QIAGEN

Technical Note

# Use of HGMD mutation data within popular variant annotation tools

Sample to Insight

Numerous free or open source variant annotation tools are available today to extract, annotate and analyze the many genomes and their identified variants coming from next generation sequencing methods.

There are many different types of information available for annotation of variants with the end goal to use that annotation to define the effect and changes in phenotype that are likely to be caused by the variant. Various information resources can act as a backend database for the annotation tools used within an annotation pipeline where the input file with an undefined collection of variants becomes directly associated with the annotation details (Figure 1).

The value derived from the annotation is directly related to the information resource selected for annotation. Cited in more than 14,500 scientific articles, HGMD is the industry leading database for published, inherited disease mutations.

In this technical note we identify a subset of popular variant annotation tools that are able to work with HGMD data and provide a step-by-step guide for the use of HGMD data by three of the tools: ANNOVAR, snpEff and VariantAnnotation – a Bioconductor package.

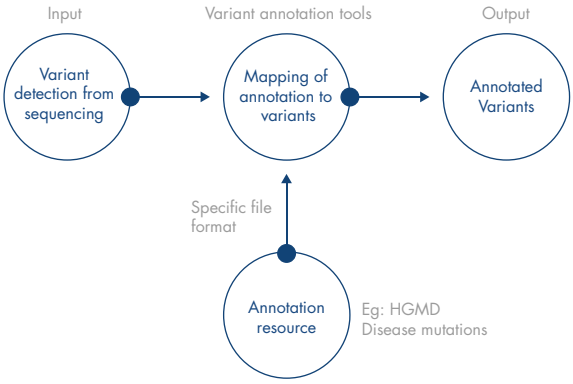



**Figure 1.** Variant annotation pipeline

## Open source variant annotation tools

A selection of popular free or open source variant annotation tools are described in Table 1.

| Tool | Code source | Annotation format supported | HGMD use described in this application note |
|---|---|---|---|
| ANNOVAR* | Perl | GFF3, VCF | Yes |
| snpEff | Java | TXT, BED, BigBed, VCF, GFF | Yes |
| Variant Annotation (Bioconductor package) | R | VCF | Yes |
| AnnTools | Python, MySQL for data storage | BED | No |
| CHAoS | Perl | BED, WIG | No |
| vcfanno | go | BED, BAM, VCF | No |
| seqminer | R | VCF, BCF, METAL | No |

*ANNOVAR is free for academic use only. Commercial use requires a license from QIAGEN.

## HGMD as an annotation resource

HGMD is a comprehensive database of published inherited disease mutations. Trained genetics experts read the published literature and extract information about germline mutations that have been shown to be associated with a specific disease or phenotype. The database is updated quarterly to ensure that the latest and most relevant information is available. As of the September 2020.1 release HGMD contained information for more than 282,000 mutations.

HGMD data is available by subscription for download in multiple formats supporting variant annotation including BED, GFF and VCF formats. Both hg19 and hg38 reference genomes are supported.

### VCF format

```
##fileformat=VCFv4.1
##Copyright=HGMD. Not for redistribution.
##source=HGMD_PRO_2016.1
##reference=GRCh38
##comment="REF and ALT sequences are both on forward strand of reference assembly"
##INFO=<ID=CLASS,Number=1,Type=String,Description="Mutation Category, https://portal.biobase-international.com/hgmd/pro/global.php#cats">
##INFO=<ID=MUT,Number=1,Type=String,Description="HGMD mutant allele">
##INFO=<ID=GENE,Number=1,Type=String,Description="Gene symbol">
##INFO=<ID=STRAND,Number=1,Type=String,Description="Gene strand">
##INFO=<ID=DNA,Number=1,Type=String,Description="DNA annotation">
##INFO=<ID=PROT,Number=1,Type=String,Description="Protein annotation">
##INFO=<ID=DB,Number=1,Type=String,Description="dbSNP identifier, build 137">
##INFO=<ID=PHEN,Number=1,Type=String,Description="HGMD primary phenotype">
#CHROM  POS ID  REF ALT QUAL    FILTER  INFO
1   942143  CM1511864   C   G   .   .   CLASS=DM?;MUT=ALT;GENE=SAMD11;STRAND=+;DNA=NM_152486.2:c.877C>G;PROT=NP_689699.2:p.P293A;DB=rs200195897;PHEN="Autism_spectrum_disorder"
1   963938  CD142720    CCT C   .   .   CLASS=DM?;MUT=ALT;GENE=KLHL17;STRAND=+;DNA=NM_198317.2:c.1375_1376delCT;PHEN="Schizophrenia"
1   1014143 CM1411641   C   T   .   .   CLASS=DM;MUT=ALT;GENE=ISG15;STRAND=+;DNA=NM_005101.3:c.163C>T;PROT=NP_005092.1:p.Q55*;PHEN="Idiopathic_basal_ganglia_calcification"
1   1014316 CI128669    C   CG  .   .   CLASS=DM;MUT=ALT;GENE=ISG15;STRAND=+;DNA=NM_005101.3:c.339dupG;PHEN="Mycobacterial_disease_mendelian_susceptibility_to"
```

### GFF3 format

```
##gff-version 3
chr1    hgmd    variant_phenotype   942143  942143  .   +   .   ID=1;accession=CM1511864;alt=G;aminoacid_change=P>A;citation_type=Primary;codon_change=CCT-GCT;codon_number=293
chr1    hgmd    variant_phenotype   963938  963940  .   +   .   ID=2;accession=CD142720;alt=C;aminoacid_change=N/A;citation_type=Primary;codon_change=N/A;codon_number=458;comm
chr1    hgmd    variant_phenotype   1014143 1014143 .   +   .   ID=3;accession=CM1411641;alt=T;aminoacid_change=Q>*;citation_type=Primary;codon_change=CAG-TAG;codon_number=55;
chr1    hgmd    variant_phenotype   1014316 1014316 .   +   .   ID=4;accession=CI128669;alt=CG;aminoacid_change=N/A;citation_type=Primary;codon_change=N/A;codon_number=113;com
chr1    hgmd    variant_phenotype   1014359 1014359 .   +   .   ID=5;accession=CM128668;alt=T;aminoacid_change=E>*;citation_type=Primary;codon_change=GAG-TAG;codon_number=127;
chr1    hgmd    variant_phenotype   1022225 1022225 .   +   .   ID=6;accession=CM148517;alt=A;aminoacid_change=G>S;citation_type=Primary,FCR;codon_change=GGC-AGC;codon_number=
chr1    hgmd    variant_phenotype   1022313 1022313 .   +   .   ID=7;accession=CM148518;alt=T;aminoacid_change=N>I;citation_type=Primary,FCR;codon_change=AAC-ATC;codon_number=
chr1    hgmd    variant_phenotype   1041582 1041582 .   +   .   ID=8;accession=CM126385;alt=T;aminoacid_change=Q>*;citation_type=Primary;codon_change=CAG-TAG;codon_number=353;
```

### BED format

```
track name="hgmd" description="HGMD Mutations" color="176,23,31" visibility=3
chr1    877522  877523  Autism_spectrum_disorder:877C>G 0   +
chr1    899317  899320  Schizophrenia:1375_1376delCT    0   +
chr1    949522  949523  Idiopathic_basal_ganglia_calcification:163C>T   0   +
chr1    949695  949696  Mycobacterial_disease_mendelian_susceptibility_to:339dupG   0   +
chr1    949738  949739  Mycobacterial_disease_mendelian_susceptibility_to:379G>T    0   +
```

## Step-by-step data analysis

Here we demonstrate the steps required to annotate an input sample with HGMD mutation data for three variant analysis tools: ANNOVAR, snpEff and VariantAnnotation.

The dataset used for the analysis is the breast cancer (primary ductal carcinoma TNM stage IIA, grade 3) HCC1187 cell line sample from the Complete Genomics public cancer data set (R. Drmanac et al, Science 327(5961), 78).

**ANNOVAR**

**Step 1:** Convert the input VCF file to ANNOVAR's specific file format using the accessory perl script convert2annovar.pl. In this example, HG00731-200-37-ASM.vcf is the input file and cgexample is the name appended to the converted output file

```
$ perl convert2annovar.pl -format vcf4 vcfBeta-HG00731-200-37-ASM.vcf -allsample -outfile cgexample
```

```
kar@sys-mkt108 /cygdrive/i/annovar
$ perl convert2annovar.pl -format vcf4 vcfBeta-HG00731-200-37-ASM.vcf -allsample -outfile cgexample
NOTICE: output files will be written to cgexample.<samplename>.avinput
NOTICE: Finished reading 10344776 lines from VCF file
NOTICE: A total of 10344658 locus in VCF file passed QC threshold, representing 3465464 SNPs (2358709 transitions and 1106755 tr
ansversions) and 6895319 indels/substitutions
NOTICE: Finished writing 3392941 SNPs (2310236 transitions and 1082705 transversions) and 581702 indels/substitutions for 1 samp
les
WARNING: Skipped 4830315 invalid alternative alleles found in input file
WARNING: Found 366 invalid reference alleles in input file
WARNING: Skipped 1658714 invalid genotype records in input file
```

**Step 2:** Annotate the converted VCF file (named cgexample.HG00731-200-37-ASM.avinput in this example) with HGMD annotations using the annotate.variation.pl script. The VCF formatted HGMD file (named HGMD_PRO_2016.1_hg19.vcf in this example) is used as the database file. In this example it is found in the humandb directory.

```
$ perl annotate_variation.pl -infoasscore -buildver hg19 -filter -dbtype vcf -vcfdbfile HGMD_PRO_2016.1_hg19.vcf cgexample.HG00731-200-37-ASM.avinput humandb/
```

```
Kar@MKT /cygdrive/d/annovar
$ perl annotate_variation.pl -infoasscore -buildver hg19 -filter -dbtype vcf -vcfdbfile HGMD_PRO_2016.
1_hg19.vcf cgexample.HG00731-200-37-ASM.avinput humandb/
NOTICE: Variants matching filtering criteria are written to cgexample.HG00731-200-37-ASM.avinput.hg19_
vcf_dropped, other variants are written to cgexample.HG00731-200-37-ASM.avinput.hg19_vcf_filtered
NOTICE: Processing next batch with 3974643 unique variants in 3974643 input lines
NOTICE: Scanning filter database humandb/HGMD_PRO_2016.1_hg19.vcf...Done
```

**Step 3:** Search the output file (named cgexample.HG00731-200-37-ASM.avinput.hg19_vcf_dropped in this example) for annotated variants in the gene of your choice. In this example we have chosen to use BRCA1 since the sample data is taken from a breast cancer cell line.

```
$ egrep -w “hgnc=BRCA1” cgexample.HG00731-200-37-ASM.avinput.hg19_vcf_dropped
```

```
Kar@MKT /cygdrive/d/annovar
$ egrep -w "GENE=BRCA1" cgexample.HG00731-200-37-ASM.avinput.hg19_vcf_dropped
vcf     CLASS=DFP;MUT=ALT;GENE=BRCA1;STRAND=-;DB=rs8176318;PHEN="Reduced_activity_association_with"     17      4119
7274    41197274        C       A       het     .       35
vcf     CLASS=DM?;MUT=ALT;GENE=BRCA1;STRAND=-;DNA=NM_007294.3:c.5152+66G>A;DB=rs3092994;PHEN="Breast_cancer"    17 4
1215825 41215825        C       T       het     .       26
vcf     CLASS=R;MUT=ALT;GENE=BRCA1;STRAND=-;DNA=NM_007294.3:c.4837A>G;PROT=NP_009225.1:p.S1613G;DB=rs1799966;PHEN="B
reast_cancer"   17      41223094        41223094        T       C       het     .       12
vcf     CLASS=DP;MUT=ALT;GENE=BRCA1;STRAND=-;DNA=NM_007294.3:c.3548A>G;PROT=NP_009225.1:p.K1183R;DB=rs16942;PHEN="Br
east_cancer_protection_against_association_with"        17      41244000        41244000        T       C       het.
43
vcf     CLASS=DP;MUT=ALT;GENE=BRCA1;STRAND=-;DNA=NM_007294.3:c.3113A>G;PROT=NP_009225.1:p.E1038G;DB=rs16941;PHEN="En
dometriosis_association_with"   17      41244435        41244435        T       C       het     .       43
vcf     CLASS=DFP;MUT=ALT;GENE=BRCA1;STRAND=-;DNA=NM_007294.3:c.2612C>T;PROT=NP_009225.1:p.P871L;DB=rs799917;PHEN="C
ervical_cancer_decreased_risk_association_with" 17      41244936        41244936        G       A       het     .  4
0
vcf     CLASS=DP;MUT=ALT;GENE=BRCA1;STRAND=-;DNA=NM_007294.3:c.1067A>G;PROT=NP_009225.1:p.Q356R;DB=rs1799950;PHEN="B
reast_and/or_ovarian_cancer_association_with"   17      41246481        41246481        T       C       het     .  4
6
vcf     CLASS=FP;MUT=ALT;GENE=BRCA1;STRAND=-;DB=rs799906;PHEN="Altered_promoter_activity"       17      41278116   4
1278116 T       C       het     .       32
vcf     CLASS=DFP;MUT=ALT;GENE=BRCA1;STRAND=-;DB=rs11655505;PHEN="Breast_cancer_descreased_risk_association_with"  1
7       41278377        41278377        G       A       het     .       49
vcf     CLASS=FP;MUT=ALT;GENE=BRCA1;STRAND=-;DB=rs799908;PHEN="Altered_promoter_activity"       17      41278916   4
1278916 A       G       het     .       16
vcf     CLASS=FP;MUT=ALT;GENE=BRCA1;STRAND=-;DB=rs4793204;PHEN="Reduced_promoter_activity"      17      41279298   4
1279298 A       G       het     .       23
```

Alternatively you can use HGMD gff file as the database file.

**Step 1:** Convert the input VCF file to ANNOVAR's specific file format using the accessory perl script convert2annovar.pl (as shown previously)

**Step 2:** Annotate the converted VCF file (named cgexample.HG00731-200-37-ASM.avinput in this example) with HGMD annotations using the annotate.variation.pl script. The GFF3 formatted HGMD file (named hgmd-hg19.gff in this example) is used as the database file. In this example it is found in the hgmdgff directory

```
$ perl annotate_variation.pl -regionanno -dbtype gff3 -gff3dbfile /hgmd-hg19.gff cgexample.HG00731-200-37-ASM.avinput --gff3attr -buildver hg19 hgmdgff
```

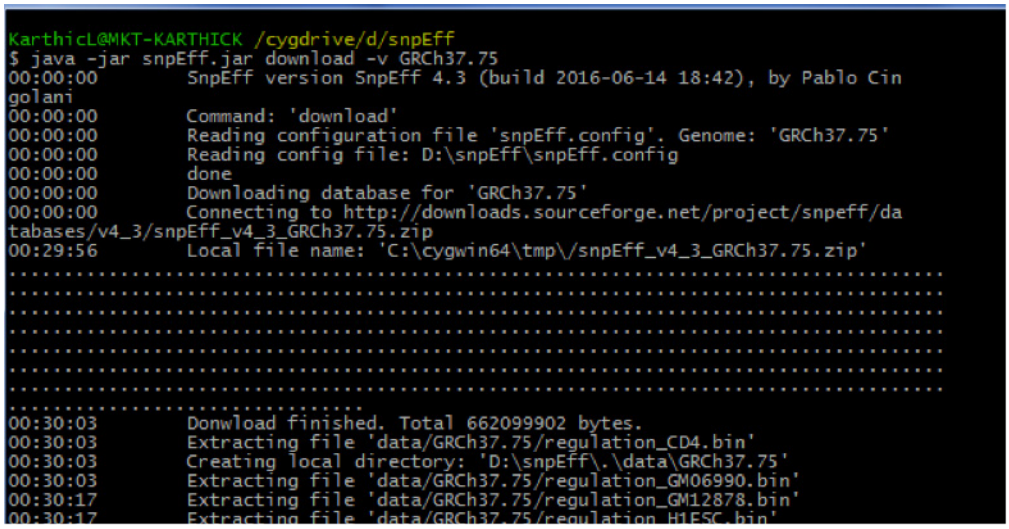

**Step 3:** Search the output file (named cgexample.HG00731-200-37-ASM.avinput.hg19_gff3 in this example) for annotated variants in the gene of your choice. In this example we have chosen to use BRCA1 since the sample data is taken from a breast cancer cell line

```
$ egrep -w "hgnc=BRCA1" cgexample.HG00731-200-37-ASM.avinput.hg19_gff3
```


## snpEff

**Step1:** Download the appropriate reference genome. In this example we are using the hg19 reference genome

```
$ java -jar snpEff.jar download -v GRCh37.75
```



**Step 2:** Annotate the input VCF file with HGMD annotations using the – interval option in snpEff to accept the HGMD file as an annotation file. In this example sample-hg00731.vcf is the input file. The BED formatted HGMD file, named hgmd-hg19.bed in this example, is used as the database file

```
$ java -Xmx4g -jar snpEff.jar -v -interval hgmd-hg19.bed GRCh37.75 sample-hg00731.vcf
```

Input:

```
KarthicL@MKT-KARTHICK /cygdrive/d/snpEff
$ java -Xmx4g -jar snpEff.jar -v -interval hgmd_20161.bed GRCh37.75 test.vcf
00:00:00        SnpEff version SnpEff 4.3 (build 2016-06-14 18:42), by Pablo Cingolani
00:00:00        Command: 'ann'
00:00:00        Reading configuration file 'snpEff.config'. Genome: 'GRCh37.75'
00:00:00        Reading config file: D:\snpEff\snpEff.config
00:00:00        done
00:00:00        Reading database for genome version 'GRCh37.75' from file 'D:\snpEff/./data/GRCh37
.75/snpEffectPredictor.bin' (this might take a while)
00:00:24        done
00:00:24        Reading interval file 'hgmd_20161.bed'
00:00:25        done (161162 intervals loaded).
00:00:25        Loading Motifs and PWMs
00:00:25        Building interval forest
```

Output:

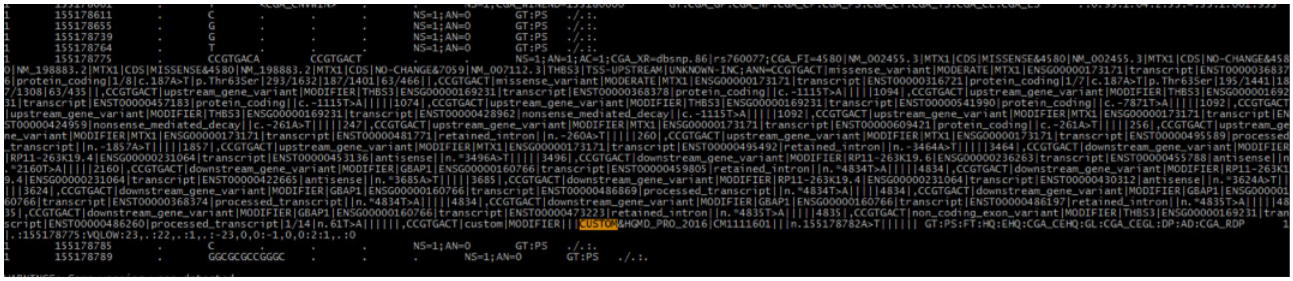

Alternatively, the VCF formatted HGMD file, named HGMD_PRO_2016.1_hg19.vf in this example, can be used as the database file

```
$ java -Xmx4g -jar snpEff.jar -v -interval HGMD_PRO_2016.1_hg19.vcf GRCh37.75 sample-hg00731.vcf
```

Input:

```
KarthicL@MKT-KARTHICK /cygdrive/d/snpEff
$ java -Xmx4g -jar snpEff.jar -v -interval HGMD_PRO_2016.1_hg19.vcf GRCh37.75 sample-hg00731.vcf
00:00:00        SnpEff version SnpEff 4.3 (build 2016-06-14 18:42), by Pablo Cingolani
00:00:00        Command: 'ann'
00:00:00        Reading configuration file 'snpEff.config'. Genome: 'GRCh37.75'
00:00:00        Reading config file: D:\snpEff\snpEff.config
00:00:00        done
00:00:00        Reading database for genome version 'GRCh37.75' from file 'D:\snpEff/./data/GRCh37.75/snpEffectPredictor.bin' (this might take a while)
00:00:24        done
00:00:24        Reading interval file 'HGMD_PRO_2016.1_hg19.vcf'
00:00:24        done (161162 intervals loaded).
```

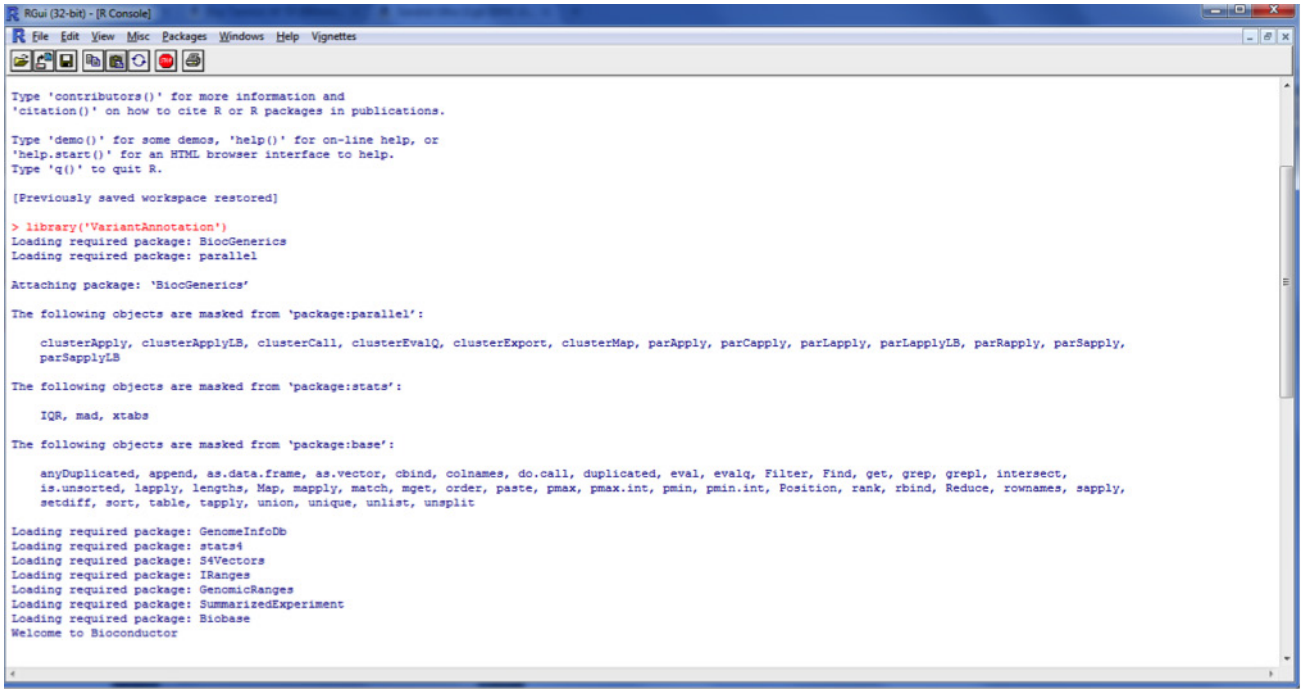
Output:



## Variant Annotation – a Bioconductor package

**Step1:** Install the VariantAnnotation package from Bioconductor

```
> library ('VariantAnnotation')
```



**Step 2:** Upload the input vcf file using the "readVcf" function. In this example sample-hg00731.vcf is the input file

```
> vcf <- readVcf("D:/sample-hg00731.vcf", "hg19")
```

```
> vcf <- readVcf("D:/sample-hg00731.vcf", "hg19" )
> vcf
class: CollapsedVCF
dim: 499882 1
rowRanges(vcf):
  GRanges with 5 metadata columns: paramRangeID, REF, ALT, QUAL, FILTER
info(vcf):
  DataFrame with 21 columns: NS, AN, AC, CGA_XR, CGA_FI, CGA_PFAM, CGA_MIRB, CGA_RPT, CGA_SDO, END, CGA_
info(header(vcf)):
             Number Type    Description
   NS        1      Integer Number of Samples With Data
   AN        1      Integer Total number of alleles in called genotypes
   AC        A      Integer Allele count in genotypes, for each ALT allele
   CGA_XR    A      String  Per-ALT external database reference (dbSNP, COSMIC, etc)
   CGA_FI    A      String  Functional impact annotation
   CGA_PFAM  .      String  PFAM Domain
   CGA_MIRB  .      String  miRBaseId
   CGA_RPT   .      String  repeatMasker overlap information
   CGA_SDO   1      Integer Number of distinct segmental duplications that overlap this locus
   END       1      Integer End position of the variant described in this record
   CGA_WINEND 1     Integer End of coverage window
   CGA_BF    1      Float   Frequency in baseline
   CGA_MEDEL 4      String  Consistent with deletion of mobile element; type,chromosome,start,end
   MATEID    1      String  ID of mate breakend
   SVTYPE    1      String  Type of structural variant
   CGA_BNDG  A      String  Transcript name and strand of genes containing breakend
   CGA_BNDGO A      String  Transcript name and strand of genes containing mate breakend
   CIPOS     2      Integer Confidence interval around POS for imprecise variants
   IMPRECISE 0      Flag    Imprecise structural variation
   MEINFO    4      String  Mobile element info of the form NAME,START,END,POLARITY
   SVLEN     .      Integer Difference in length between REF and ALT alleles
geno(vcf):
  SimpleList of length 33: GT, PS, SS, FT, GQ, HQ, EHQ, CGA_CEHQ, GL, CGA_CEGL, DP, AD, CGA_RDP, CGA_GP,
geno(header(vcf)):
             Number Type    Description
   GT        1      String  Genotype
   PS        1      Integer Phase Set
   SS        1      String  Somatic Status: Germline, Somatic, LOH, or . (Unknown)
   FT        1      String  Genotype filters
```

**Step 3:** Upload the HGMD annotations using the "readVcf" function. The VCF formatted HGMD file (named HGMD_PRO_2016.1_hg19.vcf in this example) is used as the database file

```
> hgmd <- readVcf("D:/HGMD_PRO_2016.1_hg19.vcf", "hg19")
```

**Step 4:** Optionally filter the HGMD annotations by their location within or relative to a gene using the locateVariants function and the UCSC HG19 genomic coordinates package specified as txdb. Regions are specified in the region argument and can be one of the following: CodingVariants, IntronVariants, FiveUTRVariants, ThreeUTRVariants,

IntergenicVariants, SpliceSiteVariants or PromoterVariants. Here we show an example specifying variants located within coding regions

```
> loc <- locateVariants(rowRanges(hgmd), txdb, CodingVariants())
```

```
> loc <- locateVariants(rowRanges(hgmd), txdb, CodingVariants())
'select()' returned many:1 mapping between keys and columns
> loc
GRanges object with 443700 ranges and 9 metadata columns:
        seqnames              ranges strand   | LOCATION  LOCSTART    LOCEND   QUERYID       TXID        CDSID      GENEID       PRECEDEID        FOLLOWID
           <Rle>           <IRanges>  <Rle>   | <factor> <integer> <integer> <integer> <character> <IntegerList> <character> <CharacterList> <CharacterList>
       1     chr1     [877523, 877523]      +   |   coding       877       877         1          22          28      148398
       2     chr1     [877523, 877523]      +   |   coding       832       832         1          23          28      148398
       3     chr1     [877523, 877523]      +   |   coding       880       880         1          24          28      148398
       4     chr1     [877523, 877523]      +   |   coding       829       829         1          26          28      148398
       5     chr1     [877523, 877523]      +   |   coding       274       274         1          29          28      148398
     ...      ...                  ...    ... ...      ...       ...       ...       ...         ...         ...         ...                ...              ...
  443696     chrY [16952726, 16952726]      +   |   coding      1531      1531    161162       78460      226890       22829
  443697     chrY [16952726, 16952726]      +   |   coding      2095      2095    161162       78461      226890       22829
  443698     chrY [16952726, 16952726]      +   |   coding      1114      1114    161162       78462      226890       22829
  443699     chrY [16952726, 16952726]      +   |   coding      2035      2035    161162       78463      226890       22829
  443700     chrY [16952726, 16952726]      +   |   coding      2035      2035    161162       78464      226890       22829
  -------
  seqinfo: 24 sequences from an unspecified genome; no seqlengths
```

And an example specifying variants located within promoter regions

```
> loc <- locateVariants(rowRanges(hgmd), txdb, PromoterVariants())
```

```
> loc <- locateVariants(rowRanges(hgmd), txdb, PromoterVariants())
'select()' returned many:1 mapping between keys and columns
> loc
GRanges object with 38593 ranges and 9 metadata columns:
         seqnames              ranges strand   | LOCATION  LOCSTART    LOCEND   QUERYID      TXID        CDSID      GENEID       PRECEDEID        FOLLOWID
            <Rle>           <IRanges>  <Rle>   | <factor> <integer> <integer> <integer> <integer> <IntegerList> <character> <CharacterList> <CharacterList>
      [1]     chr1 [1167659, 1167659]      +   | promoter      <NA>      <NA>        16        74                  126792
      [2]     chr1 [1167659, 1167659]      -   | promoter      <NA>      <NA>        16      4140                   51150
      [3]     chr1 [1167659, 1167659]      -   | promoter      <NA>      <NA>        16      4141                   51150
      [4]     chr1 [1167659, 1167659]      -   | promoter      <NA>      <NA>        16      4142                   51150
      [5]     chr1 [1167674, 1167674]      +   | promoter      <NA>      <NA>        17        74                  126792
      ...      ...                  ...    ... ...      ...       ...       ...       ...       ...         ...         ...                ...              ...
  [38589]     chrY [2655637, 2655637]      -   | promoter      <NA>      <NA>    161154     78581                    6736
  [38590]     chrY [2655638, 2655639]      -   | promoter      <NA>      <NA>    161155     78581                    6736
  [38591]     chrY [2655641, 2655641]      -   | promoter      <NA>      <NA>    161156     78581                    6736
  [38592]     chrY [2655719, 2655719]      -   | promoter      <NA>      <NA>    161157     78581                    6736
  [38593]     chrY [2655774, 2655774]      -   | promoter      <NA>      <NA>    161158     78581                    6736
  -------
  seqinfo: 24 sequences from an unspecified genome; no seqlengths
  '
```

**Step 5:** Annotate the input VCF file with HGMD annotations using the subsetByOverlaps function. In this example, vcf is the previously uploaded input file and hgmd is the previously uploaded HGMD annotations

```
> out <- subsetByOverlaps(hgmd,vcf)
```

```
> out<-subsetByOverlaps(hgmd,vcf)
> out
class: CollapsedVCF
dim: 200 0
rowRanges(vcf):
  GRanges with 5 metadata columns: paramRangeID, REF, ALT, QUAL, FILTER
info(vcf):
  DataFrame with 8 columns: CLASS, MUT, GENE, STRAND, DNA, PROT, DB, PHEN
info(header(vcf)):
          Number Type   Description
   CLASS  1      String Mutation Category, https://portal.biobase-international.com/hgmd/pro/global.php#cats
   MUT    1      String HGMD mutant allele
   GENE   1      String Gene symbol
   STRAND 1      String Gene strand
   DNA    1      String DNA annotation
   PROT   1      String Protein annotation
   DB     1      String dbSNP identifier, build 137
   PHEN   1      String HGMD primary phenotype
geno(vcf):
  SimpleList of length 0:
>
```

**Step 6:** View the output. Use the info(out) command to view the HGMD annotations

```
> info(out)
```

```
> info(out)
DataFrame with 200 rows and 8 columns
            CLASS         MUT        GENE      STRAND                                                DNA                PROT          DB
      <character> <character> <character> <character>                                        <character>         <character> <character>
CI148519         DM         ALT        AGRN           +                          NM_198576.3:c.1362dupC                  NA          NA
CS060109         DP         ALT     TNFRSF4           -                        NM_003327.3:c.634+25C>T                  NA   rs2298212
CM134937         DM         ALT     B3GALT6           +                          NM_080605.3:c.649G>A  NP_542172.2:p.G217S rs397514724
CM1411605        DM         ALT     B3GALT6           +                          NM_080605.3:c.766C>T  NP_542172.2:p.R256W          NA
BM1422338        DM         ALT     B3GALT6           +                          NM_080605.3:c.795A>C  NP_542172.2:p.E265D rs374677519
...             ...         ...         ...         ...                                           ...                 ...         ...
CX941936         DM         ALT         GBA           - NM_001005741.2:c.1447_1466delCTGGACGCAGTGGCACTGATinsTG                  NA          NA
CM940819         DM         ALT         GBA           -                        NM_001005741.2:c.1448T>G NP_001005741.1:p.L483R          NA
CM870010         DM         ALT         GBA           -                        NM_001005741.2:c.1448T>C NP_001005741.1:p.L483P    rs421016
CM001167         DM         ALT         GBA           -                        NM_001005741.2:c.685G>A NP_001005741.1:p.A229T          NA
CD050144         DM         ALT        LMNA           +                NM_170707.3:c.-3_12delGCCATGGAGACCCCG                  NA rs267607546
                                                                                                    PHEN
                                                                                             <character>
CI148519  "Congenital_myasthenic_syndrome_with_distal_muscle_weakness_&_atrophy"
CS060109                 "Myocardial_infarction_protection_against_association"
CM134937                                  "Ehlers-Danlos_syndrome-like"
CM1411605                "Spondyloepimetaphyseal_dysplasia_with_joint_laxity"
BM1422338                                          "Al-Gazali_syndrome"
...                                                                    ...
CX941936                                                 "Gaucher_disease"
CM940819                                                 "Gaucher_disease"
CM870010                                               "Gaucher_disease_2"
CM001167                                               "Gaucher_disease_3"
CD050144                       "Muscular_dystrophy_Emery-Dreifuss_neurogenic"
>
```

Use the rowRanges(out) command to show the genomic coordinate information for the mutations

```
> rowRanges(out)
```

```
> rowRanges(out)
GRanges object with 200 ranges and 5 metadata columns:
            seqnames              ranges strand   | paramRangeID                REF               ALT      QUAL      FILTER
               <Rle>           <IRanges>  <Rle>   |     <factor>      <DNAStringSet> <DNAStringSetList> <numeric> <character>
   CI148519        1     [ 977516,  977516]      *   |         <NA>                  T                TC      <NA>           .
   CS060109        1     [1147297, 1147297]      *   |         <NA>                  G                 A      <NA>           .
   CM134937        1     [1168307, 1168307]      *   |         <NA>                  G                 A      <NA>           .
  CM1411605        1     [1168424, 1168424]      *   |         <NA>                  C                 T      <NA>           .
  BM1422338        1     [1168453, 1168453]      *   |         <NA>                  A                 C      <NA>           .
        ...      ...                    ...    ... ...         ...                ...               ...       ...         ...
   CX941936        1 [155205024, 155205044]      *   |         <NA> CATCAGTGCCACTGCGTCCAG               CCA      <NA>           .
   CM940819        1 [155205043, 155205043]      *   |         <NA>                  A                 C      <NA>           .
   CM870010        1 [155205043, 155205043]      *   |         <NA>                  A                 G      <NA>           .
   CM001167        1 [155208001, 155208001]      *   |         <NA>                  C                 T      <NA>           .
   CD050144        1 [156084703, 156084718]      *   |         <NA>      GCCGGCCATGGAGACC               G      <NA>           .
  -------
  seqinfo: 24 sequences from hg19 genome; no seqlengths
> rowRanges(out1)
GRanges object with 184 ranges and 5 metadata columns:
                                                                                seqnames              ranges strand   | paramRangeID
                                                                                   <Rle>           <IRanges>  <Rle>   |     <factor>
                                                                  1:977510_GTGCCAT/.       1     [ 977510,  977516]      *   |         <NA>
                                                                      1:1147297_G/A        1     [1147297, 1147297]      *   |         <NA>
                                                                     1:1168306_CG/.        1     [1168306, 1168307]      *   |         <NA>
  1:1168406_GCGCCGGTGGACGTCCAGCGGGAGCACGACCCGCGCTTCGACACCGAATACCG/.       1     [1168406, 1168458]      *   |         <NA>
                                                                      1:1265154_T/C        1     [1265154, 1265154]      *   |         <NA>
                                                                                ...      ...                    ...    ... ...         ...
                                                                  1:155106697_G/A        1 [155106697, 155106697]      *   |         <NA>
                                                1:155178775_CCGTGACA/CCGTGACT        1 [155178775, 155178782]      *   |         <NA>
                                                                 1:155205043_A/.        1 [155205043, 155205043]      *   |         <NA>
                                                  1:155208001_C/<CGA_CNVWIN>        1 [155208001, 155208001]      *   |         <NA>
                                                                  1:156084704_C/.        1 [156084704, 156084704]      *   |         <NA>
```

## Obtaining access to HGMD

For more information, or to obtain a quote for a license to HGMD data for use in any of the tools profiled in this technical note, contact **bioinformaticssales@qiagen.com**.


**EMEA**
Silkeborgvej 2 · Prismet
8000 Aarhus C
**Denmark**
**Phone:** +45 7022 5509

**qiagenbioinformatics.com**

**Americas**
1700 Seaport Boulevard #3
Redwood City · CA 94063
**USA**
**Phone:** +1 (617) 945 0178

**bioinformaticssales@qiagen.com**