

# Package ‘methylGSA’

October 4, 2022

**Type** Package

**Title** Gene Set Analysis Using the Outcome of Differential Methylation

**Version** 1.15.0

**Description** The main functions for methylGSA are methylglm and methylRRA. methylGSA implements logistic regression adjusting number of probes as a covariate. methylRRA adjusts multiple p-values of each gene by Robust Rank Aggregation. For more detailed help information, please see the vignette.

**Encoding** UTF-8

**Imports** RobustRankAggreg, ggplot2, stringr, stats, clusterProfiler, missMethyl, org.Hs.eg.db, reactome.db, BiocParallel, GO.db, AnnotationDbi, shiny, IlluminaHumanMethylation450kanno.ilmn12.hg19, IlluminaHumanMethylationEPICanno.ilm10b4.hg19

**Depends** R (>= 3.5)

**Suggests** knitr, rmarkdown, testthat, enrichplot

**License** GPL-2

**URL** <https://github.com/reese3928/methylGSA>

**BugReports** <https://github.com/reese3928/methylGSA/issues>

**RoxygenNote** 6.1.0

**VignetteBuilder** knitr

**biocViews** DNAMethylation,DifferentialMethylation,GeneSetEnrichment,Regression, GeneRegulation,Pathways

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barplot	<i>Barplot for methylGSA analysis result</i>
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### Description

This function visualizes methylGSA analysis result by barplot.

### Usage

```
barplot(res, xaxis = "Size", num = 5, colorby = "padj", title = "")
```

### Arguments

<code>res</code>	A data frame which contains methylGSA analysis result.
<code>xaxis</code>	A string which specify the x-axis in the barplot. Either "Size" (number of genes in gene set) or "Count" (number of significant genes in gene set). Default is "Size". "Count" option is not available for methylglm and methylIRRA(GSEA) result.
<code>num</code>	An integer. Number of gene sets to display on the barplot. Default is 5.
<code>colorby</code>	A string. Either "pvalue" or "padj". Default is "padj".
<code>title</code>	A string. Barplot title. Default is NULL.

### Details

The implementation of the function is adapted from barplot function in enrichplot package.

### Value

ggplot object

**References**

Yu G (2018). enrichplot: Visualization of Functional Enrichment Result. R package version 1.0.2, <https://github.com/GuangchuangYu/enrichplot>.

**Examples**

```
res = data.frame(ID = c("04144", "04510", "04740", "04810", "05200"),
  Description = c("Endocytosis", "Focal adhesion",
    "Olfactory transduction",
    "Regulation of actin cytoskeleton", "Pathways in cancer"),
  Size = c(201, 200, 388, 213, 326),
  pvalue = c(0.481, 0.696, 1, 1, 1),
  padj = 1
)
barplot(res)
```

---

cpg.pval

*An example of user input cpg.pval*


---

**Description**

An example of user input cpg.pval

**Usage**

```
cpg.pval
```

**Format**

A named vector contains p-values of each probe tested

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CpG2Gene

*An example of user user-supplied mapping between CpGs and genes*


---

**Description**

An example of user user-supplied mapping between CpGs and genes

**Usage**

```
CpG2Gene
```

**Format**

A data frame contains mapping between CpGs and genes

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getAnnot	<i>Get CpG annotation</i>
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### Description

This function gets CpG IDs and their corresponding gene symbols.

### Usage

```
getAnnot(array.type, group = "all")
```

### Arguments

array.type	A string. Either "450K" or "EPIC". Default is "450K".
group	A string. "all", "body", "promoter1" or "promoter2". Default is "all". If group = "body", only CpGs on gene body will be pulled out. If group = "promoter1" or group = "promoter2", only CpGs on promoters will be pulled out. Here is the definition of "body", "promoter1" and "promoter2" according to the annotation in <code>IlluminaHumanMethylation450kanno.ilmn12.hg19</code> or <code>IlluminaHumanMethylationEPICanno.ilm10b4.hg19</code> . <ul style="list-style-type: none"> <li>• body: CpGs whose gene group correspond to "Body" or "1stExon"</li> <li>• promoter1: CpGs whose gene group correspond to "TSS1500" or "TSS200"</li> <li>• promoter2: CpGs whose gene group correspond to "TSS1500", "TSS200", "1stExon", or "5'UTR".</li> </ul>

If group = "all", all CpGs will be pulled out.

### Details

The implementation of the function is modified from `.flattenAnn` function in `missMethyl` package.

### Value

A data frame contains CpG IDs and gene symbols.

### References

- Hansen KD (2016). `IlluminaHumanMethylation450kanno.ilmn12.hg19`: Annotation for Illumina's 450k methylation arrays. R package version 0.6.0.
- Hansen KD (2017). `IlluminaHumanMethylationEPICanno.ilm10b4.hg19`: Annotation for Illumina's EPIC methylation arrays. R package version 0.6.0, [https://bitbucket.com/kasperdanielhansen/Illumina\\_EPIC](https://bitbucket.com/kasperdanielhansen/Illumina_EPIC).
- Phipson B, Maksimovic J and Oshlack A (2015). "missMethyl: an R package for analysing methylation data from Illumina's HumanMethylation450 platform." *Bioinformatics*, pp. btv560.

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getDescription	<i>Get gene set description</i>
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**Description**

This function gets description of gene sets.

**Usage**

```
getDescription(GSids, GS.type)
```

**Arguments**

GSids	A vector contains gene set IDs.
GS.type	A string. "GO", "KEGG", or "Reactome".

**Value**

A vector contains gene sets description.

**References**

Carlson M (2018). GO.db: A set of annotation maps describing the entire Gene Ontology. R package version 3.6.0.

Yu G, Wang L, Han Y, He Q (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS: A Journal of Integrative Biology, 16(5), 284-287.

Ligtenberg W (2017). reactome.db: A set of annotation maps for reactome. R package version 1.62.0.

**Examples**

```
GSids = c("GO:0007389", "GO:0000978", "GO:0043062")
Description = getDescription(GSids, "GO")
head(Description)
```

---

getGS	<i>Get Gene Sets</i>
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**Description**

This function gets gene sets information.

**Usage**

```
getGS(geneids, GS.type)
```

**Arguments**

geneids            A vector contains all gene ids of interest. Gene ids should be gene symbol.  
GS.type            A string. "GO", "KEGG", or "Reactome".

**Value**

A list contains all gene sets of interest and their corresponding genes.

**References**

Carlson M (2017). org.Hs.eg.db: Genome wide annotation for Human. R package version 3.5.0.

Ligtenberg W (2017). reactome.db: A set of annotation maps for reactome. R package version 1.62.0.

**Examples**

```
geneids = c("FKBP5", "NDUFA1", "STAT5B")  
GO.list = getGS(geneids, "KEGG")  
head(GO.list)
```

---

GS.list                            *An example of user input gene sets*

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**Description**

An example of user input gene sets

**Usage**

```
GS.list
```

**Format**

A list contains user input gene set names and their corresponding genes

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methyglm	<i>Implement logistic regression adjusting for number of probes in enrichment analysis</i>
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## Description

This function implements logistic regression adjusting for number of probes in enrichment analysis.

## Usage

```
methyglm(cpg.pval, array.type = "450K", FullAnnot = NULL,
         group = "all", GS.list = NULL, GS.idtype = "SYMBOL",
         GS.type = "GO", minsize = 100, maxsize = 500, parallel = FALSE,
         BPPARAM = bpparam())
```

## Arguments

cpg.pval	A named vector containing p-values of differential methylation test. Names should be CpG IDs.
array.type	A string. Either "450K" or "EPIC". Default is "450K". This argument will be ignored if FullAnnot is provided.
FullAnnot	A data frame provided by prepareAnnot function. Default is NULL.
group	A string. "all", "body", "promoter1" or "promoter2". Default is "all". If group = "body", only CpGs on gene body will be considered in methyglm. If group = "promoter1" or group = "promoter2", only CpGs on promoters will be considered. Here is the definition of "body", "promoter1" and "promoter2" according to the annotation in IlluminaHumanMethylation450kanno.ilmn12.hg19 or IlluminaHumanMethylationEPICanno.ilm10b4.hg19. <ul style="list-style-type: none"> <li>• body: CpGs whose gene group correspond to "Body" or "1stExon"</li> <li>• promoter1: CpGs whose gene group correspond to "TSS1500" or "TSS200"</li> <li>• promoter2: CpGs whose gene group correspond to "TSS1500", "TSS200", "1stExon", or "5'UTR".</li> </ul> <p>If group = "all", all CpGs are considered regardless of their gene group.</p>
GS.list	A list. Default is NULL. If there is no input list, Gene Ontology is used. Entry names are gene sets names, and elements correpond to genes that gene sets contain.
GS.idtype	A string. "SYMBOL", "ENSEMBL", "ENTREZID" or "REFSEQ". Default is "SYMBOL"
GS.type	A string. "GO", "KEGG", or "Reactome". Default is "GO"
minsize	An integer. If the number of genes in a gene set is less than this integer, this gene set is not tested. Default is 100.
maxsize	An integer. If the number of genes in a gene set is greater than this integer, this gene set is not tested. Default is 500.

`parallel` either TRUE or FALSE indicating whether parallel should be used. Default is FALSE

`BPPARAM` an argument provided to `bplapply`. See `register` for details.

### Details

The implementation of this function is modified from `goglm` function in `GOglm` package.

### Value

A data frame contains gene set tests results.

### References

Mi G, Di Y, Emerson S, Cumbie JS and Chang JH (2012) Length bias correction in Gene Ontology enrichment analysis using logistic regression. PLOS ONE, 7(10): e46128

Phipson, B., Maksimovic, J., and Oshlack, A. (2015). `missMethyl`: an R package for analysing methylation data from Illuminas HumanMethylation450 platform. Bioinformatics, *btv560*.

Carlson M (2017). `org.Hs.eg.db`: Genome wide annotation for Human. R package version 3.5.0.

### Examples

```
data(CpG2Genetoy)
data(cpgetoy)
data(GSlisttoy)
GS.list = GS.list[1:10]
FullAnnot = prepareAnnot(CpG2Gene)
res = methylglm(cpg.pval = cpg.pval, FullAnnot = FullAnnot,
GS.list = GS.list, GS.idtype = "SYMBOL")
head(res)
```

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methylogometh	<i>Adjusting number of probes in gene set testing using gometh or gsameth in missMethyl</i>
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### Description

This function calls `gometh` or `gsameth` function in `missMethyl` package to adjust number of probes in gene set testing

### Usage

```
methylgometh(cpg.pval, sig.cut = 0.001, topDE = NULL,
array.type = "450K", GS.list = NULL, GS.idtype = "SYMBOL",
GS.type = "GO", minsize = 100, maxsize = 500)
```



**Arguments**

cpg.pval	A named vector containing p-values of differential methylation test. Names should be CpG IDs.
sig.cut	A numeric value indicating cut-off value for significant CpG. Default is 0.001. This argument will be ignored if topDE is provided.
topDE	An integer. The top number of CpGs to be declared as significant.
array.type	A string. Either "450K" or "EPIC". Default is "450K".
GS.list	A list. Default is NULL. If there is no input list, Gene Ontology is used. Entry names are gene sets names, and elements correspond to genes that gene sets contain.
GS.idtype	A string. "SYMBOL", "ENSEMBL", "ENTREZID" or "REFSEQ". Default is "SYMBOL".
GS.type	A string. "GO", "KEGG", or "Reactome"
minsize	An integer. If the number of genes in a gene set is less than this integer, this gene set is not tested. Default is 100.
maxsize	An integer. If the number of genes in a gene set is greater than this integer, this gene set is not tested. Default is 500.

**Value**

A data frame contains gene set tests results.

**References**

Phipson, B., Maksimovic, J., and Oshlack, A. (2015). missMethyl: an R package for analysing methylation data from Illuminas HumanMethylation450 platform. *Bioinformatics*, *btv560*.

Ligtenberg W (2017). reactome.db: A set of annotation maps for reactome. R package version 1.62.0.

Carlson M (2017). org.Hs.eg.db: Genome wide annotation for Human. R package version 3.5.0.

**Examples**

```
## Not run:
library(IlluminaHumanMethylation450kanno.ilmn12.hg19)
data(cpgtoy)
res = methyIgometh(cpg.pval = cpg.pval, sig.cut = 0.001, GS.type = "KEGG",
minsize = 200, maxsize = 205)
head(res)

## End(Not run)
```

---

methyIRRA	<i>Enrichment analysis after adjusting multiple p-values of each gene by Robust Rank Aggregation</i>
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## Description

This function implements enrichment after adjusting multiple p-values of each gene by Robust Rank Aggregation.

## Usage

```
methyIRRA(cpg.pval, array.type = "450K", FullAnnot = NULL,
          group = "all", method = "ORA", sig.cut = 0.05, topDE = NULL,
          GS.list = NULL, GS.idtype = "SYMBOL", GS.type = "GO",
          minsize = 100, maxsize = 500)
```

## Arguments

cpg.pval	A named vector containing p-values of differential methylation test. Names should be CpG IDs.
array.type	A string. Either "450K" or "EPIC". Default is "450K". This argument will be ignored if FullAnnot is provided.
FullAnnot	A data frame provided by prepareAnnot function. Default is NULL.
group	<p>A string. "all", "body", "promoter1" or "promoter2". Default is "all". If group = "body", only CpGs on gene body will be considered in methyIRRA. If group = "promoter1" or group = "promoter2", only CpGs on promoters will be considered. Here is the definition of "body", "promoter1" and "promoter2" according to the annotation in IlluminaHumanMethylation450kanno.ilmn12.hg19 or IlluminaHumanMethylationEPICanno.ilm10b4.hg19.</p> <ul style="list-style-type: none"> <li>• body: CpGs whose gene group correspond to "Body" or "1stExon"</li> <li>• promoter1: CpGs whose gene group correspond to "TSS1500" or "TSS200"</li> <li>• promoter2: CpGs whose gene group correspond to "TSS1500", "TSS200", "1stExon", or "5'UTR".</li> </ul> <p>If group = "all", all CpGs are considered regardless of their gene group.</p>
method	A string. "ORA" or "GSEA". Default is "ORA"
sig.cut	A numeric value indicating FDR cut-off for significant gene in ORA. Default is 0.05. This argument will be ignored if topDE is provided or method = "GSEA" is used.
topDE	An integer. The top number of genes to be declared as significant after robust rank aggregation. This argument will be ignored if method = "GSEA" is used.
GS.list	A list. Default is NULL. If there is no input list, Gene Ontology is used. Entry names are gene sets names, and elements correspond to genes that gene sets contain.

GS.idtype	A string. "SYMBOL", "ENSEMBL", "ENTREZID" or "REFSEQ". Default is "SYMBOL".
GS.type	A string. "GO", "KEGG", or "Reactome". Default is "GO"
minsize	An integer. If the number of genes in a gene set is less than this integer, this gene set is not tested. Default is 100.
maxsize	An integer. If the number of genes in a gene set is greater than this integer, this gene set is not tested. Default is 500.

**Value**

A data frame contains gene set tests results.

**References**

Kolde, Raivo, et al. Robust rank aggregation for gene list integration and meta-analysis. *Bioinformatics* 28.4 (2012): 573-580.

Phipson, B., Maksimovic, J., and Oshlack, A. (2015). *missMethyl*: an R package for analysing methylation data from Illuminas HumanMethylation450 platform. *Bioinformatics*, btv560.

Yu, Guangchuang, et al. *clusterProfiler*: an R package for comparing biological themes among gene clusters. *OmicS: a journal of integrative biology* 16.5 (2012): 284-287.

Carlson M (2017). *org.Hs.eg.db*: Genome wide annotation for Human. R package version 3.5.0.

**Examples**

```
data(CpG2Genetoy)
data(cpghtoy)
data(GSlisttoy)
GS.list = GS.list[1:10]
FullAnnot = prepareAnnot(CpG2Gene)
res1 = methylRRA(cpg.pval = cpg.pval, FullAnnot = FullAnnot,
method = "ORA", GS.list = GS.list)
head(res1)
```

---

```
prepareAnnot
```

*Prepare user-supplied mapping between CpGs and genes.*

---

**Description**

This function prepares CpG to gene mapping which will be used by methylRRA and methylglm.

**Usage**

```
prepareAnnot(CpG2Gene, geneidtype = "SYMBOL")
```

**Arguments**

CpG2Gene	A matrix, or a data frame or a list contains CpG to gene mapping. For a matrix or data frame, 1st column should be CpG ID and 2nd column should be gene name. For a list, entry names should be gene names, and elements correspond to CpG IDs.
geneidtype	A string. "SYMBOL", "ENSEMBL", "ENTREZID" or "REFSEQ". Default is "SYMBOL".

**Value**

A data frame contains ready to use CpG to gene mapping.

**References**

Carlson M (2017). org.Hs.eg.db: Genome wide annotation for Human. R package version 3.5.0.

**Examples**

```
data(CpG2Genetoy)
FullAnnot = prepareAnnot(CpG2Gene)
head(FullAnnot)
```

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runExample	<i>methylGSA shiny app</i>
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**Description**

This is an interface for Bioconductor package methylGSA.

**Usage**

```
runExample(run = TRUE)
```

**Arguments**

run	Run the app or not. Default is TRUE
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**Value**

The shiny app will be opened in a web browser.

**Note**

In order to run the app, the following R/Bioconductor packages needs to be installed properly: shinycssloaders, DT, ggplot2, IlluminaHumanMethylation450kanno.ilmn12.hg19 (if analyzing 450K array) IlluminaHumanMethylationEPICanno.ilm10b4.hg19 (if analyzing EPIC array)

**Examples**

```
## Please note: in this example, the argument run is set to be FALSE in  
## order to pass R CMD check. However, when using the app, users are  
## expected to launch the app by runExample()  
runExample(FALSE)
```

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