

# 1. Introduction to *VariantAnnotation*

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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

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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...

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```
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 G      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): fileformat
fixed(2): FILTER 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          G      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      REF
      <Rle> <IRanges> <Rle> | <factor> <DNAStringSet>
rs7410291      22 50300078      * |      <NA>      A
rs147922003     22 50300086      * |      <NA>      C
rs114143073     22 50300101      * |      <NA>      G
              ALT      QUAL      FILTER
```

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```
      <DNAStringSetList> <numeric> <character>
rs7410291                G      100      PASS
rs147922003             T      100      PASS
rs114143073             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"
```

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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
```

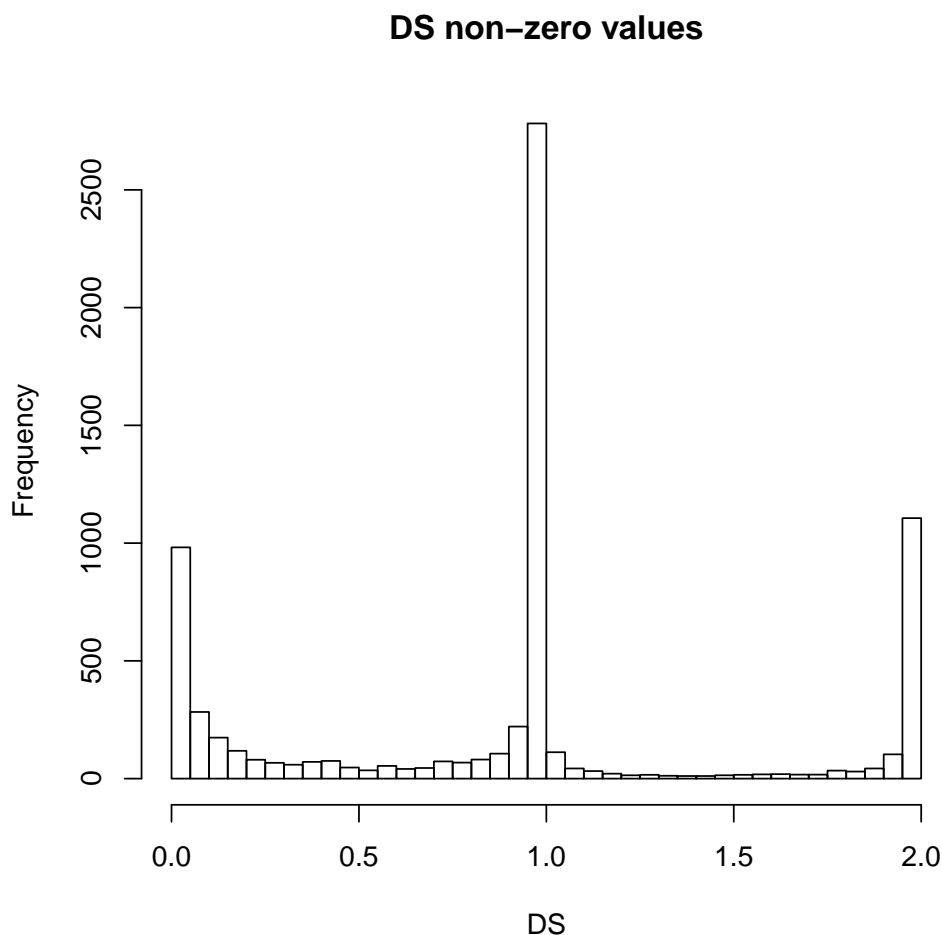
The majority of these values (86%) are zero.

```
> 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")
```

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### 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	0.989	0.9856	0.002	5e-04
rs147922003	0.0091	0.9963	0.8398	5e-04	0.0011
rs114143073	0.0098	0.9891	0.5919	7e-04	8e-04
rs141778433	0.0062	0.995	0.6756	9e-04	3e-04

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.

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```
> 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
```

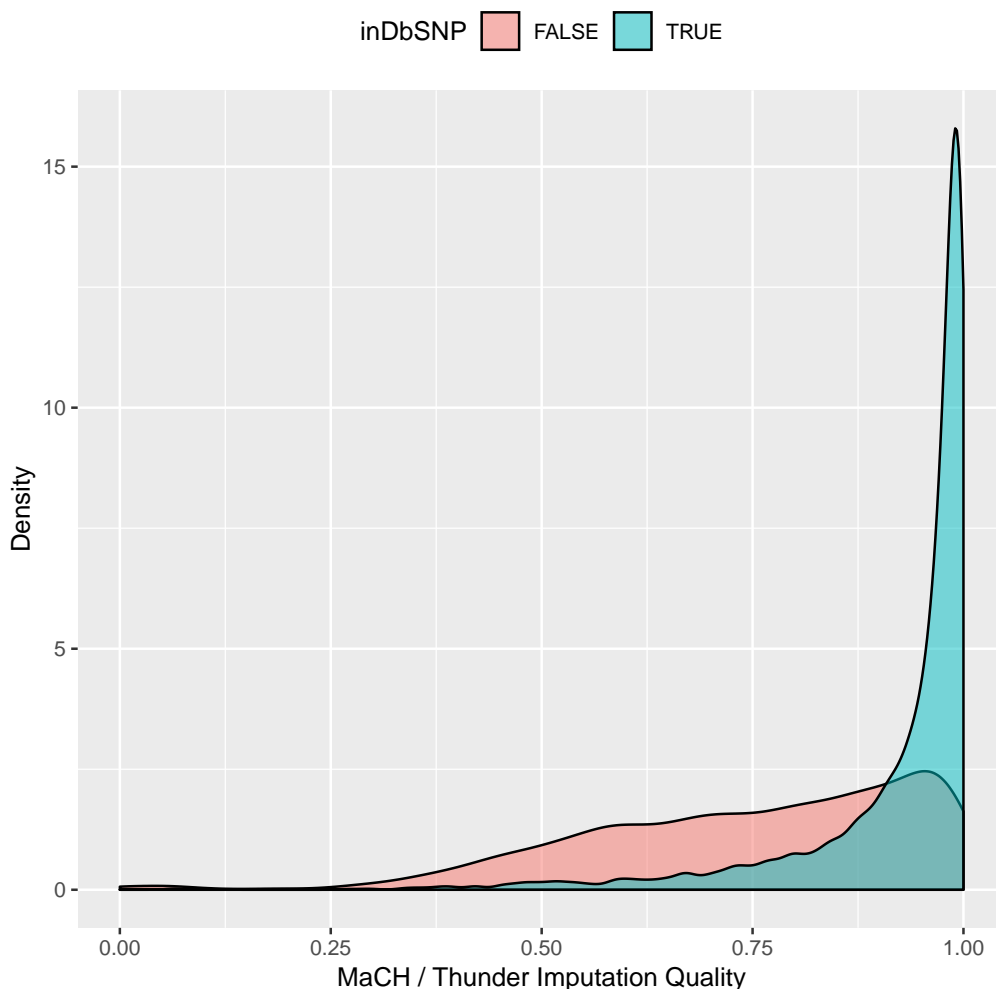
Create a data frame of quality measures of interest ...

```
> 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")
```

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## 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.



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```
> 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	ranges	strand	paramRangeID
	<Rle>	<IRanges>	<Rle>	<factor>
rs114335781	22	50301422	*	gene_79087
rs8135963	22	50301476	*	gene_79087
22:50301488_C/T	22	50301488	*	gene_79087
	REF		ALT	QUAL
	<DNAStringSet>	<DNAStringSetList>		<numeric>
rs114335781	G		A	100
rs8135963	T		C	100
22:50301488_C/T	C		T	100
	FILTER			
	<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
	<character>	<character>	<character>
LDAF	1	Float	MLE Allele Frequency Accounting for LD
AVGPOST	1	Float	Average posterior probability from MaCH/Thunder
RSQ	1	Float	Genotype imputation quality from MaCH/Thunder

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

DataFrame with 3 rows and 3 columns

	Number	Type	Description
--	--------	------	-------------

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	<character>	<character>		<character>
GT	1	String		Genotype
DS	1	Float	Genotype dosage from MaCH/Thunder	
GL	G	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.

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```
> 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	-	coding	939	939
[2]	chr22	50301476	-	coding	885	885
[3]	chr22	50301488	-	coding	873	873

	QUERYID	TXID	CDSID	GENEID	PRECEDEID
	<integer>	<character>	<IntegerList>	<character>	<CharacterList>
[1]	24	75253	218562	79087	<NA>
[2]	25	75253	218562	79087	<NA>
[3]	26	75253	218562	79087	<NA>

	FOLLOWID
	<CharacterList>
[1]	<NA>
[2]	<NA>
[3]	<NA>

-----  
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`. Vari-

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ant 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	-	<NA>
rs114264124	chr22	50302962	-	<NA>
rs149209714	chr22	50302995	-	<NA>

	REF	ALT	QUAL
	<DNAStringSet>	<DNAStringSetList>	<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	259
rs114264124	PASS	A	698	233
rs149209714	PASS	C	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	VARCODON
	<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

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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	paramRangeID		
	<Rle>	<IRanges>	<Rle>		<factor>	
22:50317001_G/GCACT	chr22	50317001	+		<NA>	
22:50317001_G/GCACT	chr22	50317001	+		<NA>	
		REF		ALT	QUAL	
		<DNAStringSet>		<DNAStringSetList>	<numeric>	
22:50317001_G/GCACT		G		GCACT	233	
22:50317001_G/GCACT		G		GCACT	233	
		FILTER		varAllele	CDSLLOC	
		<character>		<DNAStringSet>	<IRanges>	
22:50317001_G/GCACT		PASS		GCACT	808	
22:50317001_G/GCACT		PASS		GCACT	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	
		VARCODON		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.

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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>

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	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.

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```
> 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> <DNAStrSet> <DNAStrSetList> <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(elementNROWS(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
```



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```
$map
DataFrame with 9 rows and 4 columns
      snp.names      allele.1      allele.2      ignore
<character> <DNAStringSet> <DNAStringSetList> <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_sv.s.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))
```

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```
## 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 3.6.0 (2019-04-26)  
Platform: x86_64-w64-mingw32/x64 (64-bit)  
Running under: Windows Server 2012 R2 x64 (build 9600)
```

```
Matrix products: default
```

```
locale:  
[1] LC_COLLATE=C  
[2] LC_CTYPE=English_United States.1252  
[3] LC_MONETARY=English_United States.1252  
[4] LC_NUMERIC=C  
[5] LC_TIME=English_United States.1252
```

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attached base packages:

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

other attached packages:

```
[1] snpStats_1.34.0
[2] Matrix_1.2-17
[3] survival_2.44-1.1
[4] PolyPhen.Hsapiens.dbSNP131_1.0.2
[5] RSQLite_2.1.1
[6] BSgenome.Hsapiens.UCSC.hg19_1.4.0
[7] BSgenome_1.52.0
[8] rtracklayer_1.44.0
[9] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
[10] GenomicFeatures_1.36.0
[11] AnnotationDbi_1.46.0
[12] ggplot2_3.1.1
[13] SNPlocs.Hsapiens.dbSNP.20101109_0.99.7
[14] VariantAnnotation_1.30.0
[15] Rsamtools_2.0.0
[16] Biostrings_2.52.0
[17] XVector_0.24.0
[18] SummarizedExperiment_1.14.0
[19] DelayedArray_0.10.0
[20] BiocParallel_1.18.0
[21] matrixStats_0.54.0
[22] Biobase_2.44.0
[23] GenomicRanges_1.36.0
[24] GenomeInfoDb_1.20.0
[25] IRanges_2.18.0
[26] S4Vectors_0.22.0
[27] BiocGenerics_0.30.0
```

loaded via a namespace (and not attached):

```
[1] Rcpp_1.0.1           lattice_0.20-38
[3] prettyunits_1.0.2    assertthat_0.2.1
[5] digest_0.6.18        R6_2.4.0
[7] plyr_1.8.4           evaluate_0.13
[9] httr_1.4.0           pillar_1.3.1
[11] zlibbioc_1.30.0      rlang_0.3.4
[13] progress_1.2.0       lazyeval_0.2.2
[15] blob_1.1.1           rmarkdown_1.12
[17] splines_3.6.0        labeling_0.3
[19] stringr_1.4.0        RCurl_1.95-4.12
[21] bit_1.1-14           biomaRt_2.40.0
[23] munsell_0.5.0        compiler_3.6.0
[25] xfun_0.6             pkgconfig_2.0.2
[27] htmltools_0.3.6      tidyselect_0.2.5
[29] tibble_2.1.1         GenomeInfoDbData_1.2.1
[31] XML_3.98-1.19        withr_2.1.2
```

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```
[33] crayon_1.3.4          dplyr_0.8.0.1
[35] GenomicAlignments_1.20.0 bitops_1.0-6
[37] grid_3.6.0            gtable_0.3.0
[39] DBI_1.0.0             magrittr_1.5
[41] scales_1.0.0          stringi_1.4.3
[43] BiocStyle_2.12.0      tools_3.6.0
[45] bit64_0.9-7           glue_1.3.1
[47] purrr_0.3.2           hms_0.4.2
[49] yaml_2.2.0            colorspace_1.4-1
[51] BiocManager_1.30.4    memoise_1.1.0
[53] knitr_1.22
```