# 1. Introduction to *VariantAnnotation*

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## Contents



## 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 severly 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 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

`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. Multidimentional 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,]

            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.

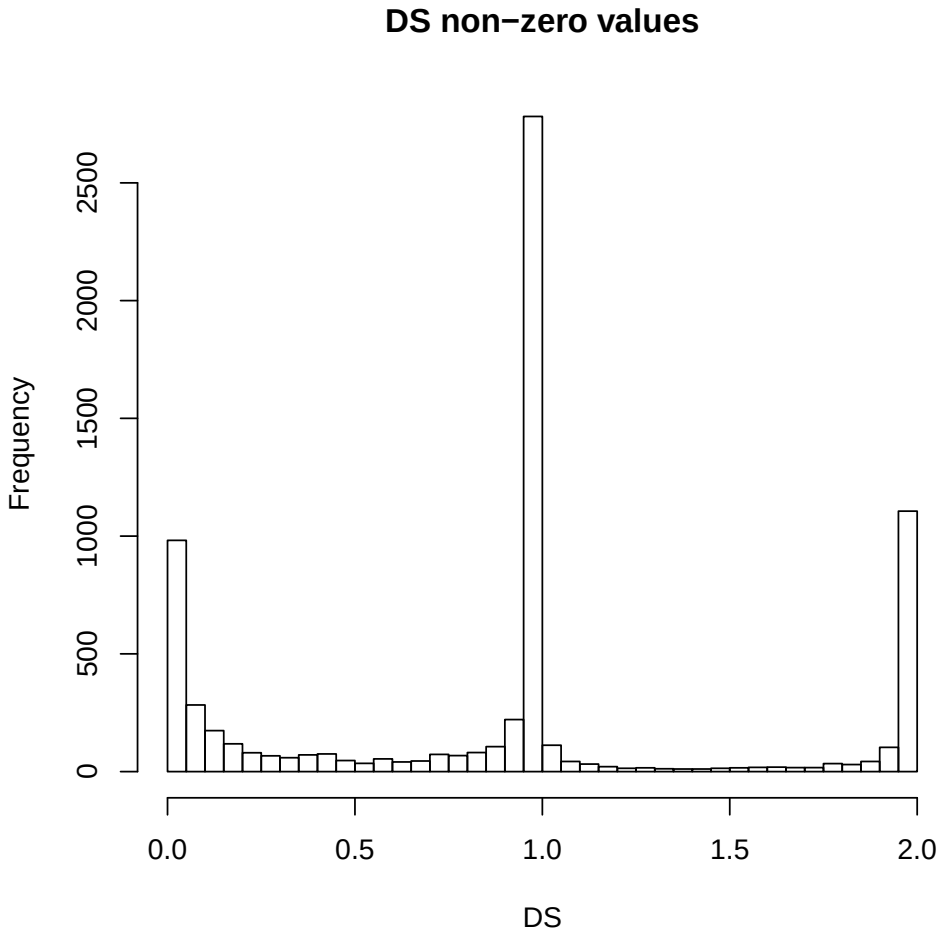
```
> fivenum(DS)

[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.

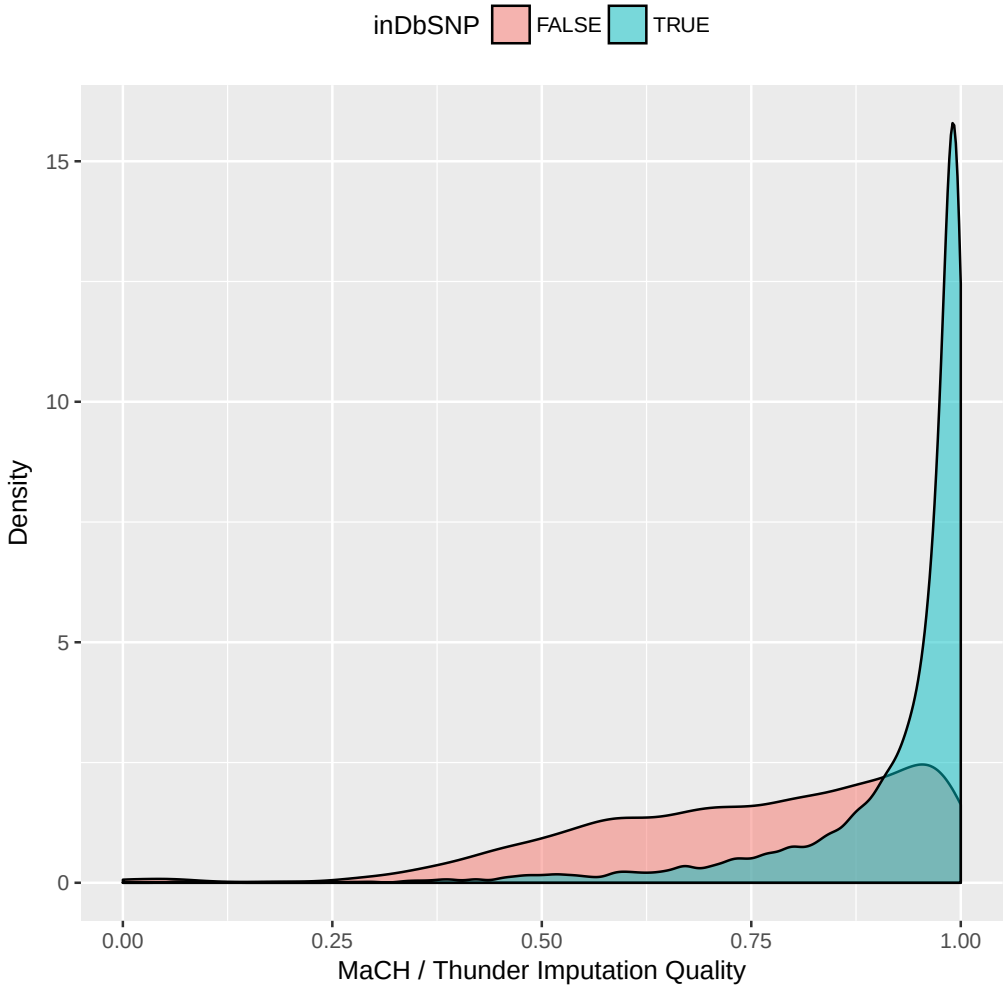
```
> info(header(vcf))[c("VT", "LDAF", "RSQ"),]

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               ranges strand | paramRangeID
                     <Rle>            <IRanges>  <Rle> |     <factor>
     rs114335781       22 [50301422, 50301422]      * |   gene_79087
       rs8135963       22 [50301476, 50301476]      * |   gene_79087
  22:50301488_C/T       22 [50301488, 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
        <character> <character>
LDAF              1       Float
AVGPOST           1       Float
RSQ               1       Float
                                          Description
                                          <character>
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
```

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

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, 50301584]      - |         <NA>
      rs114264124    chr22 [50302962, 50302962]      - |         <NA>
      rs149209714    chr22 [50302995, 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, 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       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

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
                           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.

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 `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
14  rs8139422      humdiv  rs8139422 Q6UXH1-5  182    D    E  rs8139422
15  rs8139422      humvar  rs8139422     <NA> <NA> <NA> <NA>  rs8139422
16 rs74510325      humdiv rs74510325 Q6UXH1-5  189    R    G rs74510325
17 rs74510325      humvar rs74510325     <NA> <NA> <NA> <NA> rs74510325
22 rs73891177      humdiv rs73891177 Q6UXH1-5  207    P    A rs73891177
23 rs73891177      humvar rs73891177     <NA> <NA> <NA> <NA> rs73891177
        ACC POS AA1 AA2  NT1  NT2        PREDICTION   BASEDON EFFECT
14 Q6UXH1-5 182   D   E    T    A possibly damaging alignment   <NA>
15 Q6UXH1-5 182   D   E <NA> <NA> possibly damaging      <NA>   <NA>
16 Q6UXH1-5 189   R   G    C    G possibly damaging alignment   <NA>
17 Q6UXH1-5 189   R   G <NA> <NA> possibly damaging      <NA>   <NA>
22 Q6UXH1-5 207   P   A    C    G            benign alignment   <NA>
23 Q6UXH1-5 207   P   A <NA> <NA>            benign      <NA>   <NA>
   PPH2CLASS PPH2PROB PPH2FPR PPH2TPR PPH2FDR SITE REGION PHAT DSCORE
14   neutral    0.228   0.156   0.892   0.258 <NA>   <NA> <NA>  0.951
15      <NA>    0.249   0.341   0.874    <NA> <NA>   <NA> <NA>   <NA>
16   neutral    0.475   0.131   0.858   0.233 <NA>   <NA> <NA>  1.198
17      <NA>    0.335   0.311   0.851    <NA> <NA>   <NA> <NA>   <NA>
22   neutral    0.001    0.86   0.994    0.61 <NA>   <NA> <NA> -0.225
23      <NA>    0.005   0.701   0.981    <NA> <NA>   <NA> <NA>   <NA>
```

```
   SCORE1 SCORE2 NOBS NSTRUCT NFILT PDBID PDBPOS PDBCH IDENT LENGTH
14  1.382  0.431   37       0  <NA>  <NA>   <NA>  <NA>  <NA>   <NA>
15   <NA>   <NA> <NA>    <NA>  <NA>  <NA>   <NA>  <NA>  <NA>   <NA>
16  1.338   0.14   36       0  <NA>  <NA>   <NA>  <NA>  <NA>   <NA>
17   <NA>   <NA> <NA>    <NA>  <NA>  <NA>   <NA>  <NA>  <NA>   <NA>
22  -0.45 -0.225    1       0  <NA>  <NA>   <NA>  <NA>  <NA>   <NA>
23   <NA>   <NA> <NA>    <NA>  <NA>  <NA>   <NA>  <NA>  <NA>   <NA>
   NORMACC SECSTR MAPREG DVOL DPROP BFACT HBONDS AVENHET MINDHET
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>
17    <NA>   <NA>   <NA> <NA>  <NA>  <NA>   <NA>    <NA>    <NA>
22    <NA>   <NA>   <NA> <NA>  <NA>  <NA>   <NA>    <NA>    <NA>
23    <NA>   <NA>   <NA> <NA>  <NA>  <NA>   <NA>    <NA>    <NA>
   AVENINT MINDINT AVENSIT MINDSIT TRANSV CODPOS  CPG MINDJNC PFAMHIT
14    <NA>    <NA>    <NA>    <NA>      1      2    0    <NA>    <NA>
15    <NA>    <NA>    <NA>    <NA>   <NA>   <NA> <NA>    <NA>    <NA>
16    <NA>    <NA>    <NA>    <NA>      1      0    1    <NA>    <NA>
17    <NA>    <NA>    <NA>    <NA>   <NA>   <NA> <NA>    <NA>    <NA>
22    <NA>    <NA>    <NA>    <NA>      1      0    0    <NA>    <NA>
23    <NA>    <NA>    <NA>    <NA>   <NA>   <NA> <NA>    <NA>    <NA>
   IDPMAX IDPSNP IDQMIN          COMMENTS
14 18.261 18.261 48.507 chr22:50315363_CA
15   <NA>   <NA>   <NA> chr22:50315363_CA
16 19.252 19.252 63.682 chr22:50315382_CG
17   <NA>   <NA>   <NA> chr22:50315382_CG
22  1.919   <NA> 60.697 chr22:50315971_CG
23   <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(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
```

```
$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_svs.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 3.4.1 (2017-06-30)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 16.04.2 LTS

Matrix products: default
BLAS: /home/biocbuild/bbs-3.6-bioc/R/lib/libRblas.so
LAPACK: /home/biocbuild/bbs-3.6-bioc/R/lib/libRlapack.so

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_1.27.0
 [2] Matrix_1.2-10
 [3] survival_2.41-3
 [4] PolyPhen.Hsapiens.dbSNP131_1.0.2
 [5] RSQLite_2.0
 [6] BSgenome.Hsapiens.UCSC.hg19_1.4.0
 [7] BSgenome_1.45.1
 [8] rtracklayer_1.37.3
 [9] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
[10] GenomicFeatures_1.29.8
[11] AnnotationDbi_1.39.2
[12] ggplot2_2.2.1
[13] SNPlocs.Hsapiens.dbSNP.20101109_0.99.7
[14] VariantAnnotation_1.23.8
[15] Rsamtools_1.29.0
[16] Biostrings_2.45.3
[17] XVector_0.17.0
[18] SummarizedExperiment_1.7.5
[19] DelayedArray_0.3.19
[20] matrixStats_0.52.2
[21] Biobase_2.37.2
[22] GenomicRanges_1.29.12
[23] GenomeInfoDb_1.13.4
[24] IRanges_2.11.12
[25] S4Vectors_0.15.5
[26] BiocGenerics_0.23.0

loaded via a namespace (and not attached):
 [1] progress_1.1.2           splines_3.4.1
 [3] lattice_0.20-35          colorspace_1.3-2
 [5] htmltools_0.3.6          yaml_2.1.14
 [7] blob_1.1.0               XML_3.98-1.9
 [9] rlang_0.1.1              DBI_0.7
[11] BiocParallel_1.11.5      bit64_0.9-7
[13] GenomeInfoDbData_0.99.1  plyr_1.8.4
[15] stringr_1.2.0            zlibbioc_1.23.0
[17] munsell_0.4.3            gtable_0.2.0
[19] evaluate_0.10.1          memoise_1.1.0
[21] labeling_0.3             knitr_1.16
[23] biomaRt_2.33.4           Rcpp_0.12.12
[25] backports_1.1.0          scales_0.4.1
[27] bit_1.1-12               BiocStyle_2.5.11
```

```
        [29] digest_0.6.12           stringi_1.1.5
        [31] grid_3.4.1              rprojroot_1.2
        [33] tools_3.4.1             bitops_1.0-6
        [35] magrittr_1.5            lazyeval_0.2.0
        [37] RCurl_1.95-4.8          tibble_1.3.3
        [39] pkgconfig_2.0.1         prettyunits_1.0.2
        [41] assertthat_0.2.0        rmarkdown_1.6
        [43] R6_2.2.2                GenomicAlignments_1.13.4
        [45] compiler_3.4.1
```