

Introduction to *VariantAnnotation*

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1 Introduction

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

rowData(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/T...
ERATE	1	Float	Per-marker Mutation rate from MaCH/Thunder
THETA	1	Float	Per-marker Transition rate from MaCH/Th...
CIEND	2	Integer	Confidence interval around END for impr...
CIPOS	2	Integer	Confidence interval around POS for impr...
END	1	Integer	End position of the variant described i...
HOMLEN	.	Integer	Length of base pair identical micro-hom...
HOMSEQ	.	String	Sequence of base pair identical micro-h...
SVLEN	1	Integer	Difference in length between REF and AL...
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 b...
ASN_AF	1	Float	Allele Frequency for samples from ASN b...
AFR_AF	1	Float	Allele Frequency for samples from AFR b...
EUR_AF	1	Float	Allele Frequency for samples from EUR b...
VT	1	String	indicates what type of variant the line...
SNPSOURCE	.	String	indicates if a snp was called when anal...

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): fileformat
fixed(1): ALT
info(22): LDAF AVGPOST ... 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

`rowData` 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(rowData(vcf), 3)

GRanges 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

```
---
seqlengths:
22
NA
```

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 DNStringSet 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 `DNStringSetList` (allows for multiple alternate alleles per variant) or a `DNStringSet`. When structural variants are present it will be a `CharacterList`.

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

```

DNStringSetList 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 x 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,]
```

	HG000096	HG000097	HG000099	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.

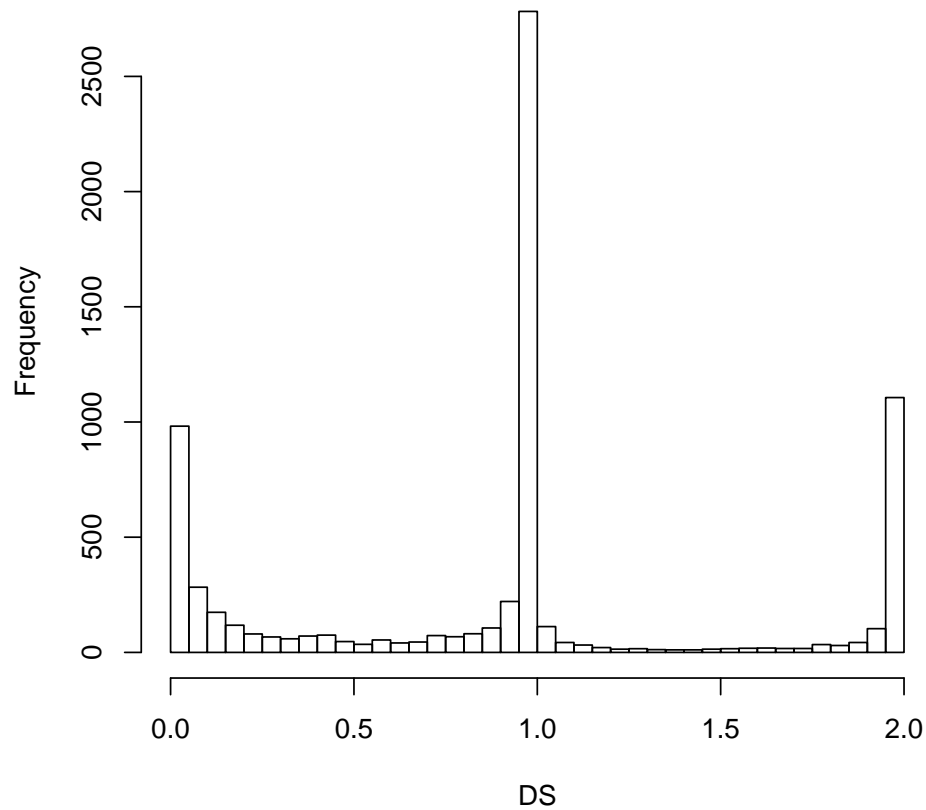
```
> length(which(DS==0))/length(DS)
```

```
[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")
```

DS non-zero values



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)
> dbsnp <- renameSeqlevels(rowData(vcf), c("22"="ch22"))
```

```
> ch22snps <- getSNPlocs("ch22")
> dbsnpchr22 <- sub("rs", "", names(dbsnpchr22)) %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

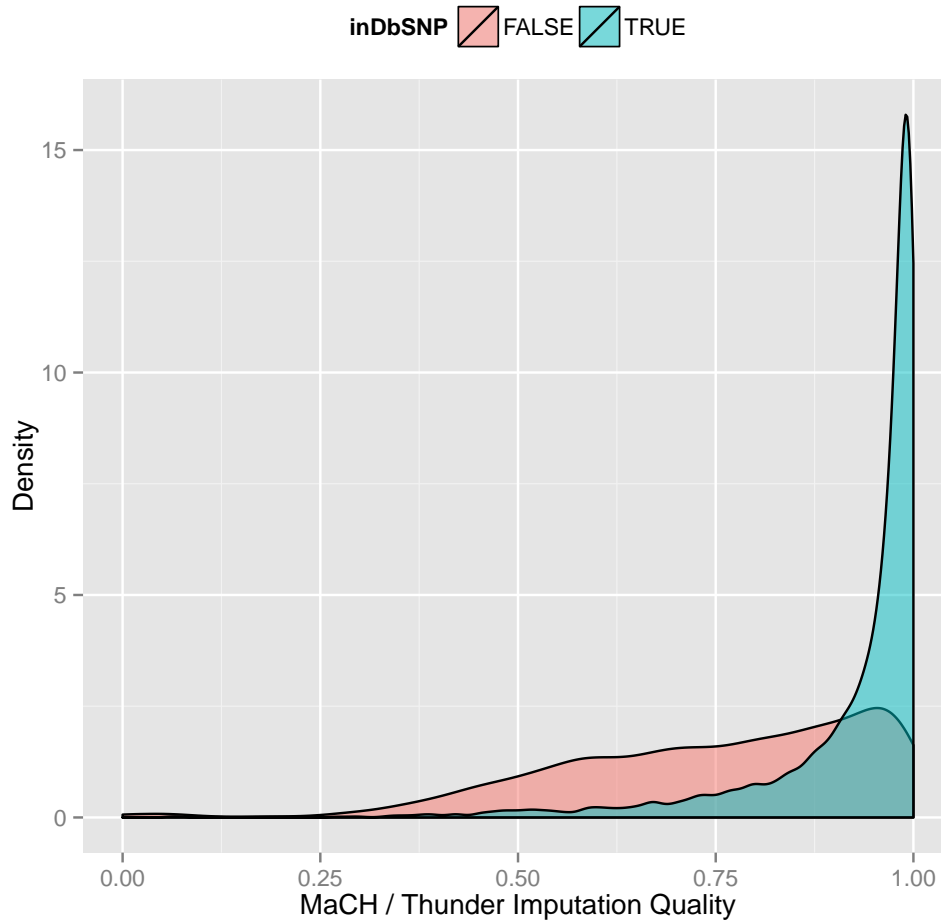
	Number	Type	
	<character>	<character>	
VT	1	String	
LDAF	1	Float	
RSQ	1	Float	
			Description
			<character>
VT			indicates what type of variant the line represents
LDAF			MLE Allele Frequency Accounting for LD
RSQ			Genotype imputation quality from MaCH/Thunder

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(rowData(vcf_rng), 3)
```

GRanges with 3 ranges and 5 metadata columns:

	seqnames <Rle>	ranges <IRanges>	strand <Rle>	paramRangeID <factor>
rs114335781	22	[50301422, 50301422]	*	gene_79087
rs8135963	22	[50301476, 50301476]	*	gene_79087
22:50301488	22	[50301488, 50301488]	*	gene_79087

	REF <DNASet<Rle>	ALT <DNASetList<Rle>	QUAL <numeric>	FILTER <character>
rs114335781	G	A	100	PASS
rs8135963	T	C	100	PASS
22:50301488	C	T	100	PASS

 seqlengths:
 22
 NA

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	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
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
```

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'. Modify the seqlevels in the VCF object with `renameSeqlevels`.

```
> library(TxDb.Hsapiens.UCSC.hg19.knownGene)
> txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene
> vcf <- renameSeqlevels(vcf, c("22"="chr22"))
> rd <- rowData(vcf)
> loc <- locateVariants(rd, txdb, CodingVariants())
> head(loc, 3)
```

GRanges with 3 ranges and 7 metadata columns:

	seqnames	ranges	strand	LOCATION	QUERYID
	<Rle>	<IRanges>	<Rle>	<factor>	<integer>
[1]	chr22	[50301422, 50301422]	*	coding	24
[2]	chr22	[50301476, 50301476]	*	coding	25
[3]	chr22	[50301488, 50301488]	*	coding	26
	TXID	CDSID	GENEID	PRECEDEID	FOLLOWID
	<integer>	<integer>	<character>	<character>	<character>
[1]	73482	217009	79087	<NA>	<NA>
[2]	73482	217009	79087	<NA>	<NA>

```
[3]      73482      217009      79087      <NA>      <NA>
---
seqlengths:
chr22
NA
```

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
956    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 with 3 ranges and 17 metadata columns:

	seqnames	ranges	strand	paramRangeID
	<Rle>	<IRanges>	<Rle>	<factor>
22:50301584	chr22	[50301584, 50301584]	-	<NA>
rs114264124	chr22	[50302962, 50302962]	-	<NA>
rs149209714	chr22	[50302995, 50302995]	-	<NA>
	REF	ALT	QUAL	FILTER
	<DNASTringSet>	<DNASTringSetList>	<numeric>	<character>
22:50301584	C	T	100	PASS
rs114264124	C	T	100	PASS

rs149209714	C	G	100	PASS
varAllele	CDSLOC	PROTEINLOC	QUERYID	
<DNAStrngSet>	<IRanges>	<IntegerList>	<integer>	
22:50301584	A [777, 777]	259	28	
rs114264124	A [698, 698]	233	57	
rs149209714	C [665, 665]	222	58	
TXID	CDSID	GENEID	CONSEQUENCE	
<character>	<integer>	<character>	<factor>	
22:50301584	73482	217009	79087	synonymous
rs114264124	73482	217010	79087	nonsynonymous
rs149209714	73482	217010	79087	nonsynonymous
REFCODON	VARCODON	REFAA	VARAA	
<DNAStrngSet>	<DNAStrngSet>	<AAStringSet>	<AAStringSet>	
22:50301584	CCG	CCA	P	P
rs114264124	CGG	CAG	R	Q
rs149209714	GGA	GCA	G	A

seqlengths:
chr22
NA

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 with 1 range and 17 metadata columns:

seqnames	ranges	strand	paramRangeID
<Rle>	<IRanges>	<Rle>	<factor>
22:50317001	chr22 [50317001, 50317001]	+	<NA>
REF	ALT	QUAL	FILTER
<DNAStrngSet>	<DNAStrngSetList>	<numeric>	<character>
22:50317001	G	GCACT	233
	PASS		
varAllele	CDSLOC	PROTEINLOC	QUERYID
<DNAStrngSet>	<IRanges>	<IntegerList>	<integer>
22:50317001	GCACT [808, 808]	270	359
TXID	CDSID	GENEID	CONSEQUENCE
<character>	<integer>	<character>	<factor>
22:50317001	72592	214765	79174
	frameshift		
REFCODON	VARCODON	REFAA	VARAA
<DNAStrngSet>	<DNAStrngSet>	<AAStringSet>	<AAStringSet>
22:50317001	GCC	GCC	A

seqlengths:

```
chr22
NA
```

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.Hapiens.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 `TranscriptDb` 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	PREDICTION	PPH2PROB
11	rs8139422	humdiv	possibly damaging	0.228
12	rs8139422	humvar	possibly damaging	0.249
13	rs74510325	humdiv	possibly damaging	0.475
14	rs74510325	humvar	possibly damaging	0.335
15	rs73891177	humdiv	benign	0.001
16	rs73891177	humvar	benign	0.005

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> <DNAStrngSet> <DNAStrngSetList> <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> <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 Expand a VCF

Coming soon ... `CollapsedVCF` and `ExpandedVCF` classes and `expand,CollapsedVCF-method`.

6.3 Write out VCF files

A VCF file can be written out from data stored in a `VCF` class. Methods to write out from more general structures are in progress.

```
> 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(in2, in3)
```

```
[1] FALSE
```

7 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/>

8 Session Information

R version 3.0.1 (2013-05-16)

Platform: i386-w64-mingw32/i386 (32-bit)

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
```

attached base packages:

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

other attached packages:

```
[1] snpStats_1.10.0
[2] Matrix_1.0-14
[3] lattice_0.20-23
[4] survival_2.37-4
[5] PolyPhen.Hsapiens.dbSNP131_1.0.2
[6] RSQLite_0.11.4
[7] DBI_0.2-7
[8] BSgenome.Hsapiens.UCSC.hg19_1.3.19
[9] BSgenome_1.28.0
[10] TxDb.Hsapiens.UCSC.hg19.knownGene_2.9.2
[11] GenomicFeatures_1.12.4
[12] AnnotationDbi_1.22.6
[13] Biobase_2.20.1
[14] ggplot2_0.9.3.1
[15] SNPlocs.Hsapiens.dbSNP.20101109_0.99.6
[16] VariantAnnotation_1.6.8
[17] Rsamtools_1.12.4
[18] Biostrings_2.28.0
[19] GenomicRanges_1.12.5
[20] IRanges_1.18.4
[21] BiocGenerics_0.6.0
```

loaded via a namespace (and not attached):

```
[1] MASS_7.3-29          RColorBrewer_1.0-5  RCurl_1.95-4.1
[4] XML_3.98-1.1         biomaRt_2.16.0      bitops_1.0-6
[7] colorspace_1.2-4     dichromat_2.0-0     digest_0.6.3
[10] grid_3.0.1           gtable_0.1.2        labeling_0.2
[13] munsell_0.4.2        plyr_1.8            proto_0.3-10
[16] reshape2_1.2.2       rtracklayer_1.20.4  scales_0.2.3
[19] stats4_3.0.1         stringr_0.6.2       tools_3.0.1
[22] zlibbioc_1.6.0
```