Package ‘DAMEfinder’

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Type Package
Title Finds DAMEs - Differential Allelicly MEthylated regions
Version 1.16.0
Description 'DAMEfinder' offers functionality for taking methtuple or bismark outputs to calculate ASM scores and compute DAMEs. It also offers nice visualization of methyl-circle plots.
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calc_asm

Calculate ASM Score

Description

This function takes in a list of samples resulting from the read_tuples function and returns a SummarizedExperiment of Allele-Specific Methylation (ASM) scores, where each row is a tuple and each column is a sample.

Usage

calc_asm(
  sampleList,
  beta = 0.5,
  a = 0.2,
  transform = modulus_sqrt,
  coverage = 5,
  verbose = TRUE
)
calc_derivedasm

Arguments

sampleList List of samples returned from read_tuples
beta The beta parameter used to calculate the weight in the ASM score. link{calc_weight} uses this parameter to penalize fully methylated or unmethylated tuples. Default = 0.5.
a The distance from 0.5 allowed, where 0.5 is a perfect MM:UU balance for a tuple. In the default mode this value is set to 0.2, and we account for the instances where the balance is between 0.3 and 0.7.
transform Transform the calculated tuple ASM scores. We use the modulus square root function which outputs the square root, while preserving the original sign.
coverage Remove tuples with total reads below coverage. Default = 5.
verbose If the function should be verbose. Default = TRUE.

Details

Calculates ASM score for a list of samples in the output format of the result of read_tuples This functions uses the following other functions: process, calcScore, calcWeight.

Value

A SummarizedExperiment of ASM scores where the rows are all the tuples and the columns the sample names.

Examples

```r
DATA_PATH_DIR <- system.file('extdata', '.', package = 'DAMEfinder')
get_data_path <- function(file_name) file.path(DATA_PATH_DIR, file_name)

tuple_files <- list.files(DATA_PATH_DIR, '.tsv.gz')
tuple_files <- get_data_path(tuple_files)
ASM <- read_tuples(tuple_files, c('CRC1', 'NORM'))
ASMscore <- calc_asm(ASM)
```

---

calc_derivedasm Calculate SNP-based ASM

Description

Combines all the GRangeslist generated in extract_bams into a RangedSummarizedExperiment object, and calculates SNP-based allele-specific methylation.

Usage

calc_derivedasm(sampleList, cores = 1, verbose = TRUE)
Arguments

sampleList List of samples returned from `extract_bams`.
cores Number of cores to thread.
verbose If the function should be verbose.

Value

`RangedSummarizedExperiment` containing in assays:

- der.ASM: matrix with SNP-based ASM
- snp.table: Matrix with SNP associated to the CpG site.
- ref.cov: Coverage of the 'reference' allele.
- alt.cov: Covariance of the 'alternative' allele.
- ref.meth: Methylated reads from the 'reference' allele.
- alt.meth: Methylated reads from the 'alternative' allele.

Examples

```r
data(extractbams_output)
derASM <- calc_derivedasm(extractbams_output[c(1,2)], cores = 1,
                           verbose = FALSE)
```

---

calc_logodds

### Description

This function calculates the log odds ratio for a CpG tuple: \((MM\timesUU)/(UM\timesMU)\), where 'M' stands for methylated and 'U' for unmethylated. 'MM' reflects the count for instances the CpG pair is methylated at both positions. The higher the MM and UU counts for that CpG pair, the higher the log odds ratio.

### Usage

```r
calc_logodds(s, eps = 1)
```

### Arguments

- **s**: A data frame that contains the MM, UU, UM, and MU counts for each CpG tuple for a particular sample. It is the resulting object of the `read_tuples`.
- **eps**: Count added to each of the MM, UU, UM and MU counts to avoid dividing by zero for example. The default is set to 1.

### Value

The same object is returned with an additional column for the log odds ratio.
**Description**

This function calculates the ASM score for every tuple in a given sample. The ASM score is a multiplication of the log odds ratio by a weight that reflects the extent of allele-specific methylation. This weight is obtained with the `calc_weight` function.

**Usage**

```r
calc_score(df, beta = 0.5, a = 0.2)
```

**Arguments**

- `df`: data frame of a sample containing all information per tuple (MM, UU, UM and MU counts, as well as the log odds ratio per tuple) needed for the ASM score.
- `beta`: parameter for the `calc_weight` function. It's the alpha and beta values for the Beta function.
- `a`: parameter for the `calc_weight` function. The weight will be the probability that the MM/(MM+UU) ratio lies between 0.5-a and 0.5+a.

**Details**

This function returns an allele-specific methylation (ASM) score for every given tuple in a sample. The ASM score is a product of the log odds ratio and a weight reflecting a measure of allele-specificity using the MM and UU counts.

**Value**

The same object with an additional column for the ASM score.

---

**Description**

This function calculates a weight which reflects MM to UU balance, where M stands for methylated and U for unmethylated. Given the MM and UU counts for a particular tuple, the weight is obtained using the `pbeta` function.

**Usage**

```r
calc_weight(MM, UU, beta = 0.5, a = 0.2)
```
Arguments

MM  The read counts for where pos1 and pos2 of the tuple were both methylated.
UU  The read counts for where pos1 and pos2 of the tuple were both unmethylated.
beta parameter for the beta distribution. In B(alpha, beta), we set alpha=beta=0.5 by default.
a parameter for how far from 0.5 we go as a measure of allele-specific methylation. The weight is the probability that the MM:(MM+UU) ratio is between 0.5-a and 0.5+a. The default is set to 0.2.

Details

For a given tuple with MM and UU counts, the weight that reflects allele-specificity is calculated as follows:

• Prior:
  \[ p(\theta|\alpha, \beta) \sim Beta(\alpha, \beta), \]
  where \( \theta = \frac{MM}{MM+UU} \) and \( \alpha = \beta = 0.5 \). \( p(\theta|\alpha, \beta) \) represents our prior belief which is that tuples are either fully methylated or fully unmethylated, rather than allele-specifically methylated which is a much rarer event.

• Likelihood:
  \[ p(x|\alpha, \beta) \propto \theta^{MM}(1-\theta)^{UU}, \]
  where \( x \) is our observation (the MM and UU counts).

• Posterior:
  \[ p(\theta|x) \propto p(x|\theta) * p(\theta|\alpha, \beta) \]
  \[ p(\theta|x) \propto \theta^{MM-0.5}(1-\theta)^{UU-0.5}, \]
  where \( \alpha = \beta = 0.5 \). This posterior also follows a beta distribution \( \sim Beta(\alpha' = MM + 0.5, \beta' = UU + 0.5) \)

Value

A number that reflects allele-specificity given MM and UU counts for a CpG pair. This is used as a weight that is multiplied by the log odds ratio to give the final ASM score of that tuple.

#calc_weight(MM=50, UU=50) #0.9999716
#calc_weight(MM=20, UU=60) #0.1646916
DAMEfinder

**DAMEfinder: Method to detect allele-specific methylation (ASM), and
differential ASM from Bisulfite sequencing data in R.**

**Description**

The package allows the user to extract an ASM score in two ways: either from a bismark bam file(s) and VCF file(s), or from the output from methtuple. Either way the final output is a list of regions with differential allele-specific methylated between groups of samples of interest. The package also provides functions to visualize ASM at the read level or the score level.

**DAMEfinder functions**

calc_asm extracts ASM for pairs of CpG sites from a methtuple file, calcDerivedasm extracts ASM at each CpG site linked to a SNP from the VCF file. Both functions generate a RangedSummarizedExperiment, which is the input for the main function find_dames, that generates a data.frame with regions exhibiting differential ASM between a number of samples.

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**dame_track**

*Plot score tracks*

**Description**

Plot score tracks

**Usage**

dame_track(
  dame,
  window = 0,
  positions = 0,
  derASM = NULL,
  ASM = NULL,
  colvec = NULL,
  plotSNP = FALSE
)
dame_track_mean

Arguments

dame: GRanges object containing a region of interest, or detected with find_dames
window: Number of CpG sites outside (up or down-stream) of the DAME should be plotted. Default = 0.
positions: Number of bp sites outside (up or down-stream) of the DAME should be plotted. Default = 0.
derASM: SummarizedExperiment object obtained from calc_derivedasm (Filtering should be done by the user)
ASM: SummarizedExperiment object obtained from calc_asm (Filtering should be done by the user)
colvec: Vector of colors (mainly useful for the SNP plot, because I add it with cowplot, so I don’t export a ggplot, optional)
plotSNP: whether to add the SNP track, only if derASM is specified. Default = FALSE

Value

Plot

Examples

```r
library(GenomicRanges)
DAME <- GRanges(19, IRanges(306443,310272))
data('readtuples_output')
ASM <- calc_asm(readtuples_output)
SummarizedExperiment::colData(ASM)$group <- c(rep('CRC',3),rep('NORM',2))
SummarizedExperiment::colData(ASM)$samples <- colnames(ASM)
dame_track(dame = DAME,
            ASM = ASM)
```

---

**dame_track_mean**

Plot means per group of score tracks.

Description

Plot means per group of score tracks.

Usage

```r
dame_track_mean(
    dame,
    window = 0,
    positions = 0,
    derASM = NULL,
    ASM = NULL,
    colvec = NULL
)
```
empirical_pval  

Arguments

dame  GRanges object containing a region of interest, or detected with find_dames
window  Number of CpG sites outside (up or down-stream) of the DAME should be plotted. Default = 0.
positions  Number of bp sites outside (up or down-stream) of the DAME should be plotted. Default = 0.
derASM  SummarizedExperiment object obtained from calc_derivedasm (Filtering should be done by the user)
ASM  SummarizedExperiment object obtained from calc_asm (Filtering should be done by the user)
colvec  Vector of colors (mainly useful for the SNP plot, because I add it with cowplot, so I don’t export a ggplot, optional)

Value

Plot

Examples

library(GenomicRanges)
DAME <- GRanges(19, IRanges(306443,310272))
data('readtuples_output')
ASM <- calc_asm(readtuples_output)
SummarizedExperiment::colData(ASM)$group <- c(rep('CRC',3),rep('NORM',2))
SummarizedExperiment::colData(ASM)$samples <- colnames(ASM)
dame_track_mean(dame = DAME,
              ASM = ASM)

empirical_pval  Calculate empirical region-level p-value

Description

This function permutes the coefficient of interest and re-runs get_tstats and regionFinder for each permutation. Code for permutations copied from the dmrseq function from the package of the same name.

Usage

empirical_pval(
  presa,
  design,
  rforiginal,
  coeff,
  cont,
smooth,
maxPerms = 10,
Q,
maxGap,
method,
...
)

Arguments

pres
SEperiment output from calc_derivedasm or calc_asm.
design
design matrix.
rforiginal
data.frame of DAMEs calculated with original design.
coeff
Coefficient of interest to permute.
cont
same as in get_tstats.
smooth
Boolean.
maxPerms
Maximum possible permutations generated. Default = 10.
Q
Quantile for cuttof.
maxGap
Same as other functions in the package.
method
lmFit method.
...
Passed to get_tstats and then to loessByCluster.

Value

Vector of empirical p-values.

extractbams_output extract_bams() output.

Description

4 Patients from a previous study (Parker et al, 2018.) with colorectal cancer were sequenced and the normal and cancerous tissue of each patient was obtained. The data includes a subset of chromosome 19.

Usage

extractbams_output
extract_bams

Format

A large list with 8 elements. Each element is a list of GRanges for each sample. Each GRanges in the list includes the location of the CpG sites contained in the reads for each SNP. The GRanges metadata table contains:

- cov.ref Number of reads of "reference" allele in that SNP
- cov.alt Number of reads of "alternative" allele in that SNP
- meth.ref Number of methylated reads of "reference" allele in that SNP
- cov.meth Number of methylated reads of "alternative" allele in that SNP
- snp The SNP containing the reads

For further details, see https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-6949/
sample names in in ArrayExpress do not necessarily match names given here!

---

extract_bams Detect allele-specific methylation from a bam file

Description

The function takes a bam (from bismark) and vcf file for each sample. For each SNP contained in the vcf file it calculates the proportion of methylated reads for each CpG site at each allele. At the end it returns (saves to working directory) a GRanges list, where each GRanges contains all the CpG sites overlapping the reads containing a specific SNP.

Usage

extract_bams(
    bamFiles,  
vcfFiles,  
sampleNames,  
referenceFile,  
coverage = 4,  
cores = 1,  
verbose = TRUE
)

Arguments

- bamFiles  List of bam files.
- vcfFiles  List of vcf files.
- sampleNames  Names of files in the list.
- referenceFile  fasta file used to generate the bam files. Or DNAStringSet with DNA sequence.
- coverage  Minimum number of reads covering a CpG site on each allele. Default = 2.
- cores  Number of cores to use. See package parallel for description of core. Default = 1.
- verbose  Default = TRUE
find_dames

Value

A list of GRanges for each sample. Each list is saved in a separate .rds file.

Examples

DATA_PATH_DIR <- system.file('extdata', '.', package = 'DAMEfinder')
geet_data_path <- function(file_name) file.path(DATA_PATH_DIR, file_name)
bamFiles <- get_data_path('NORM1_chr19_trim.bam')
vcfFiles <- get_data_path('NORM1_chr19.trim.vcf')
sampleNames <- 'NORM1'

#referenceFile
suppressPackageStartupMessages({library(BSgenome.Hsapiens.UCSC.hg19)})
genome <- BSgenome.Hsapiens.UCSC.hg19
seqnames(genome) <- gsub("chr","",seqnames(genome))
dna <- DNAStringSet(genome[[19]], use.names = TRUE)
names(dna) <- 19

GRanges_list <- extract_bams(bamFiles, vcfFiles, sampleNames, dna)

find_dames

Find DAMEs

Description

This function finds Differential Allele-specific MEthylated regions (DAMEs). It uses the regionFinder function from bumphunter, and assigns p-values either empirically or using the Simes method.

Usage

find_dames(
  sa,
  design,
  coef = 2,
  contrast = NULL,
  smooth = TRUE,
  Q = 0.5,
  pvalAssign = "simes",
  maxGap = 20,
  verbose = TRUE,
  maxPerms = 10,
  method = "ls",
  trend = FALSE,
  ...
)
**Arguments**

- **sa**: A SummarizedExperiment containing ASM values where each row correspond to a tuple/site and a column to sample/replicate.
- **design**: A design matrix created with `model.matrix`.
- **coef**: Column in design specifying the parameter to estimate. Default = 2.
- **contrast**: a contrast matrix, generated with `makeContrasts`.
- **smooth**: Whether smoothing should be applied to the t-Statistics. Default = TRUE.
- **Q**: The percentile set to get a cutoff value K. K is the value on the Qth quantile of the absolute values of the given (smoothed) t-statistics. Only necessary if `pvalAssign` = 'empirical'. Default = 0.5.
- **pvalAssign**: Choose method to assign pvalues, either 'simes' (default) or 'empirical'. This second one performs `maxPerms` number of permutations to calculate null statistics, and runs `regionFinder`.
- **maxGap**: Maximum gap between CpGs in a cluster (in bp). NOTE: Regions can be as small as 1 bp. Default = 20.
- **verbose**: If the function should be verbose. Default = TRUE.
- **maxPerms**: Maximum possible permutations generated. Only necessary if `pvalAssign` = 'empirical'. Default = 10.
- **method**: The method to be used in limma’s `lmFit`. The default is set to 'ls' but can also be set to 'robust', which is recommended on a real data set.
- **trend**: Passed to `eBayes`. Should an intensity-trend be allowed for the prior variance? Default is that the prior variance is constant, e.g. FALSE.
- **...**: Arguments passed to `get_tstats`.

**Details**

The `simes` method has higher power to detect DAMEs, but the consistency in signal across a region is better controlled with the empirical method, since it uses `regionFinder` and `getSegments` to find regions with t-statistics above a cuttof (controled with parameter Q), whereas with the 'simes' option, we initially detects clusters of CpG sites/tuples, and then test if at least 1 differential site/tuple is present in the cluster.

We recommend trying out different `maxGap` and Q parameters, since the size and the effect-size of obtained DAMEs change with these parameters.

**Value**

A data frame of detected DAMEs ordered by the p-value. Each row is a DAME and the following information is provided in the columns (some column names change depending on the `pvalAssign` choice):

- **chr**: on which chromosome the DAME is found.
- **start**: The start position of the DAME.
- **end**: The end position of the DAME.
- **pvalSimes**: p-value calculated with the Simes method.
• pvalEmp: Empirical p-value obtained from permuting covariate of interest.
• sumTstat: Sum of t-stats per segment/cluster.
• meanTstat: Mean of t-stats per segment/cluster.
• segmentL: Size of segmented cluster (from `getSegments`).
• clusterL: Size of original cluster (from `clusterMaker`).
• FDR: Adjusted p-value using the method of Benjamini, Hochberg. (from `p.adjust`).
• numup: Number of sites with ASM increase in cluster (only for Simes).
• numdown: Number of sites with ASM decrease in cluster (only for Simes).

Examples

data(readtuples_output)
ASM <- calc_asm(readtuples_output)
grp <- factor(c(rep('CRC',3),rep('NORM',2)), levels = c('NORM', 'CRC'))
mod <- model.matrix(~grp)
dames <- find_dames(ASM, mod, verbose = FALSE)

--

getMD  

MDtag parser

Description

takes a GenomicAlignments object containing the MDtag, and transforms it into a vector of characters and numbers

Usage

getMD(a)

Arguments

a  Vector of MDtags (single characters)

Value

A named list of vectors, each vector a parsed version of MDtag: - nucl.num: Numeric representation of MDtag. - MDtag: a split version of MDtag
**get_tstats**  
*Get t-Statistics*

**Description**

This function calculates a moderated t-Statistic per site or tuple using limma’s *lmFit* and *eBayes* functions. It then smooths the obtained t-Statistics using bumphunter’s *smoother* function.

**Usage**

```r
get_tstats(
  sa,  
  design,  
  contrast = NULL,  
  method = "ls",  
  trend = FALSE,  
  smooth = FALSE,  
  maxGap = 20,  
  coef = 2,  
  verbose = TRUE,  
  filter = TRUE,  
  ...  
)
```

**Arguments**

- **sa**
  - A SummarizedExperiment containing ASM values where each row and column correspond to a tuple/site and sample respectively.
- **design**
  - A design matrix created with *model.matrix*.
- **contrast**
  - A contrast matrix, generated with *makeContrasts*.
- **method**
  - The method to be used in limma’s *lmFit*. The default is set to 'ls' but can also be set to 'robust', which is recommended on a real data set.
- **trend**
  - Passed to *eBayes*. Should an intensity-trend be allowed for the prior variance? Default is that the prior variance is constant, e.g. FALSE.
- **smooth**
  - Whether smoothing should be applied to the t-Statistics. Default = FALSE. If TRUE, wherever smoothing is not possible, the un-smoothed t-stat is used instead.
- **maxGap**
  - The maximum allowed gap between genomic positions for clustering of genomic regions to be used in smoothing. Default = 20.
- **coef**
  - Column in model.matrix specifying the parameter to estimate. Default = 2. If contrast specified, column with contrast of interest.
- **verbose**
  - Set verbose. Default = TRUE.
- **filter**
  - Remove empty tstats. Default = TRUE.
- **...**
  - Arguments passed to *loessByCluster*. Only used if smooth = TRUE.
Details

The smoothing is done on genomic clusters consisting of CpGs that are close to each other. In the case of tuples, the midpoint of the two genomic positions in each tuple is used as the genomic position of that tuple, to perform the smoothing. The function takes a RangedSummarizedExperiment generated by `calc_derivedasm` or `calc_asm` containing ASM across samples, and the index of control and treatment samples.

Value

A vector of t-Statistics within the RangedSummarizedExperiment.

Examples

data(readtuples_output)
ASM <- calc_asm(readtuples_output)
grp <- factor(c(rep('CRC',3),rep('NORM',2)), levels = c('NORM', 'CRC'))
mod <- model.matrix(~grp)
tstats <- get_tstats(ASM, mod)

methyl_circle_plot

Draw methylation circle plot

Description

Draws CpG site methylation status as points, in reads containing a specific SNP. Generates one plot per bam file.

Usage

methyl_circle_plot(
  snp,
  vcfFile,
  bamFile,
  refFile,
  build = "hg19",
  dame = NULL,
  letterSize = 2.5,
  pointSize = 3,
  sampleName = "sample1",
  cpgsite = NULL,
  sampleReads = FALSE,
  numReads = 20
)
methyl_circle_plot

Arguments

- **snp**: GRanges object containing SNP location.
- **vcfFile**: vcf file.
- **bamFile**: bismark bam file path.
- **refFile**: fasta reference file path. Or DNAStringSet with DNA sequence.
- **build**: genome build used. default = "hg19"
- **dame** (optional) GRanges object containing a region to plot.
- **letterSize**: Size of alleles drawn in plot. Default = 2.5.
- **pointSize**: Size of methylation circles. Default = 3.
- **sampleName**: FIX?: this is to save the vcf file to not generate it every time you run the function.
- **cpgsite** (optional) GRanges object containing a single CpG site location of interest.
- **sampleReads**: Whether a subset of reads should be plotted. Default = FALSE.
- **numReads**: Number of reads to plot per allele, if sampleReads is TRUE. Default = 20

Value

Plot

Examples

```r
DATA_PATH_DIR <- system.file('extdata', '.', package = 'DAMEfinder')

get_data_path <- function(file_name) file.path(DATA_PATH_DIR, file_name)
bam_files <- get_data_path('NORM1_chr19_trim.bam')
vcf_files <- get_data_path('NORM1.chr19.trim.vcf')
sample_names <- 'NORM1'

#reference_file
suppressPackageStartupMessages({library(BSgenome.Hsapiens.UCSC.hg19)})
genome <- BSgenome.Hsapiens.UCSC.hg19
seqnames(genome) <- gsub("chr","",seqnames(genome))
dna <- DNAStringSet(genome[[19]], use.names = TRUE)
names(dna) <- 19

snp <- GenomicRanges::GRanges(19, IRanges::IRanges(292082, width = 1))
methyl_circle_plot(snp = snp,
  vcfFile = vcf_files,
  bamFile = bam_files,
  refFile = dna,
  sampleName = sample_names)
```
methyl_circle_plotCpG  Draw methylation circle plot without SNP

Description

Draws CpG site methylation status as points, in reads containing a specific CpG site. Generates one plot per bam file.

Usage

methyl_circle_plotCpG(
  cpgsite = cpgsite,
  bamFile = bamFile,
  pointSize = 3,
  refFile = refFile,
  dame = NULL,
  order = FALSE,
  sampleName = NULL,
  sampleReads = FALSE,
  numReads = 20
)

Arguments

cpgsite GRanges object containing a single CpG site location of interest
bamFile bismark bam file path
pointSize Size of methylation circles. Default = 3.
refFile fasta reference file path
dame (optional) GRanges object containing a region to plot
order Whether reads should be sorted by methylation status. Default= False.
sampleName Plot title.
sampleReads Whether a subset of reads should be plotted. Default = FALSE.
numReads Number of reads to plot, if sampleReads is TRUE. Default = 20

Value

Plot

Examples

DATA_PATH_DIR <- system.file('extdata', '.', package = 'DAMEfinder')
get_data_path <- function(file_name) file.path(DATA_PATH_DIR, file_name)
bam_files <- get_data_path('NORM1_chr19_trim.bam')
sample_names <- 'NORM1'
#reference_file
suppressPackageStartupMessages({library(BSgenome.Hsapiens.UCSC.hg19)})
geno <- BSgenome.Hsapiens.UCSC.hg19
seqnames(geno) <- gsub("chr","",seqnames(geno))
dna <- DNAStringSet(geno[[19]], use.names = TRUE)
names(dna) <- 19

cpg <- GenomicRanges::GRanges(19, IRanges::IRanges(292082, width = 1))
methyl_circle_plotCpG(cpgsite = cpg, bamFile = bam_files, refFile = dna)

methyl_MDS_plot

Multidimensional scaling plot of distances between methylation proportions (beta values)

Description

Same as plotMDS, except for an arc-sine transformation of the methylation proportions.

Usage

methyl_MDS_plot(x, group, top = 1000, coverage = 5, adj = 0.02, pointSize = 4)

Arguments

x          RangedSummarizedExperiment, output from calc_derivedasm or calc_asm.
group      Vector of group or any other labels, same length as number of samples.
top        Number of top CpG sites used to calculate pairwise distances.
coverage   Minimum number of reads covering a CpG site on each allele. Default = 5.
adj         Text adjustment in y-axis. Default = 0.2.
pointSize  Default = 4.

Value

Two-dimensional MDS plot.

Examples

data(readtuples_output)
ASM <- calc_asm(readtuples_output)
grp <- factor(c(rep('CRC',3),rep('NORM',2)), levels = c('NORM', 'CRC'))
methyl_MDS_plot(ASM, grp)
**modulus_sqrt**  
*Get Modulus Square Root*

**Description**

Function to calculate signed square root (aka modulus square root).

**Usage**

```r
modulus_sqrt(values)
```

**Arguments**

- `values` Vector or matrix of ASM scores where each column is a sample. These values are transformed with a square root transformation that (doesn't) preserve the sign.

**Value**

Vector or matrix of transformed scores.

---

**readtuples_output**  
*read_tuples() output.*

**Description**

3 Patients from a previous study (Parker et al, 2018.) with colorectal cancer were sequenced and the normal and cancerous tissue of each patient was obtained. The data includes a subset of chromosome 19. Here one normal sample is not included.

**Usage**

```r
readtuples_output
```

**Format**

A large list with 5 elements. Each element is a tibble with the coordinates of the pairs of CpG sites (tuples). Rest of the tibble contains:

- `MM` Number of reads with both CpG sites methylated
- `MU` Number of reads with first CpG site methylated
- `UM` Number of reads with second CpG site methylated
- `UU` Number of reads with both CpG sites unmethylated
- `cov` Coverage, total reads at tuple
- `inter_dist` Distance in bp between CpG sites

For further details, see [https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-6949/](https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-6949/) sample names in in ArrayExpress do not necessarily match names given here!
**read_tuples**  
*Read in list of methtuple files*

**Description**

This function reads in a list of files obtained from the methtuple tool. It filters out tuples based on the set minimum coverage (min_cov) and the maximum allowed distance (maxGap) between two genomic positions in a tuple.

**Usage**

```r
read_tuples(files, sampleNames, minCoverage = 2, maxGap = 20, verbose = TRUE)
```

**Arguments**

- `files` List of methtuple files.
- `sampleNames` Names of files in the list.
- `minCoverage` The minimum coverage per tuple. Tuples with a coverage < minCoverage are filtered out. Default = 2.
- `maxGap` The maximum allowed distance between two positions in a tuple. Only distances that are <= maxGap are kept. Default = 150 base pairs.
- `verbose` If the function should be verbose.

**Value**

A list of data frames, where each data frame corresponds to one file.

**Examples**

```r
DATA_PATH_DIR <- system.file('extdata', '.', package = 'DAMEfinder')
get_data_path <- function(file_name) file.path(DATA_PATH_DIR, file_name)

tuple_files <- list.files(DATA_PATH_DIR, '.tsv.gz')
tuple_files <- get_data_path(tuple_files)
ASM <- read_tuples(tuple_files, c('CRC1', 'NORM1'))
```
simes_pval  
*Calculate region-level p-value*

**Description**

This function uses the Simes method to calculate a regional-level p-value based on the single-eBayes p-values. It highly depends on the choice of `maxGap` in `find_dames`.

**Usage**

```r
simes_pval(sat, smtstat, midpt)
```

**Arguments**

- **sat**: Output from `get_tstats`.
- **smtstat**: (Smoothed) tstat vector from `get_tstats`.
- **midpt**: Coordinate vector for each CpG site/tuple.

**Details**

When used as a FDR-control method, for positively correlated P-values, Simes method is even closer to the nominal alpha level than the Bonferroni-Holm method.

**Value**

Vector of summarized pvals

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splitReads  
*Divide read names by allele*

**Description**

Takes a GenomicAlignments object and returns a list of read names divided by allele.

**Usage**

```r
splitReads(alns, v, snp)
```

**Arguments**

- **alns**: GenomicAlignments object.
- **v**: Nucleotide of reference (or alternative) allele.
- **snp**: GRanges object containing SNP location.

**Value**

A named list of vectors, each vector containing read names for each allele.
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