metrics <- data.frame(QUAL=qual(vcf), inDbSNP=dbsnpchr22,
+   VT=info(vcf)$VT, LDAF=info(vcf)$LDAF, RSQ=info(vcf)$RSQ)
```

and visualize the distribution of qualities using ggplot2. For instance, genotype imputation quality is higher for the known variants in dbSNP.

```
> library(ggplot2)
> ggplot(metrics, aes(x=RSQ, fill=inDbSNP)) +
+   geom_density(alpha=0.5) +
+   scale_x_continuous(name="MaCH / Thunder Imputation Quality") +
+   scale_y_continuous(name="Density") +
+   theme(legend.position="top")
```



## 2.2 Import data subsets

When working with large VCF files it may be more efficient to read in subsets of the data. This can be accomplished by selecting genomic coordinates (ranges) or by specific fields from the VCF file.

### 2.2.1 Select genomic coordinates

To read in a portion of chromosome 22, create a `GRanges` with the regions of interest.

```
> rng <- GRanges(seqnames="22", ranges=IRanges(
+   start=c(50301422, 50989541),
+   end=c(50312106, 51001328),
+   names=c("gene_79087", "gene_644186")))
```

When ranges are specified, the VCF file must have an accompanying Tabix index file. See `?indexTabix` for help creating an index.

```
> tab <- TabixFile(fl)
> vcf_rng <- readVcf(tab, "hg19", param=rng)
```

The `paramRangesID` column distinguishes which records came from which param range.

```
> head(rowRanges(vcf_rng), 3)
```

GRanges object with 3 ranges and 5 metadata columns:

|                 | seqnames<br><Rle> | ranges<br><IRanges>  | strand<br><Rle> | paramRangeID<br><factor> |
|-----------------|-------------------|----------------------|-----------------|--------------------------|
| rs114335781     | 22                | [50301422, 50301422] | *               | gene_79087               |
| rs8135963       | 22                | [50301476, 50301476] | *               | gene_79087               |
| 22:50301488_C/T | 22                | [50301488, 50301488] | *               | gene_79087               |

|                 | REF<br><DNASTringSet> | ALT<br><DNASTringSetList> | QUAL<br><numeric> |
|-----------------|-----------------------|---------------------------|-------------------|
| rs114335781     | G                     | A                         | 100               |
| rs8135963       | T                     | C                         | 100               |
| 22:50301488_C/T | C                     | T                         | 100               |

|                 | FILTER<br><character> |
|-----------------|-----------------------|
| rs114335781     | PASS                  |
| rs8135963       | PASS                  |
| 22:50301488_C/T | PASS                  |

```
-----
seqinfo: 1 sequence from hg19 genome; no seqlengths
```

## 2.2.2 Select VCF fields

Data import can also be defined by the `fixed`, `info` and `geno` fields. Fields available for import are described in the header information. To view the header before reading in the data, use `ScanVcfHeader`.

```
> hdr <- scanVcfHeader(fl)
> ## e.g., INFO and GENO fields
> head(info(hdr), 3)
```

DataFrame with 3 rows and 3 columns

|         | Number | Type  | Description |
|---------|--------|-------|-------------|
| LDAF    | 1      | Float |             |
| AVGPOST | 1      | Float |             |
| RSQ     | 1      | Float |             |

|         | Description                                     |
|---------|---|
| LDAF    | MLE Allele Frequency Accounting for LD          |
| AVGPOST | Average posterior probability from MaCH/Thunder |
| RSQ     | Genotype imputation quality from MaCH/Thunder   |

```
> head(geno(hdr), 3)
```

DataFrame with 3 rows and 3 columns

|    | Number | Type   | Description                       |
|----|--------|--------|-----------------------------------|
| GT | 1      | String | Genotype                          |
| DS | 1      | Float  | Genotype dosage from MaCH/Thunder |
| GL | .      | Float  | Genotype Likelihoods              |

To subset on "LDAF" and "GT" we specify them as character vectors in the `info` and `geno` arguments to `ScanVcfParam`. This creates a `ScanVcfParam` object which is used as the `param` argument to `readVcf`.



```
> ## Return all 'fixed' fields, "LAF" from 'info' and "GT" from 'geno'
> svp <- ScanVcfParam(info="LDAF", geno="GT")
> vcf1 <- readVcf(fl, "hg19", svp)
> names(geno(vcf1))

[1] "GT"
```

To subset on both genomic coordinates and fields the ScanVcfParam object must contain both.

```
> svp_all <- ScanVcfParam(info="LDAF", geno="GT", which=rng)
> svp_all

class: ScanVcfParam
vcfWhich: 1 elements
vcfFixed: character() [All]
vcfInfo: LDAF
vcfGeno: GT
vcfSamples:
```

### 3 Locating variants in and around genes

Variant location with respect to genes can be identified with the `locateVariants` function. Regions are specified in the `region` argument and can be one of the following constructors: `CodingVariants`, `IntronVariants`, `FiveUTRVariants`, `ThreeUTRVariants`, `IntergenicVariants`, `SpliceSiteVariants` or `PromoterVariants`. Location definitions are shown in Table 1.

| Location   | Details  |
|------------|--|
| coding     | falls <i>within</i> a coding region                                    |
| fiveUTR    | falls <i>within</i> a 5' untranslated region                           |
| threeUTR   | falls <i>within</i> a 3' untranslated region                           |
| intron     | falls <i>within</i> an intron region                                   |
| intergenic | does not fall <i>within</i> a transcript associated with a gene        |
| spliceSite | overlaps any portion of the first 2 or last 2 nucleotides of an intron |
| promoter   | falls <i>within</i> a promoter region of a transcript                  |

Table 1: Variant locations

For overlap methods to work properly the chromosome names (seqlevels) must be compatible in the objects being compared. The VCF data chromosome names are represented by number, i.e., '22', but the TxDb chromosome names are preceded with 'chr'. Seqlevels in the VCF can be modified with the `seqlevels` function.

```
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)
> txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
> seqlevels(vcf) <- "chr22"
> rd <- rowRanges(vcf)
> loc <- locateVariants(rd, txdb, CodingVariants())
> head(loc, 3)
```

GRanges object with 3 ranges and 9 metadata columns:

|   | seqnames  | ranges               | strand        | LOCATION    | LOCSTART        | LOCEND    |
|---|-----------|----------------------|---------------|-------------|-----------------|-----------|
|   | <Rle>     | <IRanges>            | <Rle>         | <factor>    | <integer>       | <integer> |
| 1 | chr22     | [50301422, 50301422] | -             | coding      | 939             | 939       |
| 2 | chr22     | [50301476, 50301476] | -             | coding      | 885             | 885       |
| 3 | chr22     | [50301488, 50301488] | -             | coding      | 873             | 873       |
|   | QUERYID   | TXID                 | CDSID         | GENEID      | PRECEDEID       |           |
|   | <integer> | <character>          | <IntegerList> | <character> | <CharacterList> |           |

```

1      24      75253      218562      79087
2      25      75253      218562      79087
3      26      75253      218562      79087

```

```
FOLLOWID
```

```
<CharacterList>
```

```
1
```

```
2
```

```
3
```

```
-----
```

```
seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

Locate variants in all regions with the `AllVariants()` constructor,

```
> allvar <- locateVariants(rd, txdb, AllVariants())
```

To answer gene-centric questions data can be summarized by gene regardless of transcript.

```
> ## Did any coding variants match more than one gene?
```

```
> splt <- split(mcols(loc)$GENEID, mcols(loc)$QUERYID)
```

```
> table(sapply(splt, function(x) length(unique(x)) > 1))
```

```
FALSE TRUE
```

```
965    15
```

```
> ## Summarize the number of coding variants by gene ID.
```

```
> splt <- split(mcols(loc)$QUERYID, mcols(loc)$GENEID)
```

```
> head(sapply(splt, function(x) length(unique(x))), 3)
```

```
113730 1890 23209
```

```
22     15     30
```

## 4 Amino acid coding changes

`predictCoding` computes amino acid coding changes for non-synonymous variants. Only ranges in query that overlap with a coding region in the subject are considered. Reference sequences are retrieved from either a `BSgenome` or fasta file specified in `seqSource`. Variant sequences are constructed by substituting, inserting or deleting values in the `varAllele` column into the reference sequence. Amino acid codes are computed for the variant codon sequence when the length is a multiple of 3.

The query argument to `predictCoding` can be a `GRanges` or `VCF`. When a `GRanges` is supplied the `varAllele` argument must be specified. In the case of a `VCF`, the alternate alleles are taken from `alt(<VCF>)` and the `varAllele` argument is not specified.

The result is a modified query containing only variants that fall within coding regions. Each row represents a variant-transcript match so more than one row per original variant is possible.

```
> library(BSgenome.Hsapiens.UCSC.hg19)
```

```
> coding <- predictCoding(vcf, txdb, seqSource=Hsapiens)
```

```
> coding[5:7]
```

`GRanges` object with 3 ranges and 17 metadata columns:

```

      seqnames      ranges strand | paramRangeID
      <Rle>        <IRanges> <Rle> |   <factor>
22:50301584_C/T   chr22 [50301584, 50301584] - |   <NA>
rs114264124      chr22 [50302962, 50302962] - |   <NA>
rs149209714      chr22 [50302995, 50302995] - |   <NA>
      REF          ALT          QUAL
      <DNASet> <DNASetList> <numeric>

```

```

22:50301584_C/T      C      T      100
rs114264124          C      T      100
rs149209714          C      G      100

      FILTER      varAllele      CDSLOC      PROTEINLOC
<character> <DNAStringSet> <IRanges> <IntegerList>
22:50301584_C/T      PASS      A [777, 777]      259
rs114264124          PASS      A [698, 698]      233
rs149209714          PASS      C [665, 665]      222

      QUERYID      TXID      CDSID      GENEID
<integer> <character> <IntegerList> <character>
22:50301584_C/T      28      75253      218562      79087
rs114264124          57      75253      218563      79087
rs149209714          58      75253      218563      79087

      CONSEQUENCE      REFCODON      VARCHODON
<factor> <DNAStringSet> <DNAStringSet>
22:50301584_C/T      synonymous      CCG      CCA
rs114264124          nonsynonymous      CGG      CAG
rs149209714          nonsynonymous      GGA      GCA

      REFAA      VARAA
<AAStringSet> <AAStringSet>
22:50301584_C/T      P      P
rs114264124          R      Q
rs149209714          G      A

```

```
-----
seqinfo: 1 sequence from hg19 genome; no seqlengths
```

Using variant rs114264124 as an example, we see varAllele A has been substituted into the refCodon CGG to produce varCodon CAG. The refCodon is the sequence of codons necessary to make the variant allele substitution and therefore often includes more nucleotides than indicated in the range (i.e. the range is 50302962, 50302962, width of 1). Notice it is the second position in the refCodon that has been substituted. This position in the codon, the position of substitution, corresponds to genomic position 50302962. This genomic position maps to position 698 in coding region-based coordinates and to triplet 233 in the protein. This is a non-synonymous coding variant where the amino acid has changed from R (Arg) to Q (Gln).

When the resulting varCodon is not a multiple of 3 it cannot be translated. The consequence is considered a frameshift and varAA will be missing.

```
> ## CONSEQUENCE is 'frameshift' where translation is not possible
> coding[mcols(coding)$CONSEQUENCE == "frameshift"]
```

GRanges object with 2 ranges and 17 metadata columns:

```

      seqnames      ranges strand |
      <Rle>      <IRanges> <Rle> |
22:50317001_G/GCACT chr22 [50317001, 50317001] + |
22:50317001_G/GCACT chr22 [50317001, 50317001] + |

      paramRangeID      REF      ALT
      <factor> <DNAStringSet> <DNAStringSetList>
22:50317001_G/GCACT      <NA>      G      GCACT
22:50317001_G/GCACT      <NA>      G      GCACT

      QUAL      FILTER      varAllele      CDSLOC
      <numeric> <character> <DNAStringSet> <IRanges>
22:50317001_G/GCACT      233      PASS      GCACT [808, 808]
22:50317001_G/GCACT      233      PASS      GCACT [628, 628]

      PROTEINLOC      QUERYID      TXID      CDSID
      <IntegerList> <integer> <character> <IntegerList>
22:50317001_G/GCACT      270      359      74357      216303

```

```

22:50317001_G/GCACT      210      359      74358      216303
                        GENEID CONSEQUENCE      REFCODON
                        <character> <factor> <DNAStringSet>
22:50317001_G/GCACT      79174 frameshift      GCC
22:50317001_G/GCACT      79174 frameshift      GCC
                        VARCHODON      REFAA      VARAA
                        <DNAStringSet> <AAStringSet> <AAStringSet>
22:50317001_G/GCACT      GCACTCC
22:50317001_G/GCACT      GCACTCC
-----
seqinfo: 1 sequence from hg19 genome; no seqlengths

```

## 5 SIFT and PolyPhen Databases

From `predictCoding` we identified the amino acid coding changes for the non-synonymous variants. For this subset we can retrieve predictions of how damaging these coding changes may be. SIFT (Sorting Intolerant From Tolerant) and PolyPhen (Polymorphism Phenotyping) are methods that predict the impact of amino acid substitution on a human protein. The SIFT method uses sequence homology and the physical properties of amino acids to make predictions about protein function. PolyPhen uses sequence-based features and structural information characterizing the substitution to make predictions about the structure and function of the protein.

Collated predictions for specific dbSNP builds are available as downloads from the SIFT and PolyPhen web sites. These results have been packaged into *SIFT.Hsapiens.dbSNP132.db* and *PolyPhen.Hsapiens.dbSNP131.db* and are designed to be searched by rsid. Variants that are in dbSNP can be searched with these database packages. When working with novel variants, SIFT and PolyPhen must be called directly. See references for home pages.

Identify the non-synonymous variants and obtain the rsids.

```

> nms <- names(coding)
> idx <- mcols(coding)$CONSEQUENCE == "nonsynonymous"
> nonsyn <- coding[idx]
> names(nonsyn) <- nms[idx]
> rsids <- unique(names(nonsyn)[grep("rs", names(nonsyn), fixed=TRUE)])

```

Detailed descriptions of the database columns can be found with `?SIFTDbColumns` and `?PolyPhenDbColumns`. Variants in these databases often contain more than one row per variant. The variant may have been reported by multiple sources and therefore the source will differ as well as some of the other variables.

It is important to keep in mind the pre-computed predictions in the SIFT and PolyPhen packages are based on specific gene models. SIFT is based on Ensembl and PolyPhen on UCSC Known Gene. The TxDb we used to identify the coding snps was based on UCSC Known Gene so we will use PolyPhen for predictions. PolyPhen provides predictions using two different training datasets and has considerable information about 3D protein structure. See `?PolyPhenDbColumns` or the PolyPhen web site listed in the references for more details.

Query the PolyPhen database,

```

> library(PolyPhen.Hsapiens.dbSNP131)
> pp <- select(PolyPhen.Hsapiens.dbSNP131, keys=rsids,
+             cols=c("TRAININGSET", "PREDICTION", "PPH2PROB"))
> head(pp[!is.na(pp$PREDICTION), ])

```

|    | RSID       | TRAININGSET | OSNPID     | OACC     | OPOS | OAA1 | OAA2 | SNPID      |
|----|------------|-------------|------------|----------|------|------|------|------------|
| 13 | rs8139422  | humdiv      | rs8139422  | Q6UXH1-5 | 182  | D    | E    | rs8139422  |
| 14 | rs8139422  | humvar      | rs8139422  | <NA>     | <NA> | <NA> | <NA> | rs8139422  |
| 15 | rs74510325 | humdiv      | rs74510325 | Q6UXH1-5 | 189  | R    | G    | rs74510325 |
| 16 | rs74510325 | humvar      | rs74510325 | <NA>     | <NA> | <NA> | <NA> | rs74510325 |

```

21 rs73891177      humdiv rs73891177 Q6UXH1-5 207    P    A rs73891177
22 rs73891177      humvar rs73891177      <NA> <NA> <NA> <NA> rs73891177
    ACC POS AA1 AA2 NT1 NT2      PREDICTION      BASEDON EFFECT
13 Q6UXH1-5 182   D   E   T   A possibly damaging alignment <NA>
14 Q6UXH1-5 182   D   E <NA> <NA> possibly damaging <NA> <NA>
15 Q6UXH1-5 189   R   G   C   G possibly damaging alignment <NA>
16 Q6UXH1-5 189   R   G <NA> <NA> possibly damaging <NA> <NA>
21 Q6UXH1-5 207   P   A   C   G      benign alignment <NA>
22 Q6UXH1-5 207   P   A <NA> <NA>      benign <NA> <NA>
    PPH2CLASS PPH2PROB PPH2FPR PPH2TPR PPH2FDR SITE REGION PHAT DSCORE
13 neutral 0.228 0.156 0.892 0.258 <NA> <NA> <NA> 0.951
14 <NA> 0.249 0.341 0.874 <NA> <NA> <NA> <NA> <NA>
15 neutral 0.475 0.131 0.858 0.233 <NA> <NA> <NA> 1.198
16 <NA> 0.335 0.311 0.851 <NA> <NA> <NA> <NA> <NA>
21 neutral 0.001 0.86 0.994 0.61 <NA> <NA> <NA> -0.225
22 <NA> 0.005 0.701 0.981 <NA> <NA> <NA> <NA> <NA>
    SCORE1 SCORE2 NOBS NSTRUCT NFILT PDBID PDBPOS PDBCH IDENT LENGTH
13 1.382 0.431 37 0 <NA> <NA> <NA> <NA> <NA> <NA>
14 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
15 1.338 0.14 36 0 <NA> <NA> <NA> <NA> <NA> <NA>
16 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
21 -0.45 -0.225 1 0 <NA> <NA> <NA> <NA> <NA> <NA>
22 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
    NORMACC SECSTR MAPREG DVOL DPROP BFACT HBONDS AVENHET MINDHET
13 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
14 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
15 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
16 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
21 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
22 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
    AVENINT MINDINT AVENSIT MINDSIT TRANSV CODPOS CPG MINDJNC PFAMHIT
13 <NA> <NA> <NA> <NA> 1 2 0 <NA> <NA>
14 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
15 <NA> <NA> <NA> <NA> 1 0 1 <NA> <NA>
16 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
21 <NA> <NA> <NA> <NA> 1 0 0 <NA> <NA>
22 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
    IDPMAX IDPSNP IDQMIN COMMENTS
13 18.261 18.261 48.507 chr22:50315363_CA
14 <NA> <NA> <NA> chr22:50315363_CA
15 19.252 19.252 63.682 chr22:50315382_CG
16 <NA> <NA> <NA> chr22:50315382_CG
21 1.919 <NA> 60.697 chr22:50315971_CG
22 <NA> <NA> <NA> chr22:50315971_CG

```

## 6 Other operations

### 6.1 Create a SnpMatrix

The 'GT' element in the FORMAT field of the VCF represents the genotype. These data can be converted into a *SnpMatrix* object which can then be used with the functions offered in *snpStats* and other packages making use of the *SnpMatrix* class.

The `genotypeToSnpMatrix` function converts the genotype calls in `geno` to a `SnpMatrix`. No `dbSNP` package is used in this computation. The return value is a named list where 'genotypes' is a `SnpMatrix` and 'map' is a `DataFrame` with SNP names and alleles at each loci. The `ignore` column in 'map' indicates which variants were set to NA (missing) because they met one or more of the following criteria,

- variants with >1 ALT allele are set to NA
- only single nucleotide variants are included; others are set to NA
- only diploid calls are included; others are set to NA

See `?genotypeToSnpMatrix` for more details.

```
> res <- genotypeToSnpMatrix(vcf)
> res

$genotypes
A SnpMatrix with 5 rows and 10376 columns
Row names: HG00096 ... HG00101
Col names: rs7410291 ... rs114526001

$map
DataFrame with 10376 rows and 4 columns
      snp.names      allele.1      allele.2      ignore
      <character> <DNAStringSet> <DNAStringSetList> <logical>
1      rs7410291          A          G      FALSE
2      rs147922003        C          T      FALSE
3      rs114143073        G          A      FALSE
4      rs141778433        C          T      FALSE
5      rs182170314        C          T      FALSE
...      ...          ...          ...      ...
10372 rs187302552        A          G      FALSE
10373  rs9628178        A          G      FALSE
10374  rs5770892        A          G      FALSE
10375 rs144055359        G          A      FALSE
10376 rs114526001        G          C      FALSE
```

In the map `DataFrame`, `allele.1` represents the reference allele and `allele.2` is the alternate allele.

```
> allele2 <- res$map[["allele.2"]]
> ## number of alternate alleles per variant
> unique(elementLengths(allele2))

[1] 1
```

In addition to the called genotypes, genotype likelihoods or probabilities can also be converted to a `SnpMatrix`, using the `snpStats` encoding of posterior probabilities as byte values. To use the values in the 'GL' or 'GP' FORMAT field instead of the called genotypes, use the `uncertain=TRUE` option in `genotypeToSnpMatrix`.

```
> fl.gl <- system.file("extdata", "gl_chr1.vcf", package="VariantAnnotation")
> vcf.gl <- readVcf(fl.gl, "hg19")
> geno(vcf.gl)
```

```
List of length 3
names(3): GT DS GL
```

```
> ## Convert the "GL" FORMAT field to a SnpMatrix
> res <- genotypeToSnpMatrix(vcf.gl, uncertain=TRUE)
> res
```

```
$genotypes
A SnpMatrix with 85 rows and 9 columns
```

Row names: NA06984 ... NA12890

Col names: rs58108140 ... rs200430748

\$map

DataFrame with 9 rows and 4 columns

|   | snp.names   | allele.1      | allele.2          | ignore    |
|---|-------------|---------------|-------------------|-----------|
|   | <character> | <DNAStrngSet> | <DNAStrngSetList> | <logical> |
| 1 | rs58108140  | G             | A                 | FALSE     |
| 2 | rs189107123 | C             |                   | TRUE      |
| 3 | rs180734498 | C             | T                 | FALSE     |
| 4 | rs144762171 | G             |                   | TRUE      |
| 5 | rs201747181 | TC            |                   | TRUE      |
| 6 | rs151276478 | T             |                   | TRUE      |
| 7 | rs140337953 | G             | T                 | FALSE     |
| 8 | rs199681827 | C             |                   | TRUE      |
| 9 | rs200430748 | G             |                   | TRUE      |

```
> t(as(res$genotype, "character"))[c(1,3,7), 1:5]
```

|             | NA06984     | NA06986     | NA06989     | NA06994     | NA07000     |
|-------------|-------------|-------------|-------------|-------------|-------------|
| rs58108140  | "Uncertain" | "Uncertain" | "A/B"       | "Uncertain" | "Uncertain" |
| rs180734498 | "Uncertain" | "Uncertain" | "Uncertain" | "Uncertain" | "Uncertain" |
| rs140337953 | "Uncertain" | "Uncertain" | "Uncertain" | "Uncertain" | "Uncertain" |

```
> ## Compare to a SnpMatrix created from the "GT" field
```

```
> res.gt <- genotypeToSnpMatrix(vcf.gl, uncertain=FALSE)
```

```
> t(as(res.gt$genotype, "character"))[c(1,3,7), 1:5]
```

|             | NA06984 | NA06986 | NA06989 | NA06994 | NA07000 |
|-------------|---------|---------|---------|---------|---------|
| rs58108140  | "A/B"   | "A/B"   | "A/B"   | "A/A"   | "A/A"   |
| rs180734498 | "A/B"   | "A/A"   | "A/A"   | "A/A"   | "A/B"   |
| rs140337953 | "B/B"   | "B/B"   | "A/B"   | "B/B"   | "A/B"   |

```
> ## What are the original likelihoods for rs58108140?
```

```
> geno(vcf.gl)$GL["rs58108140", 1:5]
```

```
$NA06984
```

```
[1] -4.70 -0.58 -0.13
```

```
$NA06986
```

```
[1] -1.15 -0.10 -0.84
```

```
$NA06989
```

```
[1] -2.05 0.00 -3.27
```

```
$NA06994
```

```
[1] -0.48 -0.48 -0.48
```

```
$NA07000
```

```
[1] -0.28 -0.44 -0.96
```

For variant rs58108140 in sample NA06989, the "A/B" genotype is much more likely than the others, so the *SnpMatrix* object displays the called genotype.

## 6.2 Write out VCF files

A VCF file can be written out from data stored in a VCF class.

```
> fl <- system.file("extdata", "ex2.vcf", package="VariantAnnotation")
> out1.vcf <- tempfile()
> out2.vcf <- tempfile()
> in1 <- readVcf(fl, "hg19")
> writeVcf(in1, out1.vcf)
> in2 <- readVcf(out1.vcf, "hg19")
> writeVcf(in2, out2.vcf)
> in3 <- readVcf(out2.vcf, "hg19")
> identical(rowRanges(in1), rowRanges(in3))

[1] TRUE

> identical(geno(in1), geno(in2))

[1] TRUE
```

## 7 Performance

---

Targeted queries can greatly improve the speed of data input. When all data from the file are needed define a `yieldSize` in the `TabixFile` to iterate through the file in chunks.

```
readVcf(TabixFile(fl, yieldSize=10000))
```

`readVcf` can be used with a `ScanVcfParam` to select any combination of INFO and GENO fields, samples or genomic positions.

```
readVcf(TabixFile(fl), param=ScanVcfParam(info='DP', geno='GT'))
```

While `readvcf` offers the flexibility to define combinations of INFO, GENO and samples in the `ScanVcfParam`, sometimes only a single field is needed. In this case the lightweight read functions (`readGT`, `readInfo` and `readGeno`) can be used. These functions return the single field as a matrix instead of a VCF object.

```
readGT(fl)
```

The table below highlights the speed differences of targeted queries vs reading in all data. The test file is from 1000 Genomes and has 494328 variants, 1092 samples, 22 INFO, and 3 GENO fields and is located at <ftp://ftp-trace.ncbi.nih.gov/1000genomes/ftp/release/20101123/>. `yieldSize` is used to define chunks of 100, 1000, 10000 and 100000 variants. For each chunk size three function calls are compared: `readGT` reading only GT, `readVcf` reading both GT and ALT and finally `readVcf` reading in all the data.

```
library(microbenchmark)
fl <- "ALL.chr22.phase1_release_v3.20101123.snps_indels_svsvs.genotypes.vcf.gz"
ys <- c(100, 1000, 10000, 100000)

## readGT() input only 'GT':
fun <- function(fl, yieldSize) readGT(TabixFile(fl, yieldSize))
lapply(ys, function(i) microbenchmark(fun(fl, i), times=5))

## readVcf() input only 'GT' and 'ALT':
fun <- function(fl, yieldSize, param)
  readVcf(TabixFile(fl, yieldSize), "hg19", param=param)
param <- ScanVcfParam(info=NA, geno="GT", fixed="ALT")
lapply(ys, function(i) microbenchmark(fun(fl, i, param), times=5))

## readVcf() input all variables:
fun <- function(fl, yieldSize) readVcf(TabixFile(fl, yieldSize), "hg19")
lapply(ys, function(i) microbenchmark(fun(fl, i), times=5))
```



| n records | readGT | readVcf (GT and ALT) | readVcf (all) |
|-----------|--------|----------------------|---------------|
| 100       | 0.082  | 0.128                | 0.501         |
| 1000      | 0.609  | 0.508                | 5.878         |
| 10000     | 5.972  | 6.164                | 68.378        |
| 100000    | 78.593 | 81.156               | 693.654       |

Table 2: Targeted queries (time in seconds)

## 8 References

---

Wang K, Li M, Hakonarson H, (2010), ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Research, Vol 38, No. 16, e164.

McLaren W, Pritchard B, RiosD, et. al., (2010), Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. Bioinformatics, Vol. 26, No. 16, 2069-2070.

SIFT home page : <http://sift.bii.a-star.edu.sg/>

PolyPhen home page : <http://genetics.bwh.harvard.edu/pph2/>

## 9 Session Information

---

R version 3.2.2 (2015-08-14)

Platform: x86\_64-pc-linux-gnu (64-bit)

Running under: Ubuntu 14.04.3 LTS

locale:

```
[1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
[3] LC_TIME=en_US.UTF-8      LC_COLLATE=C
[5] LC_MONETARY=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
[7] LC_PAPER=en_US.UTF-8     LC_NAME=C
[9] LC_ADDRESS=C             LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
```

attached base packages:

```
[1] stats4      parallel  stats      graphics  grDevices  utils
[7] datasets   methods   base
```

other attached packages:

```
[1] snpStats_1.20.0
[2] Matrix_1.2-2
[3] survival_2.38-3
[4] PolyPhen.Hsapiens.dbSNP131_1.0.2
[5] RSQLite_1.0.0
[6] DBI_0.3.1
[7] BSgenome.Hsapiens.UCSC.hg19_1.4.0
[8] BSgenome_1.38.0
[9] rtracklayer_1.30.1
[10] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
[11] GenomicFeatures_1.22.2
```

```
[12] AnnotationDbi_1.32.0
[13] ggplot2_1.0.1
[14] SNPlocs.Hsapiens.dbSNP.20101109_0.99.7
[15] VariantAnnotation_1.16.3
[16] Rsamtools_1.22.0
[17] Biostrings_2.38.0
[18] XVector_0.10.0
[19] SummarizedExperiment_1.0.0
[20] Biobase_2.30.0
[21] GenomicRanges_1.22.0
[22] GenomeInfoDb_1.6.1
[23] IRanges_2.4.1
[24] S4Vectors_0.8.0
[25] BiocGenerics_0.16.0
```

loaded via a namespace (and not attached):

```
[1] Rcpp_0.12.1          futile.logger_1.4.1
[3] plyr_1.8.3           bitops_1.0-6
[5] futile.options_1.0.0 tools_3.2.2
[7] zlibbioc_1.16.0      biomaRt_2.26.0
[9] digest_0.6.8         lattice_0.20-33
[11] gtable_0.1.2         proto_0.3-10
[13] stringr_1.0.0        grid_3.2.2
[15] XML_3.98-1.3         BiocParallel_1.4.0
[17] reshape2_1.4.1       lambda.r_1.1.7
[19] magrittr_1.5         splines_3.2.2
[21] MASS_7.3-44          scales_0.3.0
[23] GenomicAlignments_1.6.1 colorspace_1.2-6
[25] BiocStyle_1.8.0      labeling_0.3
[27] stringi_1.0-1        munsell_0.4.2
[29] RCurl_1.95-4.7
```