

Introduction to GeneBreak

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Contents

1	Running GeneBreak	2
1.1	Detect breakpoints from copy-number data	2
1.1.1	Loading cghCall object	2
1.1.2	Loading data from a dataframe	3
1.2	Breakpoint selection by filtering	4
1.3	Identification of genes affected by breakpoints	4
1.3.1	Loading gene annotation data	4
1.3.2	Feature-to-gene mapping	5
1.3.3	Detection of gene-associated breakpoints	6
1.4	Cohort-based breakpoint statistics	7
1.4.1	Detection of recurrent breakpoint genes	7
1.4.2	Detection of recurrent breakpoint locations	7
1.5	Visualization of breakpoint frequencies	8
2	Storage of R objects	10
3	Downloading Gene Annotations	10
4	Session Information	12

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1 Running GeneBreak

The `GeneBreak` package aims to systematically identify genes recurrently affected by copy number aberration-associated breakpoint locations that indicate underlying DNA breaks and thereby genes involved in structural variants. This is a short tutorial on how to use the `GeneBreak` package. It describes an example workflow which uses copy number aberration (CNA) data obtained by analysis of 200 array-CGH (Agilent 180k) samples from advanced colorectal cancers. We used the `CGHcall` package that can be obtained via Bioconductor (www.bioconductor.org). First, we will start with loading the package.

```
> library(GeneBreak)
```

1.1 Detect breakpoints from copy-number data

Copy number data can be loaded in two ways. We recommend the usage of Bioconductor packages `CGHcall` or `QDNAseq` to process CNA data from array-CGH or sequencing data respectively. The obtained `cghCall`/`QDNAseq` object can directly serve as input for the `GeneBreak` pipeline. Alternatively, a `data.frame` with exactly these five columns: "Chromosome", "Start", "End" and "Feature-Name" (usually probe or bin identifier) followed by columns with sample data can be provided. In this tutorial we will use a built-in dataset that contains CNA data from chromosome 20:

1.1.1 Loading `cghCall` object

To load and run the example dataset, which is an object of class `CGHcall`, the `CGHcall` package needs to be installed.

```
> # Install the "CGHcall" package from Bioconductor:
> # if (!requireNamespace("BiocManager", quietly=TRUE))
  > # install.packages("BiocManager")
> # BiocManager::install("CGHcall")
```

Load the example dataset from `GeneBreak`:

```
> library(CGHcall)
> data( "copynumber.data.chr20" )
```

Inspection of the loaded data shows an R object of class `cghCall` that contains CNA data from 3653 features (array-CGH probes in this case) and 200 samples.

```
> copynumber.data.chr20
```

```
cghCall (storageMode: lockedEnvironment)
assayData: 3653 features, 200 samples
  element names: calls, copynumber, probamp, probgain, probloss, probnorm, segmented
```

```

protocolData: none
phenoData
  sampleNames: sample_1 sample_2 ... sample_200 (200 total)
  varLabels: Cellularity
  varMetadata: labelDescription
featureData
  featureNames: A_16_P03469195 A_14_P136138 ... A_18_P13856091
  (3653 total)
  fvarLabels: Chromosome Start End
  fvarMetadata: labelDescription
experimentData: use 'experimentData(object)'
Annotation:

```

To generate an object of class `CopyNumberBreakpoints` with breakpoint locations, run `getBreakpoints()`. This will obtain the required information from the `cghCall` object and determine the breakpoint locations.

```
> breakpoints <- getBreakpoints( data = copynumber.data.chr20 )
```

```
Breakpoint detection started...
```

```
> breakpoints
```

```
--- Object Info ---
```

```
This is an object of class "CopyNumberBreakPoints"
```

```
3653 features by 200 samples
```

```
A total of 1035 breakpoints
```

```
See accessOptions(object) for how to access data in this object
```

Inspection of the generated object shows that we have copy number data of 3653 features from 200 samples. A total of 1035 individual breakpoint locations were identified.

1.1.2 Loading data from a dataframe

If the CNA data has not been generated by `CGHcall` or `QDNaseq`, there is a possibility of using a `data.frame()` as input for `GeneBreak`. This allows breakpoint analysis of data from any copy number detection pipeline by importing a text file into `getBreakpoints()`.

Here we show how to use two `data.frames()` with `segment` and (optionally) `calls` values as input for `getBreakpoints` instead of a `cghCall/QDNaseq` object.

```

> library(CGHcall)
> cgh <- copynumber.data.chr20
> segmented <- data.frame( Chromosome=chromosomes(cgh), Start=bpstart(cgh),
+ End=bpstart(cgh), FeatureName=rownames(cgh), segmented(cgh))
> called <- data.frame( Chromosome=chromosomes(cgh), Start=bpstart(cgh),
+ End=bpstart(cgh), FeatureName=rownames(cgh), calls(cgh))
> breakpoints <- getBreakpoints( data = segmented, data2 = called )

```

Note: the first five column names of the data.frame must exactly be "Chromosome", "Start", "End" and "FeatureName".

1.2 Breakpoint selection by filtering

Next, breakpoints can be filtered by stringent criteria. Different filters can be set (see `?bpFilter` for more details). Default setting is "CNA-ass" which means that breakpoints flanked by copy number neutral segments will be filtered out. Note: you need discrete copy number calls (loss,neutral, gain, etc) for this option.

```
> breakpointsFiltered <- bpFilter( breakpoints, filter = "CNA-ass" )
```

Applying BP selection...

```
> breakpointsFiltered
```

```
--- Object Info ---
```

```
This is an object of class "CopyNumberBreakPoints"
```

```
3653 features by 200 samples
```

```
A total of 985 breakpoints
```

```
See accessOptions(object) for how to access data in this object
```

Inspection of the output shows that 985 CNA-associated breakpoint locations remain following the filter step.

1.3 Identification of genes affected by breakpoints

Identification of genes affected by breakpoints requires execution of the following three steps.

1.3.1 Loading gene annotation data

We need to load gene annotations to be able to identify genes affected by breakpoints in the next step. Gene annotation for human reference genome hg18 (and hg19, hg38) are built-in, but also user-defined annotations can be used. The required columns for this data.frame are "Gene", "Chromosome", "Start" and "End".

```
> data( "ens.gene.ann.hg18" )
```

This shows the content of the first six rows of the hg18 gene annotation dataframe:

```
> head( ens.gene.ann.hg18 )
```

	Gene	EnsID	Chromosome	Start	End	band	strand
21297	MIRN1302-2	ENSG00000221311	1	20229	20366	p36.33	1
21	FAM138E	ENSG00000222027	1	24417	25944	p36.33	-1
827	FAM138E	ENSG00000222003	1	24417	25944	p36.33	-1
828	FAM138A	ENSG00000222003	1	24417	25944	p36.33	-1
829	OR4F5	ENSG00000177693	1	58954	59871	p36.33	1
830	OR4F29	ENSG00000177799	1	357522	358460	p36.33	1

1.3.2 Feature-to-gene mapping

Here, the loaded gene annotation information will be added to the GeneBreak object and feature-to-gene mapping will be performed.

```
> breakpointsAnnotated <- addGeneAnnotation( breakpointsFiltered, ens.gene.ann.hg18 )
```

```
Adding of gene annotation started on 659 genes by 200 samples
0% ... 25% ... 50% ... 75% ... Adding gene annotation DONE
```

To show the names of associated features of e.g. the "PCMTD2" gene, give:

```
> featuresPerGene ( breakpointsAnnotated , geneName = "PCMTD2" )
```

```
Gene chosen: PCMTD2
```

```
[1] "A_14_P125849" "A_16_P21189265" "A_16_P21189294" "A_16_P34766035"
```

Gene-associated feature information has been added to breakpointsAnnotated. Visualisation shows:

```
> geneFeatures <- geneInfo( breakpointsAnnotated )
> head( geneFeatures[ ,
+ c("Gene", "Chromosome", "Start", "End", "featureTotal",
+ "featureNames", "remarks") ] )
```

	Gene	Chromosome	Start	End	featureTotal
1366	DEFB125	20	16351	25296	2
1376	DEFB126	20	71231	74391	2
1383	DEFB127	20	86122	87804	2
1393	DEFB128	20	116527	118264	1
1396	DEFB129	20	155899	158523	2
1402	DEFB132	20	186377	189735	2

	featureNames	remarks
1366	A_14_P136138,A_16_P03469215	D
1376	A_14_P122034,A_14_P106962	D
1383	A_14_P106962,A_16_P41238845	D
1393	A_16_P41238870	C
1396	A_14_P113156,A_16_P03469327	D
1402	A_14_P200562,A_16_P41239011	D

Possible "remarks" that describe gene position with respect to feature positions are: "A": genes located upstream of the first chromosomal feature (no gene-associated features) "B": genes located downstream of the last chromosomal feature (no gene-associated features) "C": in case of array-CGH probes, the whole gene is located between two features "C": in case of sequencing data, the whole gene is located between start and end of one bin "D": gene represented by one or multiple features "E": gene represented by one or multiple features, but the end of the gene is not covered by any feature "X": no feature covers the chromosome of the gene

1.3.3 Detection of gene-associated breakpoints

In the next step, gene-associated breakpoints will be identified by using `bpGenes()`.

```
> breakpointGenes <- bpGenes( breakpointsAnnotated )
```

```
Running bpGenes: 659 genes and 200 samples
0% ... 25% ... 50% ... 75% ... bpGenes DONE
A total of 1029 gene breaks in 241 genes detected
```

This is an example of the output when selected for broken genes:

```
> result_BreakpointGenes <- geneInfo ( breakpointGenes )
> head( result_BreakpointGenes[ which ( result_BreakpointGenes$sampleCount > 0 ) ,
+   c( "Gene", "Chromosome", "Start", "End", "featureTotal", "nrOfBreakLocations",
+     "sampleCount", "sampleNamesWithBreakpoints" ) ] )
```

	Gene	Chromosome	Start	End	featureTotal	nrOfBreakLocations	
1414	C20orf96	20	199504	219390	3	1	
1633	SRXN1	20	575270	581890	2	1	
1643	SCRT2	20	590241	604823	2	1	
1683	RSP04	20	887098	930904	4	1	
1694	PSMF1	20	1041906	1097022	5	3	
1808	NSFL1C	20	1370807	1396417	3	1	
	sampleCount						sampleNamesWithBreakpoints
1414	1						sample_60
1633	1						sample_128
1643	1						sample_128
1683	1						sample_1
1694	5	sample_16, sample_42, sample_105, sample_180, sample_195					
1808	1						sample_64

This table shows the genes (rows) and the number of gene-associated features in "featureTotal". The column "nrOfBreakLocations" indicates the number of identified breakpoint locations in the gene across all samples. As a consequence, this is a subset of, and limited by, the total number of gene-associated features. The total of samples that harbor a breakpoint in the gene is given in the column "sampleCount".

1.4 Cohort-based breakpoint statistics

Following identification of (gene) breakpoints per profile, breakpoint events that are significantly recurring across samples will be determined by dedicated statistical analysis. This can be performed at "gene" (breakpoint gene) and/or "feature" (breakpoint location) level. Two different methods of FDR-type correction for multiple testing can be used, the standard Benjamini-Hochberg FDR-type correction ("BH") or dedicated Benjamini-Hochberg FDR-type correction ("Gilbert").

1.4.1 Detection of recurrent breakpoint genes

The gene-based statistical analysis includes correction for covariates that may influence the probability to be a breakpoint gene including number of breakpoints in a profile, number of gene-associated features and gene length by gene-associated feature coverage. Multiple testing can be applied by the powerful dedicated Benjamini-Hochberg FDR-type correction ("Gilbert") that accounts for the discreteness of the null-distribution. (Reference: Gilbert PB, Appl Statist. 2005;54:143-58) NOTE: when running `bpStats()` warnings can be generated by a function (`glm.fit`) of a dependency package, this does not harm the analysis.

```
> breakpointStatistics <- bpStats( breakpointGenes,  
+   level = "gene", method = "Gilbert" )
```

Applying statistical test over 200 samples for: gene breakpoints: Gilbert test...

This will return an object of class `CopyNumberBreakPointGenes`. By using `recurrentGenes()` we can observe the recurrent affected genes with associated P-value and FDR.

```
> head( recurrentGenes( breakpointStatistics ) )
```

```
A total of 19 recurrent breakpoint genes (at FDR < 0.1)
```

	Gene	sampleCount	featureTotal	pvalue	FDR
13886	PCMTD2	64	4	1.350385e-103	1.848343e-101
13898	C20orf69	33	3	5.522293e-44	3.860197e-42
4268	BFSP1	8	5	3.941447e-07	3.148759e-05
5473	ABHD12	10	9	5.756361e-05	3.687639e-03
4780	C20orf26	7	18	2.748743e-04	1.204846e-02
4102	KIF16B	7	19	4.054266e-04	1.322722e-02

1.4.2 Detection of recurrent breakpoint locations

With this step, statistics at breakpoint location (feature) level will be added to the object of class `CopyNumberBreakPointGenes`. Here, we recommend to use the less computationally intensive standard Benjamini-Hochberg FDR-type correction for multiple testing, because the breakpoint probability is equal across features per profile, which means that all positions correspond to the same null-distribution.

```
> breakpointStatistics <- bpStats(
+   breakpointStatistics, level = "feature", method = "BH" )
```

Applying statistical test over 200 samples for feature breakpoints: BH test...

```
> breakpointStatistics
```

```
--- Object Info ---
```

```
This is an object of class "CopyNumberBreakPointGenes"
```

```
3653 features by 200 samples
```

```
A total of 985 breakpoints
```

```
A total of 1029 gene breaks in 241 genes
```

```
A total of 19 recurrent breakpoint genes (FDR < 0.1)
```

```
A total of 29 recurrent breakpoints (FDR < 0.1)
```

```
See accessOptions(object) for how to access data in this object
```

By using `featureInfo()` we can observe the features and whether they were identified as breakpoints including the calculated FDR values:

```
> head( featureInfo( breakpointStatistics ) )
```

	Chromosome	Start	End	featureInterval	sampleCount			
A_16_P03469195	20	8747	8793	0	0			
A_14_P136138	20	18580	18639	9833	0			
A_16_P03469215	20	25530	25589	6950	0			
A_16_P21047338	20	32699	32743	7169	0			
A_16_P41238750	20	39125	39184	6426	0			
A_16_P03469235	20	50422	50481	11297	0			
	sampleNamesWithBreakpoints	nrOfBreakLocations	pvalue	FDR				
A_16_P03469195		0	1	1				
A_14_P136138		0	1	1				
A_16_P03469215		0	1	1				
A_16_P21047338		0	1	1				
A_16_P41238750		0	1	1				
A_16_P03469235		0	1	1				

1.5 Visualization of breakpoint frequencies

Breakpoint locations and frequencies can be visualized using `bpPlot()`:

```
> bpPlot( breakpointStatistics, fdr.threshold = 0.1 )
```

```
Plotting breakpoint frequencies ...
```

```
Plotting Chromosome: 20
```

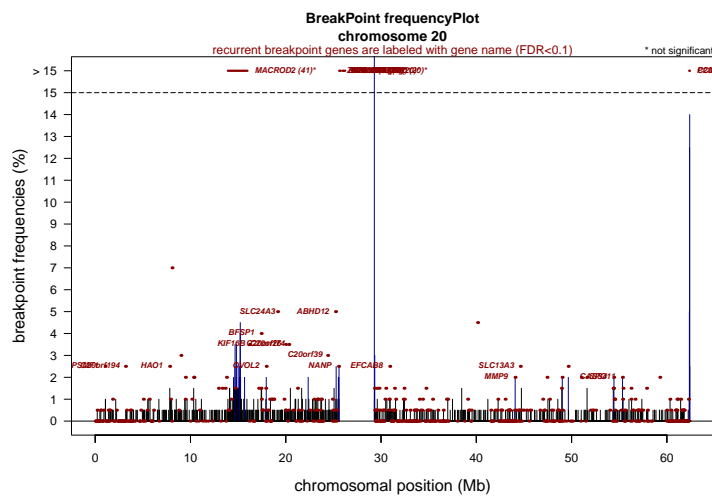



Figure 1: Graphical representation of CNA-associated chromosomal breakpoint frequencies and their distribution over chromosomes 20. The X-axis depicts the genomic position in Mb. The Y-axis depicts the chromosomal breakpoint frequencies across the series of 200 CRC samples. Breakpoint frequencies are indicated on array-CGH probe-level (vertical black bars) and on gene-level (horizontal black bars). Recurrent breakpoint genes (FDR<0.1) are named. When the gene breakpoint frequency exceeded 15% (horizontal dashed line), the breakpoint frequency (%) follows the gene name.

2 Storage of R objects

At any time during the analysis, the GeneBreak objects (and any R objects for that matter) can be saved to disk with: `saveRDS`, and in the future be read from the local file with `loadRDS`

3 Downloading Gene Annotations

This section describes the steps taken to create the gene annotations used in this package. It may serve as a start for creating your own if required for whatever reason.

```
> # gene annotations obtained via BiomaRt.
> # HUGO gene names (HGNC symbol), Ensembl_ID and chromosomal location
>
> # Used (and most) recent releases:
> # HG18: release54
> # HG19: release75
> # HG38: release80 (date: 150629)
>
> library(biomaRt)
> ensembl54 = useMart(
+   host = 'may2009.archive.ensembl.org',
+   biomaRt = 'ENSEMBL_MART_ENSEMBL',
+   dataset = "hsapiens_gene_ensembl"
+ )
> ensembl75 = useMart(
+   host = 'feb2014.archive.ensembl.org',
+   biomaRt = 'ENSEMBL_MART_ENSEMBL',
+   dataset = "hsapiens_gene_ensembl"
+ )
> ensembl80 = useMart(
+   "ensembl",
+   dataset = "hsapiens_gene_ensembl"
+ )
> createAnnotationFile <- function( biomaRtVersion ) {
+   biomaRt_result <- getBM(
+     attributes = c(
+       "hgnc_symbol", "ensembl_gene_id", "chromosome_name",
+       "start_position", "end_position", "band", "strand"
+     ),
+     mart = biomaRtVersion
+   )
+   biomaRt_result[,3] <- as.vector( biomaRt_result[,3] )
+   idx_x <- biomaRt_result$chromosome_name == "X"
```

```

+   idx_y <- biomart_result$chromosome_name == "Y"
+   biomart_result$chromosome_name[ idx_x ] <- "23"
+   biomart_result$chromosome_name[ idx_y ] <- "24"
+
+   biomart_genes <- biomart_result[ which(biomart_result[,1] != "" &
+     biomart_result[,3] %in% c(1:24)) , ]
+   colnames(biomart_genes)[1:5] <- c("Gene", "EnsID", "Chromosome", "Start", "End")
+
+   cat(
+     c( "Biomart version:", biomartVersion@host,
+       "including:", dim(biomart_genes)[1], "genes\n"
+     )
+   )
+
+   return( biomart_genes )
+ }
> ens.gene.ann.hg18 <- createAnnotationFile( ensembl54 )
> ens.gene.ann.hg19 <- createAnnotationFile( ensembl75 )
> ens.gene.ann.hg38 <- createAnnotationFile( ensembl80 )
>

```

4 Session Information

The version number of R and packages loaded for generating the vignette were:

R version 4.3.1 (2023-06-16)

Platform: x86_64-pc-linux-gnu (64-bit)

Running under: Ubuntu 22.04.3 LTS

Matrix products: default

BLAS: /home/biocbuild/bbs-3.18-bioc/R/lib/libRblas.so

LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.10.0

locale:

```
[1] LC_CTYPE=en_US.UTF-8      LC_NUMERIC=C
[3] LC_TIME=en_GB             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
```

time zone: America/New_York

tzcode source: system (glibc)

attached base packages:

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

other attached packages:

```
[1] GeneBreak_1.32.0      GenomicRanges_1.54.0 GenomeInfoDb_1.38.0
[4] IRanges_2.36.0        S4Vectors_0.40.0     CGHcall_2.64.0
[7] snowfall_1.84-6.2     snow_0.4-4           CGHbase_1.62.0
[10] marray_1.80.0         limma_3.58.0         Biobase_2.62.0
[13] BiocGenerics_0.48.0  DNACopy_1.76.0       impute_1.76.0
[16] QDNaseq_1.38.0
```

loaded via a namespace (and not attached):

```
[1] crayon_1.5.2          statmod_1.5.0
[3] listenv_0.9.0         future.apply_1.11.0
[5] RCurl_1.98-1.12       Biostrings_2.70.0
[7] bitops_1.0-7          compiler_4.3.1
[9] codetools_0.2-19     XVector_0.42.0
[11] future_1.33.0         BiocParallel_1.36.0
[13] R.oo_1.25.0           digest_0.6.33
[15] R.utils_2.12.2        parallelly_1.36.0
[17] parallel_4.3.1        GenomeInfoDbData_1.2.11
[19] R.methodsS3_1.8.2     tools_4.3.1
```

```
[21] matrixStats_1.0.0      globals_0.16.2
[23] Rsamtools_2.18.0        zlibbioc_1.48.0
```