# Package 'GeneDMRs'

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Type Package

Title Gene-based differentially methylated regions analysis

Version 1.0

**Imports** clusterProfiler, corrplot, dplyr, genomation, Hmisc, KEGG.db, org.Hs.eg.db (for human), org.Mm.eg.db (for mouse), org.Ss.eg.db (for pig), pheatmap, plotrix, qqman, RCircos, RColorBrewer, VennDiagram,

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**Description** GeneDMRs is an R package to detect the differentially methylated regions based on genes, gene body, CpG islands and gene body interacted with CpG island features. The output *coverage* file (i.e. *.bismark.cov*) of Bismark software for reduced-representation bisulfite sequencing (RRBS) can be directly used in the GeneDMRs package. Additionally, the methylation calling of whole genome bisulfite sequencing (WGBS) can be used if they are in the same format as the output *coverage* file of Bismark. *Bed* file (i.e. *.bed*) of refseq and cpgi can be directly used for the methylation levels in different gene or CpG island regions, and then filtered for the significant methylated genes or CpG islands. With the annotation of promoter, exon, intron, CpG island and CpG island shore based on R package genomation, gene body or CpG island feature regions and their interactive regions can also be analyzed by GeneDMRs package.

License GPL-3

RoxygenNote 6.0.1

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downloaded from UCSC
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# **Description**

This function reads the *bed* file of refseq or cpgi and sorts them by chromosome and position. The dataset of the example are the mouse reference genes and CpG island information that are downloaded from UCSC website (<a href="http://genome.ucsc.edu/cgi-bin/hgTables">http://genome.ucsc.edu/cgi-bin/hgTables</a>). The R package genomation used here can divide the *refseq.bed* file into several gene body features, e.g. promoter, exon, intron regions and the *cpgi.bed* file into CpG island features, e.g. CpG island and CpG island shore.

# # refseq.bed file

chr1 1342029	13419921 50	4 1 134203505	.34235457 0	[ -	NM_00129: 2	1930 4376,230	0),	- 0,3601
chr1 1342029	13419921 50	4 1 134234733	.34234856 0	!	NM_00129: 2	1928 4376,194	0	- 0,3544
8, chr1 1342029	13419921 50	4 1 134234355	.34235457 0	!	NM_001283 3	2945 4376,432	0	- 0,3480
0,36013, chr1 1342029	13419921		.34235457		NM_00103	,	0	- 0,3480
0,36013,	30	13 1234333	Ū	•	•	1575,550	,,230,	0,5400

```
NM_001008533
                          134199214
                                                               134235457
        134202950
                                             134234355
                                                                                                                                                            0,3480
                                                                                                                       4376,1443,
0.
chr1
                           58713285 58733227 NM_009805
                                                                                                                                          58726436 5873236
                          5 374,427,106,136,975,
58713285 58758882 NM_207653
                                                                                                    0,13020,15770,17866,18967,
        0
2
                                                                                                                                         58726436 5875392
chr1
2 0 10 374,427,106,136,74,55,50,82,508,5099,
7866,18967,22372,26977,27679,39053,40498,
chr1 58711490 58758882 NM_001289704 0 +
                                                                                                                                          0,13020,15770,1
                                                                                                                                          58726436 5875392
Chr1 58711490 58758882 NM_001289704 0 + 58726436 5875392 2 0 10 102,427,106,136,74,55,50,82,508,5099, 0,14815,17565,1 9661,20762,24167,28772,29474,40848,42293, chr1 8359738 9299877 NM_001290390 0 - 8363474 8803943 0 21 3895,111,93,153,72,117,39,130,131,83,103,42,41,58,57,135,54,54,64,82,1745, 0,54464,85288,88241,103655,105698,194986,223466,235673,24 7331,265040,318068,318198,319334,322234,423024,444178,522422,639447,843440,938
                                                                                                                                         0,14815,17565,1
                          8359738 9299877 NM_027671
                                                                                                                                         8363474 8803943
chr1
                                                                                                    0
0 21 3895,111,93,153,72,117,39,130,131,83,103,42,44,58,57,135,

54,54,64,82,1745, 0,54464,85288,88241,103655,105698,194986,223466,235673,24

7331,265040,318068,318198,319334,322234,423024,444178,522422,639447,843440,938
```

#### # cpgi.bed file

chr1	84934572	84935054	CpG:_47	
chr1	63176547	63177427	CpG:_78	
chr1	12543517	4	125435976	CpG:_67
chr1	18336892	6	183369826	CpG:_93
chr1	3531624	3531843	CpG:_27	
chr1	3670619	3671074	CpG:_34	
chr1	3671654	3672156	CpG:_45	
chr1			CpG:_165	
chr1	4496947	4497608	CpG:_47	
chr1	4571641	4572075	CpG:_44	

### Usage

Bedfile\_read(paths = paste(system.file(package = "GeneDMRs"), "/methdata", sep=""), bedfile = "refseq", suffix = ".txt", feature = FALSE, featurewrite = FALSE)

# **Arguments**

paths	The path of bed file, with default the package path.
bedfile	The file name of <i>bed</i> file like "refseq" or "cpgi". This file is
	downloaded from UCSC website, with default "refseq".
suffix	The suffix of bed file, e.g. ".gz", ".zip" and so on (some files are in
	text .txt format, then ".txt" or ".txt.gz"), with default ".txt".
feature	TRUE or FALSE value indicating whether to read the bed file with
	the features, with default FALSE. If feature = TRUE, the output of
	this function will contain the features e.g. promoter, exon, intron or
	CpG island, CpG island shore based on R package genomation.
featurewrite	TRUE or FALSE value indicating whether to write out the feature
	file to the given path, with default FALSE.

### Value

A data frame contains four columns of chromosome, start position, end position. If feature = TRUE, the data frame is five columns with the added feature such as genebody or cpgfeature.

#### Reference

Akalin A, Franke V, Vlahovicek K, Mason C, Schubeler D (2014). "genomation: a toolkit to summarize, annotate and visualize genomic intervals." *Bioinformatics*. doi: 10.1093/bioinformatics/btu775, http://bioinformatics.oxfordjournals.org/content/early/2014/12/04/bioinformatics.btu775.long.

### **Examples**

```
inputrefseqfile <- Bedfile_read()
inputrefseqfile <- Bedfile_read(paths = paste(system.file(package = "GeneDMRs"),
   "/methdata", sep=""), bedfile = "refseq", suffix = ".txt", feature = FALSE)
inputcpgifile <- Bedfile_read(paths = paste(system.file(package = "GeneDMRs"), "/methdata",
   sep=""), bedfile = "cpgi", suffix = ".txt", feature = FALSE)
inputgenebodyfile <- Bedfile_read(bedfile = "refseq", feature = TRUE, featurewrite = TRUE)
inputcpgifeaturefile <- Bedfile_read(bedfile = "cpgi", feature = TRUE, featurewrite = FALSE)</pre>
```

Circos\_plot Plot the circos

### **Description**

This function outputs the circos plot for the methylation level and the density of gene, CpG island and CpG island shore on different chromosomes based on R RCircos package. All the files used in this function should contain chromosome, start position, and end position information that are required for R RCircos package.

### **Usage**

Circos\_plot(inputcytofile, inputmethfile\_QC, inputrefseqfile, inputcpgifeaturefile, labelname = regiongeneall significant, linecolor = NULL)

#### **Arguments**

inputcytofile The output of Cytofile\_read() which contains the chromosome

information.

inputrefseqfile The output of Bedfile\_read() which contains the gene information. inputcpgifeaturefile The output of Bedfile\_read() which contains the CpG island and

CpG island shore information.

labelname The label of gene names which could be the significant genes after

Significant filter(), with default regiongeneall significant with

differentially methylated genes. Sometimes,

regiongenealls\_significant will have some errors because it has unannotated chromosome name like chrUn\_JH584304 or

chrUn\_NW\_018084826v1. Thus, these chromosome names should be removed. If the labelname is from selfdefinedfile, then the file

should contain the headers with chr (chromosome), start (start

position), end (end position) and id (gene name).

linecolor The colors of the lines plot for different methylation levels, with

default NULL (black). If the linecolor is used, the length of colors

should correspond to the length of groups.

#### Value

A circus figure with chromosomes, gene labels, the densities of the genes (track 3), CpG islands (track 4) and CpG island shores (track 5) and the methylation levels of different groups from the outermost circle to the innermost circle.

#### Reference

Hongen Zhang, Paul Meltzer, and Sean Davis. RCircos: an R package for Circos 2D track plots. BMC Bioinformatics, 2013, 14:244.

# **Examples**

Circos\_plot(inputcytofile, inputmethfile\_QC, inputrefseqfile, inputcpgifeaturefile)
Circos\_plot(inputcytofile, inputmethfile\_QC, inputrefseqfile, inputcpgifeaturefile, labelname = selfdefinedfile, linecolor = c("blue", "orange", "green"))

Correlation plot

Plot the methylation correlation

### **Description**

This function outputs the correlation plot for the methylation level of different samples or groups based on R package corrplot, Hmisc and RColorBrewer.

#### **Usage**

Correlation\_plot(inputmethfile\_QC, unmeth\_exclude = TRUE)

# **Arguments**

inputmethfile QC The input file with methylation levels, with default inputmethfile

after quality control.

unmeth\_exclude TRUE or FALSE value indicating whether to exclude the

unmethylated sites or regions, with default TRUE

#### Value

A correlation figure.

### **Examples**

Correlation\_plot(inputmethfile\_QC)

Correlation plot(siteall)

Correlation\_plot(regiongenealls)

Correlation\_plot(genefeatureall\_cpgfeature)

Correlation plot(genefeatureall cpgfeature, unmeth exclude = FALSE)

Chomosome\_pieplot

Pie plot based on different chromosomes

## **Description**

This function outputs the pie plot for the percentages of sites or regions in different chromosomes.

# Usage

Chromosome\_pieplot(genefeatureall\_cpgfeature\_significantcpgisland, genefeatureall\_cpgfeature\_significantshore = NULL, methydirection = "both", title = "Pie plot for chromosome")

#### **Arguments**

genefeatureall\_cpgfeature\_significantcpgisland

The input file with chromosomes, which can be files with/without

Significant\_filter().

genefeatureall\_cpgfeature\_significantshore

Another input file with chromosomes,

e.g.

genefeatureall\_cpgfeature\_significant shore file for comparison, with

default NULL.

methydirection

The methylation direction when the input file contains the methylation

difference column i.e. methdiff after Logic\_regression(), which can be "hypo", "hyper" and "both", with the default "both" for both directions. Figure titles, with the default "Pie

plot for chromosome".

title

#### Value

A pie figure in different chromosomes.

### **Examples**

Chromosome\_pieplot(genefeatureall\_cpgfeature\_significantcpgisland, title = "")

Chromosome\_pieplot(genefeatureall\_cpgfeature\_significantcpgisland, title = "CpGisland")

Chromosome\_pieplot(genefeatureall\_cpgfeature\_significantcpgisland, genefeatureall\_cpgfeature\_significantshore = genefeatureall\_cpgfeature\_significantshore, title = c("CpGisland","Shore"))

Chromosome\_pieplot(siteall, title = "All cytosine sites") # Only consider the annotated chromosomes and the unannotated chromosomes will be discarded #

Chromosome\_pieplot(siteall\_Qvalue, title = "All cytosine sites")

Chromosome\_pieplot(siteall\_significant, title = "Significant cytosine sites")

Chromosome\_pieplot(siteall\_Qvalue, methydirection = "hyper", title = "Hyper-methylated distribution"))

Chromosome\_pieplot(siteall\_significant, methydirection = "hypo", title = "Hypo-methylated pie plot"))

Chromosome\_pieplot(regiongeneall\_Qvalue, methdirection = "hyper", title = "Hypermethylated genes")

Cytofile\_read

Read the cyto file

# **Description**

This function reads the chromosome information from *cyto* file (*cytoBandIdeo.txt*) and sort them by chromosome and position. The dataset of the example is the mouse genome information downloaded from UCSC website

(http://hgdownload.cse.ucsc.edu/goldenPath/mm10/database/cytoBandIdeo.txt.gz).

# Usage

Cytofile\_read(paths = paste(system.file(package = "GeneDMRs"), "/methdata", sep=""), cytofile = "cytoBandIdeo", suffix = ".txt.gz")

#### **Arguments**

paths The path of input file, with default the package path.

cytofile The name of input cyto file that is downloaded from UCSC website,

with default "cytoBandIdeo".

suffix The suffix of input cyto file, e.g. ".gz", ".zip" and so on (some files

are in text .txt format, then ".txt" or ".txt.gz"), with default

".txt.gz".

#### Value

A data frame contains chromosome, start position, end position.

#### **Examples**

inputcytofile <- Cytofile\_read()

inputcytofile <- Cytofile\_read(paths = paste(system.file(package = "GeneDMRs"), "/methdata",
sep=""), cytofile = "cytoBandIdeo", suffix = ".txt.gz")</pre>

DMC feature	Annotate the differentially methylated cytosine
DIVIC_leature	(DMC) to features

# **Description**

This function annotates the differentially methylated cytosine (DMC) after statistical test Logic regression() to gene body or CpG island features.

### Usage

DMC\_feature(siteall\_significant, featureid = TRUE, featurefile = list(inputgenebodyfile, inputcpgifeaturefile))

### **Arguments**

siteall\_significant The input file with DMC sites.

featureid TRUE or FALSE value indicating whether to include the feature id

or not, with the default TRUE. The feature id will output the id of

first file of the featurefile list e.g. the id of inputgenebodyfile.

featurefile The input feature files e.g. input genebodyfile and

inputcpgifeaturefile, with default two files in a list as featurefile = list(inputgenebodyfile, inputcpgifeaturefile), and it can also be one

file without a list e.g. featurefile = inputgenebodyfile.

### Value

A data frame contains DMC sites with features.

### **Examples**

siteall\_significant\_feature <- DMC\_feature(siteall\_significant, featurefile = list(inputgenebodyfile, inputcpgifeaturefile))

siteall\_significant\_feature <- DMC\_feature(siteall\_significant, featureid = FALSE, featurefile = list(inputgenebodyfile, inputcpgifeaturefile))

siteall\_significant\_feature <- DMC\_feature(siteall\_significant, featureid = TRUE, featurefile = inputgenebodyfile)

DMC methfile QC	Merge the methylation file after quality control
<b>- -</b>	with DMCs

### **Description**

This function merges the methylation file after quality control of all samples with the DMCs after Significant\_filter().

### **Usage**

DMC methfile QC(inputmethfile QC, siteall significant)

### **Arguments**

inputmethfile QC Input methylation file after quality control.

Input DMCs file. siteall\_significant

#### Value

A data frame by merging two input files of inputmethfile\_QC and siteall\_significant.

### **Examples**

DMC\_inputmethfile\_QC <- DMC\_methfile\_QC(inputmethfile\_QC, siteall\_significant)

Enrich\_plot

Enrich plot for GO terms and pathways

### **Description**

This function outputs the groups, GO terms and pathways plot for the enrichment based on R package clusterProfiler, org.Mm.eg.db (Mouse for example) and KEGG.db.

#### **Usage**

Enrich plot(regiongenealls significant, adjustpvaluecut = 0.1, enrichterm = "pathway", Dbannotation = "org.Mm.eg.db", keggorganism = "mmu", listnum = 20, title = "Enrichment for significant gene", expressionfile\_significant = NULL, expressionfile\_genetype = NULL)

### **Arguments**

Differentially methylated genes. regiongenealls\_significant

Threshold of the adjusted P values for the enrichment, adjustpvaluecut

with default 0.1.

The term need to be analyzed, which can be "GOgroup", enrichterm

"GO", "pathway", with default "pathway".

TRUE or FALSE value indicating whether to divide the category

> enrichments into two categories, i.e. hypo/hyper methylated or down/up regulated, with default TRUE.

Dbannotation

Annotation dataset, with default "org.Mm.eg.db" of

mouse.

keggorganism Species name for KEGG enrichment, with default "mmu"

of mouse.

listnum The list of display number, with default 20. title The title of figure, with default "Enrichments for

significant gene".

expressionfile significant An additional file for differentially expressed genes,

which includes gene name and Log fold change (LogFC). This is an optional file for GO terms and pathways, with

default NULL.

expressionfile genetype The gene type of expressionfile\_significant file, which

can be "REFSEQ", "ENTREZID", "SYMBOL", or other gene types that can be used in clusterProfiler, with default

NULL.

#### Value

A dot-plot figure of enrichment.

### **Examples**

Enrich\_plot(regiongenealls\_significant, enrichterm = "GOgroup", Dbannotation = "org.Mm.eg.db", title = "Biological process for significant gene")

Enrich\_plot(regiongenealls\_significant, enrichterm = "GO", title = "Go term for significant
gene")

Enrich\_plot(regiongenealls\_significant, enrichterm = "GO", Dbannotation = "org.Hs.eg.db", title = "Go term for significant gene") # for human data #

Enrich\_plot(regiongenealls\_significant, adjustpvaluecut = 0.2, enrichterm = "pathway", keggorganism = "mmu", title = "Pathway for significant gene")

Enrich\_plot(regiongenealls\_significant, enrichterm = "pathway", category = FALSE, keggorganism = "hsa", title = "Pathway for significant gene") # for human data #

expressionfile\_significant <- read.table(paste(system.file(package = "GeneDMRs"), "/methdata/DEgenes.txt", sep=""), header = T) # read DEgene file #

Enrich\_plot(regiongenealls\_significant, adjustpvaluecut = 0.2, enrichterm = "GO", Dbannotation = "org.Mm.eg.db", title = "Go term for significant gene in two categories", expressionfile significant = expressionfile significant, expressionfile genetype = "SYMBOL")

Enrich\_plot(regiongenealls\_significant, enrichterm = "pathway", keggorganism = "mmu", title = "Pathway for significant gene in two categories", expressionfile\_significant = expressionfile\_significant, expressionfile\_genetype = "SYMBOL")

Feature\_pieplot

Pie plot based on different features

#### **Description**

This function outputs the pie plot of feature percentages in gene body or CpG island mainly for DMC sites with features.

### **Usage**

Feature\_pieplot(siteall\_significant\_feature, methdirection = "both", title = "Pie plot for feature", threeDplot = TRUE)

### **Arguments**

features.

methydirection The methylation direction when the input file contains the

methylation difference column i.e. methdiff after Logic\_regression(), which can be "hypo", "hyper" and "both", with the default "both" for both directions.

Figure titles, with the default "Pie plot for chromosome"

title Figure titles, with the default "Pie plot for chromosome". threeDplot TRUE or FALSE value indicating whether to pie plot in

three dimensions based on R pacakge plotrix, with the

default TRUE.

#### Value

A pie figure in different features.

#### **Examples**

Feature\_pieplot(siteall\_significant\_feature)

Feature pieplot(siteall significant feature, methdirection = "hypo")

Feature\_pieplot(siteall\_significant\_feature, title = c("Gene body", "CpG island"))

Feature\_pieplot(siteall\_significant\_feature, title = c("Pie plot for Gene body", "Pie plot for CpG island"), threeDplot = FALSE)

Feature\_pieplot(siteall\_significant\_feature, methdirection = "hyper", title = c("Pie plot for Gene body", "Pie plot for CpG island"))

	Gene-based differentially methylated regions
GeneDMRs	analysis (GeneDMRs) and install the
	dependencies

### **Description**

GeneDMRs is an R package to detect the differentially methylated regions based on genes (DMG), gene body (DMP, DME, DMI), CpG islands and gene body interacted with CpG island features (e.g. DMG/DMP/DME/DMI\_CpG island and DMG/DMP/DME/DMI\_CpG island shore). This function can install the other R packages for the dependencies of GeneDMRs.

#### **Usage**

GeneDMRs(Dbannotation = "org.Mm.eg.db")

### **Arguments**

Dbannotation The annotation dataset for enrichment, with default "org.Mm.eg.db"

of mouse.

#### Value

A list of required R packages.

# **Examples**

GeneDMRs(Dbannotation = "org.Mm.eg.db")

Group\_boxplot

Boxplot the methylation levels for groups

### **Description**

This function outputs the methylation levels of all the groups in boxplot without considering other features.

### **Usage**

Group boxplot(regiongeneall, ttest = TRUE, title = "Group boxplot", col = NULL)

### **Arguments**

regiongeneall The input file with group methylation levels.

ttest TRUE or FALSE value indicating whether to perform the Student t-

test, with default TRUE.

title The figure title, with default "Group boxplot among genes".

col The boxplot colors, with default NULL.

#### Value

A boxplot figure with groups.

### **Examples**

Group\_boxplot(regiongeneall)

Group\_boxplot(genebodypromoterall, title = "Three groups among genes in promoter region")

Group\_boxplot(regiongeneall, ttest = FALSE, title = "Three groups among genes", col = c("red", "green", "blue"))

on CpG island features
------------------------

### **Description**

This function outputs the methylation levels in boxplot for one or more groups based on CpG island features, e.g. CpG island and CpG island shore features.

### Usage

Group\_cpgfeature\_boxplot(genefeatureall\_cpgfeature, groupnum = "all", ttest = TRUE, cpgfeaturelable = c("CpGisland", "Shore"), title = c("Group1", "Group2", "Group3"), col = NULL)

### **Arguments**

island features.

groupnum Group number, with default "all" for all of the groups.

TRUE or FALSE value indicating whether to perform the

Student t-test, with default TRUE.

cpgfeaturelable CpG island features, with default "CpGisland" and

"Shore". Only one CpG island feature can also be

available, e.g. "CpGisland".

title The figure title, with default "Group1", "Group2" and

"Group3".

col The boxplot colors, with default NULL.

#### Value

A boxplot figure with groups and CpG island features.

### **Examples**

Group cpgfeature boxplot(genefeatureall cpgfeature, groupnum = 1)

Group\_cpgfeature\_boxplot(genefeatureall\_cpgfeature, groupnum = "all", ttest = TRUE, cpgfeaturelable = c("CpGisland", "Shore"), title = c("Group1", "Group2", "Group3"), col = c("blue", "red", "green"))

Genebody_cpgfeature_boxplot  Boxplot the methylation lev based on CpG island feature	
--	--

### **Description**

This function outputs the methylation levels in boxplot for one or more features of gene body based on CpG island features, e.g. CpG island and CpG island shore features.

### **Usage**

```
Genebody_cpgfeature_boxplot(genefeatureall_cpgfeature, genebodyname = c("promoters","exons","introns","TSSes"), ttest = TRUE, cpgfeaturelable = c("CpGisland", "Shore"), title = c("Promoter", "Exon", "Intron", "TSS"), col = NULL)
```

### **Arguments**

CpG island features.

genebodyname The name of gene body features e.g. promoter, exon,

intron and TSSes, with default "promoters", "exons",

"introns", "TSSes".

ttest TRUE or FALSE value indicating whether to perform the

Student t-test, with default TRUE.

cpgfeaturelable CpG island features, with default "CpGisland" and

"Shore".

title The figure title, with default "Promoter", "Exon", "Intron"

and "TSS".

col The boxplot colors, with default NULL.

#### Value

A boxplot figure with gene body and CpG island features.

### **Examples**

```
{\tt Genebody\_cpgfeature\_boxplot(genefeatureall\_cpgfeature)}
```

```
Genebody_cpgfeature_boxplot(genefeatureall_cpgfeature, genebodyname = c("promoters","exons"), ttest = TRUE, cpgfeaturelable = c("CpGisland", "Shore"), title = c("Promoter", "Exon"), col = c("blue", "red"))
```

```
Genebody_cpgfeature_boxplot(genefeatureall_cpgfeature, genebodyname = c("promoters","exons","introns","TSSes"), ttest = TRUE, cpgfeaturelable = c("CpGisland", "Shore"), title = c("Promoters", "Exons", "Introns", "TSSes"), col = c("blue", "red", "green", "purple"))
```

Heatmap\_plot

Heat map plot for chromosomes and features

### **Description**

This function outputs the heat map plot for methylation level in different chromosomes of differentially methylated genes with features based on R package pheatmap.

#### **Usage**

Heatmap\_plot(regiongeneall\_significant, featurename = NULL, title = "Methylation level", display\_numbers = FALSE, number\_format = "%.0f", cluster\_rows = FALSE, cluster\_cols = TRUE, gaps\_row = c(1,2), gaps\_col = NULL)

### **Arguments**

methylated genes or the genes in different gene body

features.

feature name of the output file from Significant\_filter() for

genefeatureall\_cpgfeature file, that is "CpGisland" or

"Shore", with default NULL.

title The figure title, with the default "Methylation level".

display numbers TRUE or FALSE value indicating whether to display the

methylation value in the figure, with default FALSE.

number format The displayed number of the methylation value in round

format.

cluster rows TRUE or FALSE value indicating whether to cluster the

row, with the default FALSE.

cluster cols TRUE or FALSE value indicating whether to cluster the

column, with the default TRUE.

gaps\_row TRUE or FALSE value indicating whether to divide the

row, with the default c(1,2) that divide the rows into three

parts by row 1 and row 2.

gaps\_col TRUE or FALSE value indicating whether to divide the

column, with the NULL.

#### Value

A heat map figure with methylation levels.

#### **Examples**

Heatmap\_plot(regiongeneall\_significant)

Heatmap\_plot(genefeatureall\_cpgfeature\_significantcpgisland, featurename = "CpGisland", display numbers = FALSE, title = "Methylation level (%) for genes with CpG island")

Heatmap\_plot(genefeatureall\_cpgfeature\_significantshore, featurename = "Shore", title = "Methylation level (%) for genes with shores")

Heatmap\_plot(genefeatureall\_cpgfeature\_significantshore, featurename = "Shore", title = "Methylation level (%) for genes with shores", cluster\_cols = FALSE)

Heatmap\_plot(regiongeneall\_significant, title = "Methylation level (%) for genes", display numbers = FALSE)

Heatmap\_plot(regiongeneall\_significant, title = "Methylation level (%) for genes", display\_numbers = FALSE, cluster\_rows = TRUE, gaps\_row = NULL)

Logic_regression	Logistical regression analysis for each region or each cytosine site
	or each cylosine sile

### **Description**

This function tests each region or each cytosine site by logistical regression model to achieve the P values and then be adjusted to Q values to account for multiple hypothesis testing.

#### **Usage**

Logic\_regression(genefeatureall\_cpgfeature, covariates = NULL, adjustedmethod = "fdr", diffgroup = NULL)

### **Arguments**

genefeatureall\_cpgfeature The input file with methylation levels to be tested.

covariates Extra covariates used in the model, with the default

NULL.

adjustedmethod The methods to adjust P values to Q values, with the

default "fdr" method. The adjustedmethod could be "holm", "Hochberg", "hommel", "bonferroni", "BH",

"BY", "none" methods as well.

diffgroup Methylation difference between two groups, with the

default NULL, that is the max group - min group. The two

groups can be manually selected e.g. diffgroup =

c("group1", "group2").

#### Value

A data frame of region gene or region cpgi or those regions with different features or cytosine sites, by accompanying with P values, Q values and methylation differences.

#### **Examples**

```
regiongeneall_Qvalue <- Logic_regression(regiongenealls)
regioncpgall_Qvalue <- Logic_regression(regioncpgall , adjustedmethod = "fdr")
regiongenebodyall_Qvalue <- Logic_regression(regioncpgall , diffgroup = c("group1", "group2"))
regiongeneall_cpgfeature_Qvalue <- Logic_regression(regiongeneall_cpgfeature)
genefeatureall_cpgfeature_Qvalue <- Logic_regression(genefeatureall_cpgfeature)
genefeatureall_Qvalue <- Logic_regression(genefeatureall, adjustedmethod = "bonferroni")
siteall_Qvalue <- Logic_regression(siteall, adjustedmethod = "fdr") # for each cytosine site #
```

siteall\_Qvalue <- Logic\_regression(siteall, adjustedmethod = "fdr", diffgroup = c("group1", "group2"))

Manhattan\_plot

Manhattan plot for all cytosines or regions

# **Description**

This function outputs the Manhattan plot for all cytosines or regions in different chromosomes with significant line based on R package qqman.

### **Usage**

 $\label{eq:manhattan_plot} Manhattan_plot(siteall_Qvalue, chrlabs = NULL, col = c("black", "grey"), ylab = "-log(Q value)", suggestiveline = -log10(1e-02), genomewideline = -log10(1e-03))$ 

### **Arguments**

siteall\_Qvalue The input file with Q values from DMR\_test(), e.g.

regiongeneall\_Qvalue, genefeatureall\_cpgfeature\_Qvalue

or others with Q values.

chrlabs The label of chromosomes, with default NULL. col The color of plots, with default black and grey.

suggestiveline The significant line, with default 0.01.

genomewideline The genome-wide significant line, with default 0.001.

#### Value

A Manhattan figure with Q values.

#### **Examples**

Manhattan\_plot(siteall\_Qvalue, ylab = "-log(Q-value)")

Manhattan\_plot(regiongenealls\_Qvalue, chrlabs = c(1:18,"X"), col = c("green","orange"), genomewideline = -log10(1e-02))

Manhattan\_plot(genefeatureall\_cpgfeature\_Qvalue, ylab = c("-log(Q value) for CpG island", "-log(Q value) for Shore"), col = c("red","blue"), suggestiveline = -log10(5e-02), genomewideline = -log10(1e-02))

Methfile\_read

Read the methylation file

### **Description**

This function reads all of the methylation files and generates one file with all samples including methylated read coverages (Cs) and unmethylated read coverages (Ts). It can automatically test

how many samples and how many replicates in each group and the distribute them from 1\_1, 1\_2 to the final file by headers. The methylation files should be the standard *coverage* file (i.e. *.bismark.cov*) outputted from Bismark software. The dataset of the example is the Reduced representation bisulfite sequencing (RRBS) data of DNA methylation for mouse myeloid progenitor tissue from GEO (Accession number: GSE62392) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62392).

#1 1.gz after rename the coverage file (i.e. .bismark.cov) of the first replicate of first group

```
chr1 3020877 3020877
                        97.46835 77
chr1 3020891
              3020891
                        92.40506
                                       6
chr1 3020946 3020946
                        88.67925 47
chr1 3020988 3020988
chr1 3021013 3021013
                        98.64865 73
                                       1
                       100.00000 74
                                      0
chr1 3094122 3094122
                         0.00000
chr1 3094126 3094126 100.00000
                                      0
chr1 3150008 3150008 100.00000
                                      0
chr1 3150022 3150022 100.00000
                                      0
chr1 3150068 3150068 100.00000
```

### Usage

```
Methfile_read(paths = paste(system.file(package = "GeneDMRs"), "/methdata", sep=""), suffix = ".gz")
```

# **Arguments**

paths	The path of methylation file, with default the package path.
suffix	The suffix of methylation file, e.g. ".gz", ".zip" and so on (some
	files are in text .txt format, then ".txt" or ".txt.gz"), with default
	".gz".

# Value

A data frame contain chromosome, position, and Cs & Ts for different replicates and groups.

# **Examples**

```
inputmethfile <- Methfile_read()
inputmethfile <- Methfile_read(paths = paste(system.file(package = "GeneDMRs"),
"/methdata", sep=""), suffix = ".gz")</pre>
```

# # inputmethfile

#chr	posi	Cs1_1	Ts1_1	Cs1_2	Ts1_2	Cs1_3	Ts1_3	Cs2_1	Ts2_1	Cs2_2	Ts2_2
chr1	3020877	77	2	77	7	49	2	31	4	68	0
chr1	3020891	73	6	78	6	49	2	33	2	68	0
chr1	3020946	47	6	96	17	71	9	52	5	71	12
chr1	3020988	73	1	58	0	57	6	55	2	61	2
chr1	3021013	74	0	56	2	59	4	49	8	63	0
chr1	3531651	11	1	25	0	13	1	7	0	15	1
chr1	3531658	12	0	25	0	12	2	7	0	16	0
chr1	3531671	12	0	25	0	13	1	6	1	16	0
chr1	3531676	12	0	25	0	14	0	7	0	16	0
chr1	3531680	12	0	22	3	10	3	7	0	14	1

N/	Δt	htı	lΔ	OC
10	CL			$\sim$

Quality control for the input methylation file

# **Description**

This function discards the cytosine sites with low read coverage (quantile) or high read coverage (quantile).

### **Usage**

Methfile\_QC(inputmethfile, low\_coveragenum = 10, high\_coveragenum = NULL, low\_quantile = NULL, high\_quantile = 99.9, coveragewrite = TRUE)

### **Arguments**

inputmethfile The input of methylation file after Methfile read().

low\_coveragenum The minimum read coverage to be discarded, with default 10.

The maximum read coverage to be discarded, with default

NULL.

low\_quantile The minimum quantile of read coverage to be discarded, with

default NULL.

high\_quantile The maximum quantile of read coverage to be discarded, with

default 99.99.

coveragewrite TRUE or FALSE value indicating whether to write out the read

coverage file to the given path, with default TRUE.

#### Value

A data frame contain chromosome, position, and Cs & Ts for different replicates and groups after quality control.

#### **Examples**

inputmethfile\_QC <- Methfile\_QC(inputmethfile)

inputmethfile\_QC <- Methfile\_QC(inputmethfile, low\_coveragenum = 20, high\_quantile = 99.99)

inputmethfile\_QC <- Methfile\_QC(inputmethfile, low\_coveragenum = 10, high\_coveragenum = 100, coveragewrite = FALSE)

Methmean\_region

Calculate the methylation mean for regions

#### **Description**

This function outputs the methylation mean for different groups based on gene and CpG island regions by matching with cytosine. It is also for gene body of promoter, exon, intron and TSSes

regions, cgpi feature of CpG island and CpG island shores and their interactive regions e.g. promoter CpG island.

### Usage

Methmean\_region(inputmethfile\_QC, inputrefseqfile, cpgifeaturefile = NULL, chrnum = "all", posistart = NULL, posiend = NULL, featureid = NULL, featurename = NULL)

#### **Arguments**

inputmethfile\_QC The input of methylation file after quality control.

inputrefseqfile The input file with regions e.g. inputrefseqfile/inputcpgifile with 4

columns or input genebody file/input cpgifeature file with 5 columns.

cpgifeaturefile The input of CpG island feature file e.g. inputcpgifeaturefile, with

default NULL. If provided, the output file is methylation mean of inputrefsegfile or inputgenebodyfile with CpG island and CpG

island shore features.

chrnum The chromosome number or all chromosomes (all) or all

chromosomes with unannotated sites (alls), with default "all".

posistart Start position if requested, with default NULL. End position if requested, with default NULL.

featureid NCBI ID of specific gene or all the genes, with default NULL. The

CpG id can also be used like "cpgi1" or "shore2".

featurename Different gene body features of promoter, exon, intron and TSSes.

The CpG island features can also be used that are "CpGisland" and

"Shores".

#### Value

A data frame of the methylation mean of provided regions with/without different features.

### **Examples**

Methmean\_region(inputmethfile\_QC, inputrefseqfile, chrnum = "alls", featureid = c("NM 001244353", "NM 001244864")) # find sepecific gene by NCBI ID #

Methmean\_region(inputmethfile\_QC, inputrefseqfile, chrnum = "chr1", posistart = 21800, posiend = 21900)

regiongenechr <- Methmean\_region(inputmethfile\_QC, inputrefseqfile, chrnum =
c("chr1","chr2"))</pre>

regiongeneall <- Methmean\_region(inputmethfile\_QC, inputrefseqfile, chrnum = "all")

DMC\_regiongeneall <- Methmean\_region(DMC\_inputmethfile\_QC, inputrefseqfile, chrnum = "all") # Calculate DMC first and then recalculate the methylation mean by replacing the RRBS cytosine sites #

regiongenealls <- Methmean\_region(inputmethfile\_QC, inputrefseqfile, chrnum = "alls") # alls include unannotated CpG site like chrUn\_NW\_018084826v1 #

```
Methmean_region(inputmethfile_QC,inputcpgifile,"chr1", 21800, 21900) # acturally
regiongenepart = regioncpgpart #
regioncpgchr <- Methmean_region(inputmethfile_QC, inputcpgifile, chrnum = c("chr1","chr2"))
regioncpgall <- Methmean_region(inputmethfile_QC, inputcpgifile, chrnum = "all")
regioncpgalls <- Methmean region(inputmethfile QC, inputcpgifile)
regiongenebodychr <- Methmean_region(inputmethfile_QC, inputgenebodyfile, chrnum =
c("chr1","chr2"))
regiongenebodyall <- Methmean_region(inputmethfile_QC, inputgenebodyfile, chrnum = "all")
regiongenebodyalls <- Methmean_region(inputmethfile_QC, inputgenebodyfile)
regioncpgifeaturechr <- Methmean region(inputmethfile QC, inputcpgifeaturefile, chrnum =
c("chr1","chr2"))
regioncpgifeatureall <- Methmean region(inputmethfile QC, inputcpgifeaturefile, chrnum =
regioncpgifeaturealls <- Methmean region(inputmethfile QC, inputcpgifeaturefile)
partgenebody <- Methmean region(inputmethfile QC, inputgenebodyfile, featureid =
"NM 001244353")
partgenebodyexon <- Methmean region(inputmethfile QC, inputgenebodyfile, featureid =
"NM 001244353", featurename = "exons")
partgenebodyall <- Methmean region(inputmethfile QC, inputgenebodyfile, featureid =
"NM_001244353", featurename = c("promoters","exons","introns","TSSes"))
genebodypromoterall <- Methmean_region(inputmethfile_QC, inputgenebodyfile, featureid =
"all", featurename = "promoters")
genebodyexonall <- Methmean_region(inputmethfile_QC, inputgenebodyfile, featureid = "all",</pre>
featurename = "exons")
genefeatureall <- Methmean region(inputmethfile QC, inputgenebodyfile, featureid = "all",
featurename = c("promoters", "exons", "introns", "TSSes")) #long time #
partcpgi <- Methmean region(inputmethfile QC, inputcpgifeaturefile, featureid = "cpgi1")
partshore <- Methmean region(inputmethfile QC, inputcpgifeaturefile, featureid = "shore10")
cpgislandall <- Methmean region(inputmethfile QC, inputcpgifeaturefile, featureid = "all",
featurename = "CpGisland")
cpgshoreall <- Methmean region(inputmethfile QC, inputcpgifeaturefile, featureid = "all",
featurename = "Shores") #long time #
cpgfeatureall <- Methmean_region(inputmethfile_QC, inputcpgifeaturefile, featureid = "all",
featurename = c("CpGisland", "Shores") #long time #
```

```
genebodychr_promoter <- Methmean_region(inputmethfile_QC, inputgenebodyfile, chrnum =
"chr1", featureid = "all", featurename = "promoters")
cpgchr_island <- Methmean_region(inputmethfile_QC, inputcpgifeaturefile, chrnum = "chr1",
featureid = "all", featurename = "CpGisland")
# when the cpgifeaturefile = inputcpgifeaturefile is provided #
regiongenechr_cpgfeature <- Methmean_region(inputmethfile_QC, inputrefseqfile,
cpgifeaturefile = inputcpgifeaturefile, chrnum = c("chr1","chr2"))
regiongeneall cpgfeature <- Methmean region(inputmethfile QC, inputrefseqfile,
cpgifeaturefile = inputcpgifeaturefile, chrnum = "all")
regiongenealls cpgfeature <- Methmean region(inputmethfile QC, inputrefseqfile,
cpgifeaturefile = inputcpgifeaturefile, chrnum = "alls")
genebodypromoterall cpgfeature <- Methmean region(inputmethfile QC, inputgenebodyfile,
cpgifeaturefile = inputcpgifeaturefile, featureid = "all", featurename = "promoters")
genebodyexonall_cpgfeature <- Methmean_region(inputmethfile_QC, inputgenebodyfile,
cpgifeaturefile = inputcpgifeaturefile, featureid = "all", featurename = "exons")
genefeatureall_cpgfeature <- Methmean_region(inputmethfile_QC, inputgenebodyfile,
cpgifeaturefile = inputcpgifeaturefile, featureid = "all", featurename =
c("promoters", "exons", "introns", "TSSes")) #long time #
# windows #
```

windowfileall <- Methmean\_region(inputmethfile\_QC, windowfile, chrnum = "all")
windowfilealls <- Methmean\_region(inputmethfile\_QC, windowfile, chrnum = "alls")

Methmean_site	Calculate the methylation mean for cytosine sites
	sties

# **Description**

This function outputs the methylation mean for each cytosine site. It will calculate methylation difference along each group.

#### Usage

Methmean\_site(inputmethfile\_QC)

### **Arguments**

inputmethfile\_QC The input of methylation file after quality control.

#### Value

A data frame of the methylation mean of provided cytosine sites.

# **Examples**

siteall <- Methmean\_site(inputmethfile\_QC)</pre>

Quick_DMCs	Quick use the GeneDMRs package for
	differentially methylated cytosine sites

# **Description**

This function outputs the differentially methylated cytosine sites (DMCs).

# Usage

```
Quick_DMCs(paths = paste(system.file(package = "GeneDMRs"), "/methdata", sep=""), suffixmeth = ".gz")
```

# **Arguments**

paths The path of input file, with default the package path.

suffixmeth The suffix of methylation file, e.g. ".gz", ".zip" and so on (some

files are in text .txt format, then ".txt" or ".txt.gz"), with default

".gz".

### Value

Outputs DMC results.

### **Examples**

```
allDMCs <- Quick_DMCs()
```

Quick_GeneDMRs	Quick use the GeneDMRs package for gene based differentially methlated regions

### **Description**

This function outputs a series of results and figures for gene based regions' methylation analysis.

### Usage

```
Quick_GeneDMRs(paths = paste(system.file(package = "GeneDMRs"), "/methdata", sep=""), suffixmeth = ".gz", bedfile = "refseq", suffixbed = ".txt", Dbannotation = "org.Mm.eg.db", keggorganism = "mmu")
```

### **Arguments**

paths The path of input file, with default the package path.

suffixmeth The suffix of methylation file, e.g. ".gz", ".zip" and so on (some

files are in text .txt format, then ".txt" or ".txt.gz"), with default

".gz".

bedfile The file name of bed file for "refseq". This file is downloaded

from UCSC website, with default "refseq".

suffixbed The suffix of bed file, e.g. ".gz", ".zip" and so on (some files are

in text .txt format, then ".txt" or ".txt.gz"), with default ".txt".

Dbannotation The annotation dataset for enrichment, with default

"org.Mm.eg.db" of mouse.

keggorganism The species name for KEGG enrichment, with default "mmu" of

mouse.

#### Value

Outputs a series of DMG results.

### **Examples**

```
allDMGs <- Quick_GeneDMRs()
allDMGs_mouse <- Quick_GeneDMRs(Dbannotation = "org.Mm.eg.db", keggorganism = "mmu")
```

Sample_boxplot	Boxplot the methylation levels or read numbers
	in different samples

# **Description**

This function outputs the methylation levels or read numbers of the selected genes or all the genes in the different samples.

#### **Usage**

Sample\_boxplot(inputmethfile, inputrefseqfile, Meth\_plot = TRUE, ylab = "Methylation level", refseqname = NULL, col = NULL)

### **Arguments**

inputmethfile The input file with methylation levels.

inputrefseqfile The input of gene regions.

Meth plot TRUE or FALSE value indicating whether to plot the methylation

levels, with default TRUE, otherwise to plot the read numbers.

ylab The label of y axis, with default "Methylation level".

refsequame NCBI ID of specific gene, with default NULL.

The boxplot colors, with default NULL.

#### Value

A boxplot figure with all the samples.

### **Examples**

```
Sample boxplot(inputmethfile QC, inputrefsegfile)
```

Sample boxplot(inputmethfile QC, inputrefseqfile, refseqname = "NM 001244864")

Sample boxplot(inputmethfile QC, inputrefsegfile, refsegname = c("NM 001244864", "NM 001244534"))

Sample boxplot(inputmethfile QC, inputrefsegfile, , ylab = "Methylation level (%)", refsegname = c("NM 001244864", "NM 001143697", "NM 213902"), col = c("red", "green", "blue"))

Sample\_boxplot(inputmethfile\_QC, inputrefseqfile, Meth\_plot = FALSE, ylab = "Read number", col = c("red", "blue"))

Sample boxplot(inputmethfile QC, inputrefseqfile, Meth plot = FALSE, ylab = "Read number", refsegname = c("NM 001244864", "NM 001244534"))

Sample boxplot(inputmethfile QC, inputrefseqfile, Meth plot = FALSE, ylab = "Read number", refseqname = c("NM\_001244864", "NM\_001143697", "NM\_213902"), col = c("red", "green", "blue"))

Significant filter

Filter the significant regions or cytosine sites

#### **Description**

This function filters significant regions or cytosine sites based on Q value and methylation difference.

#### **Usage**

Significant filter(genefeatureall cpgfeature Qvalue, qvalue = 0.01, methdiff = 0, featureout = 1)

#### **Arguments**

genefeatureall\_cpgfeature\_Qvalue

qvalue

methdiff

The input file with Q values and methylation

differences need to be filtered.

Threshold of Q values that Q values larger than this will be discarded, with default 0.01. Threshold of methylation differences that methylation differences less than this will be

discarded, with the default 0.

featureout

Which feature will be filtered, with default 1. When featureout = 2, it means that the second feature will be filtered and outputted.

#### Value

A data frame of the significant regions or cytosine sites.

# **Examples**

```
genefeatureall_cpgfeature_significantcpgisland <-
Significant_filter(genefeatureall_cpgfeature_Qvalue)

genefeatureall_cpgfeature_significantshore <-
Significant_filter(genefeatureall_cpgfeature_Qvalue, featureout = 2)

genefeatureall_cpgfeature_significantcpgisland <-
Significant_filter(genefeatureall_cpgfeature_Qvalue, qvalue = 0.001, methdiff = 0.01, featureout = 1)

regiongeneall_cpgfeature_significantcpgisland <-
Significant_filter(regiongeneall_cpgfeature_Qvalue, methdiff = 0.05, featureout = 1)

regiongeneall_significant <- Significant_filter(regiongeneall_Qvalue)

regiongenealls_significant <- Significant_filter(regiongenealls_Qvalue, methdiff = 0.1)

siteall_significant <- Significant_filter(siteall_Qvalue)

siteall_significant <- Significant_filter(siteall_Qvalue, qvalue = 0.001, methdiff = 0.1)
```

Vann plat	Venn plot for the common CpG island and CpG			
Venn_plot	island shore			

# Description

This function outputs the venn plot for the common CpG island and CpG island shore regions that are covered by methylated cytosine sites based on R package VennDiagram.

#### **Usage**

```
Venn_plot(genefeatureall_cpgfeature_Qvalue, title = "Venn plot", fillcolor =
c("cornflowerblue","green"))
```

# **Arguments**

genefeatureall\_cpgfeature\_Qvalue title fillcolor

The input file with two features.
Figure title, with default "Venn plot".
Filled color, with default "cornflowerblue" and "green"

#### Value

A venn figure in two features.

# **Examples**

```
Venn_plot(genefeatureall_cpgfeature)
```

Venn\_plot(genefeatureall\_cpgfeature\_Qvalue)

Venn plot(genefeatureall cpgfeature Qvalue, fillcolor = c("red", "blue"))

Volcano\_plot

Volcano plot for all the cytosines

### **Description**

This function outputs the volcano plot for all the cytosines with Q values and methylation differences.

# Usage

Volcano\_plot(siteall\_Qvalue, title = "Volcano for Q value and methylation difference", qvalue = 0.01, methdiffpercentage = c(5, 10, 15, 20, 15), pointcolor = c("red", "purple", "orange", "yellow", "blue", "green"))

# **Arguments**

siteall\_Qvalue The input file with Q values and methylation differences.

title Figure title, with default "Volcano for Q value and methylation

difference".

qvalue Threshold of Q values that Q values less than this will be colored,

with default 0.01.

methdiffpercentage Threshold of methylation level (%) differences that methylation

differences larger than this will be colored, with default 5, 10, 15,

20, 25.

pointcolor Point plot color, with default "red", "purple", "orange", "yellow",

"blue", "green".

#### Value

A volcano figure.

### **Examples**

```
Volcano_plot(siteall_Qvalue)
```

Volcano\_plot(siteall\_Qvalue, pointcolor = c("red", "blue", "yellow", "purple", "orange", "green"))

Volcano\_plot(siteall\_Qvalue, title = "Volcano plot", qvalue = 0.001, methdiffpercentage = c(10, 15, 20, 30, 40), pointcolor = c("red", "purple", "orange", "yellow", "blue", "green"))

Window\_divide

Divide the genome to windows

### **Description**

This function outputs the window regions of the whole genome.

# Usage

Window\_divide(inputcytofile, windowbp = 1000000)

### **Arguments**

inputcytofile

The input *cyto* file with chromosome information.

windowbp

Window length in base pair (bp) to be divided, with default 1,000,000.

# Value

A data frame with window regions.

# **Examples**

```
windowfile <- Window_divide(inputcytofile)
```

windowfile <- Window\_divide(inputcytofile, windowbp = 10000)