Package 'GeneDMRs'

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Type Package

Title Gene-based differentially methylated regions analysis

Version 1.0

Imports clusterProfiler, corrplot, dplyr, ffbase, genomation, org.Hs.eg.db (for human), org.Mm.eg.db (for mouse), org.Ss.eg.db (for pig), org.Bt.eg.db (for cattle), pheatmap, plotrix, qqman, RCircos, 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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Bedfile_read	Read the standard bedfile of refseq or cpgi downloaded from UCSC

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 (http://genome.ucsc.edu/cgi-bin/hgTables). 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
                134199214
                                       134235457
                                                              NM_001291930
                                                                                                            134202950
                                                                                     n
                134199214
                                       134234856
                                                              NM_001291928
                                                                                                            134202950
chr1
                                                                                     0
chr1
                134199214
                                       134235457
                                                              NM_001282945
                                                                                     0
                                                                                                            134202950
                134199214
                                       134235457
chr1
                                                              NM_001039510
                                                                                     0
                                                                                                            134202950
                                                              NM_001008533
chr1
                134199214
                                       134235457
                                                                                                            134202950
chr1
                58713285 58733227 NM_009805
                                                                                     58726436 5873236
                5 374,427,106,136,975,
58713285 58758882 NM_207653
                                                              0,13020,15770,17866,18967,
0 + 58726436 5875392
2
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
2 0 10 102,427,106,136,74,55,50,82,508,5099,
9661,20762,24167,28772,29474,40848,42293,
chr1 8359738 9299877 NM_001290390 0 -
                                                                                     0,14815,17565,1
                                                                                     8363474 8803943 0
                                                                                                                        21
522422,639447,843440,938394,
```

```
63176547 63177427 cpg:_78
125435174 125435976
               125435174
                                                          CpG:_67
chr1
chr1
               183368926
                                     183369826
                                                          CpG:_93
chr1
               3531624
                          3531843
                                    CpG:_27
CpG:_34
                          3671074
               3670619
chr1
chr1
               3671654
                          3672156
                                     CpG:_45
               4491701 4493673
4496947 4497608
                                    CpG:_165
CpG:_47
chr1
chr1
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 bed 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.

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

inputcpgifile <- Bedfile_read(paths = paste(system.file(package = "GeneDMRs"), "/methdata",
sep=""), bedfile = "cpgi", suffix = ".txt", feature = FALSE)</pre>

inputgenebodyfile <- Bedfile read(bedfile = "refseq", feature = TRUE, featurewrite = TRUE)

inputcpgifeaturefile <- Bedfile_read(bedfile = "cpgi", feature = TRUE, featurewrite = FALSE)

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.

inputmethfile_QC The input file with methylation levels after quality control.
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.

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.

The methylation direction when the methydirection 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) to features	DMC_feature	Annotate the differentially methylated cytosine		
		(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))</pre>

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 with DMCs
	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.

siteall_significant Input DMCs file.

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 and org.Mm.eg.db (Mouse for example).

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

adjustpvaluecut Threshold of the adjusted P values for the enrichment,

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.

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

of mouse.

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

significant gene".

An additional file for differentially expressed genes, expressionfile significant

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

default NULL.

The gene type of expressionfile significant file, which expressionfile_genetype

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", Dbannotation = "org.Mm.eg.db", 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", Dbannotation = "org.Mm.eg.db", keggorganism = "mmu", title = "Pathway for significant gene")

Enrich_plot(regiongenealls_significant, enrichterm = "pathway", category = FALSE, keggorganism = "hsa", Dbannotation = "org.Hs.eg.db", 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", Dbannotation = "org.Mm.eg.db", 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

GeneDMRs

threeDplot

methylation difference column i.e., methdiff after

Logic_regression(), which can be "hypo", "hyper" and "both", with the default "both" for both directions.

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

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"))

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"))

Group_cpgfeature_boxplot	Boxplot the methylation levels for groups based on CpG island features
	on cpo isiana jeatures

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

The input file with group methylation levels and CpG genefeatureall_cpgfeature

island features.

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

TRUE or FALSE value indicating whether to perform the

Student t-test, with default TRUE.

CpG island features, with default "CpGisland" and cpgfeaturelable

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

available, e.g., "CpGisland".

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

"Group3".

The boxplot colors, with default NULL. col

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 levels for gene body based on CpG island features
-----------------------------	---

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".

The boxplot colors, with default NULL.

Value

A boxplot figure with gene body and CpG island features.

```
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.

featurename 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		
Logic_regression	or each cytosine site		

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

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.

```
regiongeneall_Qvalue <- Logic_regression(regiongeneall)

regiongenealls_Qvalue <- Logic_regression(regiongenealls)

regioncpgall_Qvalue <- Logic_regression(regioncpgall, adjustedmethod = "fdr")

regiongenebodyall_Qvalue <- Logic_regression(regiongenebodyall, 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"))

covariateinfo <- data.frame(Timepoint = c("week3","week3","week12","week12","week12"), Diet = c("Low","High","Low","High","High"))

regiongeneall_Qvalue <- Logic_regression(regiongeneall, covariates = covariateinfo)
```

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

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)")

 $\label{eq:manhattan_plot} Manhattan_plot(regiongenealls_Qvalue, chrlabs = c(1:18,"X"), col = c("green","orange"), genomewideline = -log10(1e-02))$

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))

М	et	hti	le.	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 2
chr1 3020891 3020891 92.40506 73 6
chr1 3020946 3020946 88.67925 47 6
chr1 3020988 3020988 98.64865 73 1
chr1 3021013 3021013 100.00000 74 0
chr1 3094122 3094122 0.00000 0 1
chr1 3094126 3094126 100.00000 1 0
chr1 3150008 3150008 100.00000 3 0
chr1 3150022 3150022 100.00000 1 0
```

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.
control_paths	The path of control groups, with default NULL.
case_paths	The path of case groups, with default NULL.
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.

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

```
# if only case and control group (n = 2) paths are provided #
```

controls <- c("C:/Users/GeneDMRs/methdata/1_1.gz", C:/Users/GeneDMRs/methdata/1_2.gz", "C:/Users/GeneDMRs/methdata/1_3.gz")

```
cases <- c("C:/Users/GeneDMRs/methdata/2_1.gz", "C:/Users/GeneDMRs/methdata/2_1.gz")
```

inputmethfile <- Methfile_read(control_paths = controls, case_paths = cases)

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

Methfile_QC

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, samplenum_QC = "all")

Arguments

inputmethfile	The input of methylation file after Methfile_read().
low_coveragenum	The minimum read coverage to be discarded, with default 10.
high_coveragenum	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.
samplenum_QC	The sample numbers under quanlity control (e.g.,
	samplenum_QC = 3 means that if three of five samples at one
	cytosine site have unqualified read coverage, then this site will
	be discarded), with default "all" samples.

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, samplenum QC = 3)

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 inputgenebodyfile/inputcpgifeaturefile 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 inputrefseqfile 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. posiend 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.

```
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"))
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, inputrefsegfile, 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, chrnum = "alls")
regiongenebodychr <- Methmean region(inputmethfile QC, inputgenebodyfile, chrnum =
c("chr1","chr2"))
regiongenebodyall <- Methmean_region(inputmethfile_QC, inputgenebodyfile, chrnum = "all")
regiongenebodyalls <- Methmean region(inputmethfile QC, inputgenebodyfile, chrnum =
"alls")
regioncpgifeaturechr <- Methmean_region(inputmethfile_QC, inputcpgifeaturefile, chrnum =
c("chr1","chr2"))
regioncpgifeatureall <- Methmean region(inputmethfile QC, inputcpgifeaturefile, chrnum =
"all")
regioncpgifeaturealls <- Methmean region(inputmethfile QC, inputcpgifeaturefile, chrnum =
"alls")
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",
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

GeneDMRs

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
	sues

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.
control_paths The path of control groups, with default NULL.
case_paths The path of case groups, with default NULL.

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()
```

if only case and control group (n = 2) paths are provided

 $controls <- c ("C:/Users/GeneDMRs/methdata/1_1.gz", C:/Users/GeneDMRs/methdata/1_2.gz", "C:/Users/GeneDMRs/methdata/1_3.gz")\\$

cases <- c("C:/Users/GeneDMRs/methdata/2_1.gz", "C:/Users/GeneDMRs/methdata/2_1.gz")

allDMCs <- Quick DMCs(control paths = controls, case paths = cases)

Quick_GeneDMRs	Quick use the GeneDMRs package for gene based differentially methlated regions
	basea aijjerentiatty methiatea 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.
control_paths	The path of control groups, with default NULL.
case_paths	The path of case groups, with default NULL.

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".

suffix bed 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(Dbannotation = "org.Mm.eg.db", keggorganism = "mmu")

# if only case and control group (n = 2) paths are provided #

controls <- c("C:/Users/GeneDMRs/methdata/1_1.gz", C:/Users/GeneDMRs/methdata/1_2.gz",
"C:/Users/GeneDMRs/methdata/1_3.gz")

cases <- c("C:/Users/GeneDMRs/methdata/2_1.gz", "C:/Users/GeneDMRs/methdata/2_1.gz")

allDMGs <- Quick_GeneDMRs(paths = "C:/Users/GeneDMRs/methdata", control_paths = controls, case_paths = cases)
```

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.

col The boxplot colors, with default NULL.

Value

A boxplot figure with all the samples.

Examples

Sample_boxplot(inputmethfile_QC, inputrefseqfile)

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

Sample_boxplot(inputmethfile_QC, inputrefseqfile, refseqname = c("NM_001244864", "NM_001244534"))

Sample_boxplot(inputmethfile_QC, inputrefseqfile, , ylab = "Methylation level (%)", refseqname = $c("NM_001244864", "NM_001143697", "NM_213902")$, 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", refseqname = 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

methdiff

genefeatureall_cpgfeature_Qvalue The input file with Q values and methylation

differences need to be filtered.

qvalue 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.

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)
```

Venn_plot	Venn plot for the common CpG island and CpG island shore
	istanta sitoi e

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

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.

GeneDMRs

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

 $\label{eq:volcano_plot} Volcano_plot(siteall_Qvalue, title = "Volcano for Q value and methylation difference", qvalue = 0.01, methdiffpercentage = c(5, 10, 15, 20, 15), pointcolor = c("red", "purple", "orange", "yellow", "blue", "green"))$

Arguments

title

siteall_Qvalue The input file with Q values and methylation differences.

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.

```
windowfile <- Window_divide(inputcytofile)
windowfile <- Window_divide(inputcytofile, windowbp = 10000)</pre>
```