

1. Introduction to *VariantAnnotation*

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May 19, 2019

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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

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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 AVGPPOST ... 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> <DNAStrngSet>  
rs7410291      22 50300078      * | <NA>      A  
rs147922003    22 50300086      * | <NA>      C  
rs114143073    22 50300101      * | <NA>      G  
              ALT      QUAL      FILTER
```

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```
      <DNAStrngSetList> <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 `DNAStrngSet` and `qual` is a numeric vector.

```
> ref(vcf)[1:5]
A DNAStrngSet 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 `DNAStrngSetList` (allows for multiple alternate alleles per variant) or a `DNAStrngSet`. When structural variants are present it will be a `CharacterList`.

```
> alt(vcf)[1:5]
DNAStrngSetList 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, ]  
      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.

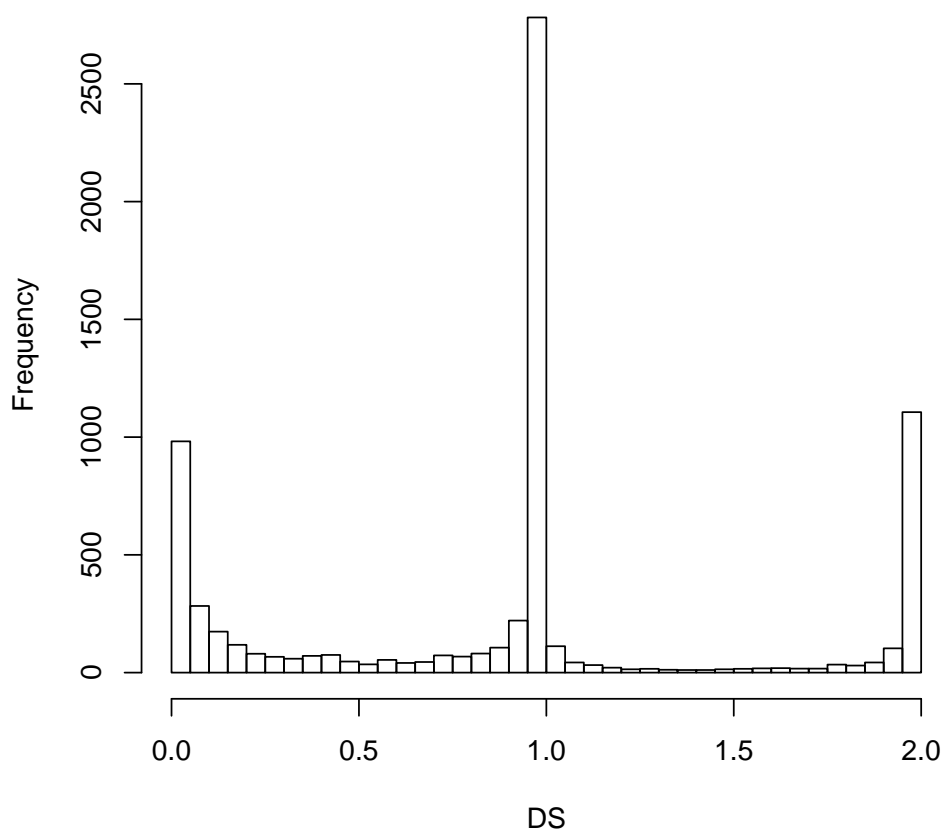
```
> length(which(DS==0))/length(DS)  
[1] 0.8621627
```

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")
```

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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 | 0.989 | 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
```

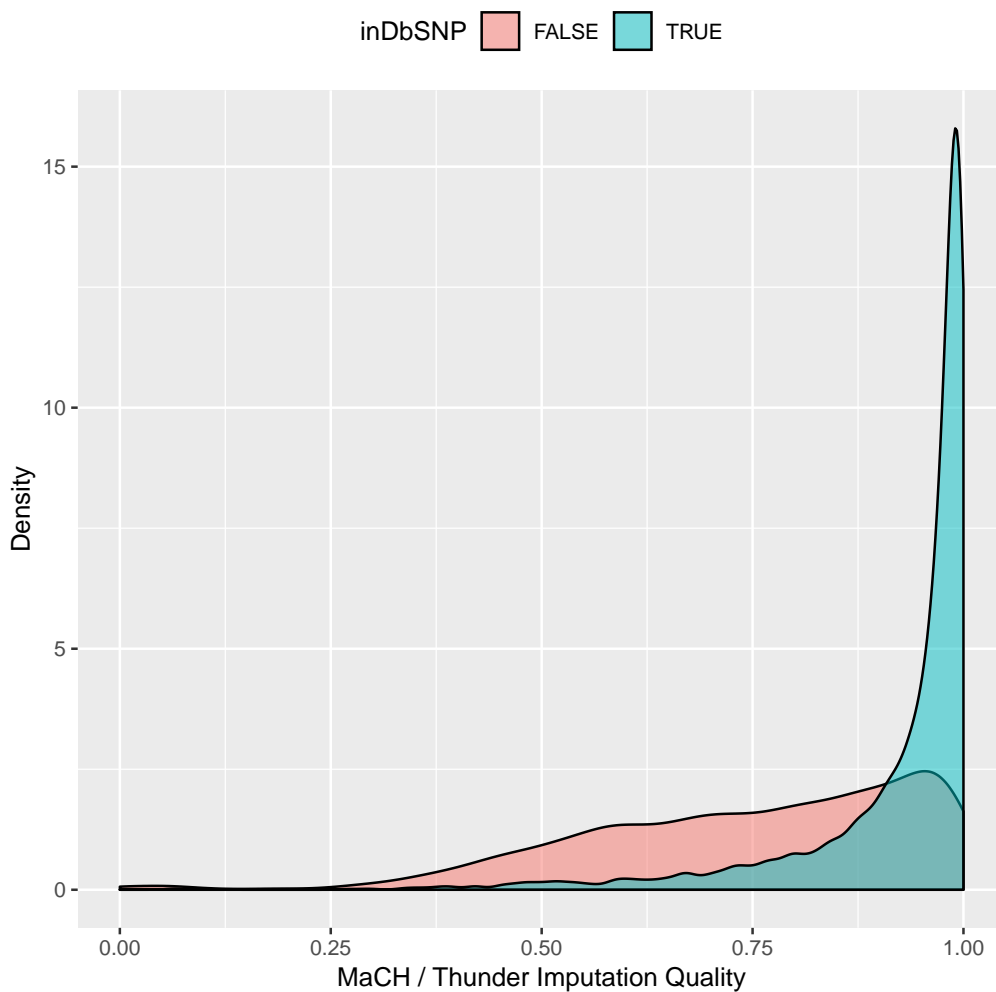
Create a data frame of quality measures of interest ...

```
> 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
      <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
```

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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?
> spl <- split(mcols(loc)$GENEID, mcols(loc)$QUERYID)
> table(sapply(spl, function(x) length(unique(x)) > 1))

FALSE TRUE
  965   15

> ## Summarize the number of coding variants by gene ID.
> spl <- split(mcols(loc)$QUERYID, mcols(loc)$GENEID)
> head(sapply(spl, 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      CDSLOC
<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> <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> <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> <NA>
14 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
15 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
16 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
21 <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA> <NA>
22 <NA> <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> <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(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.

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6.2 Write out VCF files

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

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

[1] TRUE

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

[1] TRUE
```

7 Performance

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

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

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

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

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

```
readGT(fl)
```

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

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

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

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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-pc-linux-gnu (64-bit)  
Running under: Ubuntu 18.04.2 LTS  
  
Matrix products: default  
BLAS: /home/biocbuild/bbs-3.9-bioc/R/lib/libRblas.so  
LAPACK: /home/biocbuild/bbs-3.9-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
```

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```
[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_1.34.0
[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_1.30.1
[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_1.4.0
[11] zlibbioc_1.30.0    rlang_0.3.4
[13] progress_1.2.2     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_0.7           pkgconfig_2.0.2
```

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```
[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
[33] crayon_1.3.4         dplyr_0.8.1
[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_1.23
```