Package ‘methylMnM’

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Description To give the exactly p-value and q-value of MeDIP-seq and MRE-seq data for different samples comparation.
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methylMnM-package

MeDIP-Seq and MRE-Seq data analysis

Description

M&M was developed for analyzing data derived from methylated DNA immunoprecipitation (MeDIP) experiments followed by sequencing (MeDIP-Seq) and the digestions with the methyl-sensitive restriction enzymes (MRE-Seq). Nevertheless, functionalities like the quality controls may be applied to other types of sequencing data (e.g. ChIP-Seq). MeDIP-MRE (methylMnM) test which combine the two differential techniques (MeDIP-seq and MRE-seq) data to detecting the differentially methylation level of CpG.

Details

Package: methylMnM
Type: Package
Version: 1.0
Date: 2012-12-01
License: GPL-3
LazyLoad: yes
Depends: R (>= 2.12.0)

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang
Maintainer: Yan Zhou <zhouy1016@163.com>

References

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang, 2012
**calcFactornew**  

**Normalization factor.**

**Description**

Amends of TMM normalization for our method.

**Usage**

```
calcFactornew(obs, ref, m, k, logratioTrim=.3, sumTrim=0.05,  
doWeighting=TRUE, Acutoff=-1e10)
```

**Arguments**

- `obs`: Counts of treatment sample.
- `ref`: Counts of control sample.
- `m`: The number of CpG in each bin.
- `k`: The number of MRE-CpG in each bin.
- `logratioTrim`: amount of trim to use on log-ratios ("M" values)
- `sumTrim`: amount of trim to use on the combined absolute levels ("A" values)
- `doWeighting`: logical, whether to compute (asymptotic binomial precision) weights
- `Acutoff`: cutoff on "A" values to use before trimming

**Value**

A real value larger than 0.

**Author(s)**

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

**Examples**

```
d <- matrix( rpois(1000, lambda=5), nrow=200 )  
mu <- rep(1,nrow=200 )  
k <- rep(1,nrow=200 )  
f <- calcFactornew(d[,2], d[,1], m, k, logratioTrim=.3, sumTrim=0.05,  
doWeighting=TRUE, Acutoff=-1e10)
```
calculatecount

Description

Call C programs to R for calculate MeDIP-seq or CpG count of each bin.

Usage

calculatecount(data2, data3, cpg2, cpg3, datalength, cpglength, count=rep(0,cpglength))

Arguments

data2 Start position of each tag.
data3 End position of each tag.
cpg2 Start position of each bin.
cpg3 End position of each bin.
datalength The number of tags
cpglength The number of bins
count Read count of each bin.

Value

Read count of each bin.

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

data<-matrix(1:800, nrow=400)
data[,2]<-data[,1]+37
cpg<-matrix(1:20, nrow=10)
cpg[,1]<-seq(0,360,length=10)
cpg[,2]<-seq(40,400,length=10)
f <- calculatecount(data[,1], data[,2], cpg[,1], cpg[,2], length(data[,1]), length(cpg[,2]), count=rep(0,length(cpg[,2])))
calculatecount1 \hspace{1cm} Call C programs to R.

Description

Call C programs to R for calculate MRE-seq "+" direction count of each bin.

Usage

\begin{verbatim}
calculatecount1(data2, data3, cpg2, cpg3, datalength, cpglength, count=rep(0,cpglength))
\end{verbatim}

Arguments

- \textbf{data2} \hspace{1cm} Start position of each tag.
- \textbf{data3} \hspace{1cm} End position of each tag.
- \textbf{cpg2} \hspace{1cm} Start position of each bin.
- \textbf{cpg3} \hspace{1cm} End position of each bin.
- \textbf{datalength} \hspace{1cm} The number of tags
- \textbf{cpglength} \hspace{1cm} The number of bins
- \textbf{count} \hspace{1cm} Count of MRE-seq "+" direction of each bin.

Value

Count of MRE-seq "+" direction of each bin.

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

\begin{verbatim}
data<-matrix( 1:400, nrow=200)
cpg<-matrix( 1:40, nrow=20)
cpg[,1]<-seq(0,380,length=20)
cpg[,2]<-seq(20,400,length=20)
f <- calculatecount1(data[,1], data[,2], cpg[,1], cpg[,2], length(data[,1]), length(cpg[,2]), count=rep(0,length(cpg[,2])))
\end{verbatim}
calculatecountneg  

Call C programs to R.

Description

Call C programs to R for calculate MRE-seq "-" direction count of each bin.

Usage

```r
calculatecountneg(data2, data3, cpg2, cpg3, datalength, cpglength, count=rep(0,cpglength))
```

Arguments

- `data2`: Start position of each tag.
- `data3`: End position of each tag.
- `cpg2`: Start position of each bin.
- `cpg3`: End position of each bin.
- `datalength`: The number of tags
- `cpglength`: The number of bins
- `count`: Count of MRE-seq "-" direction of each bin.

Value

Count of MRE-seq "-" direction of each bin.

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

```r
data<-matrix( 1:400, nrow=200 )
cpg<-matrix( 1:40, nrow=20)
cpg[,1]<-seq(0,380,length=20)
cpg[,2]<-seq(20,400,length=20)
f <-calculatecountneg(data[,1], data[,2], cpg[,1], cpg[,2], length(data[,1]), length(cpg[,2]), count=rep(0,length(cpg[,2])))
```
**Description**

The function is used to normalize copy number variation.

**Usage**

```r
cNVnorm(CNVfile, bincount)
```

**Arguments**

- **CNVfile**: The path of copy number variation file.
- **bincount**: Count of all bins.

**Value**

Count of all bins after CNV normalization.

**Author(s)**

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

**Examples**

```r
datafile <- system.file("extdata", package = "methylMnM")
filepath <- datafile[1]
file1 <- paste(filepath, "/all_CpGsite_chr18.txt", sep="")
CpGsite <- read.table(file1, header=FALSE, skip=0, nrows=200, as.is=TRUE)
winbin <- CpGsite[1:100, 1:4]
winbin[, 2] <- seq(0, 49500, 500)
winbin[, 3] <- winbin[, 2] + 500
winbin[, 4] <- rpois(100, lambda=5)
cnv <- winbin[1:5,]
cnv[, 2] <- c(0, 10000, 20000, 30000, 40000)
cnv[, 3] <- cnv[, 2] + 10000
ncnv <- cnv[, 1]
cnv[, 4] <- c(1.2, 1.6, 1.2, 1)
CNVfile <- paste(setwd(getwd()), "/CNVfile.bed", sep = "")
write.table(cnv, CNVfile, quote=FALSE, row.names = FALSE, col.names = FALSE)
f <- CNVnormal(CNVfile, winbin)
```
countcpgbin

Compute the total CpG number of each bin with each CpG site.

Description

The function is used to compute the total CpG number of each bin with each CpG site.

Usage

```r
countcpgbin(file.cpgsite, file.blacklist=NULL, file.bin=NULL, writefile=NULL, reportfile=NULL, binlength=500)
```

Arguments

- `file.cpgsite` The path of cpg site file or sequence tag file.
- `file.blacklist` The path of blacklist file (If we do not use the file, there will be defaulted as NULL).
- `file.bin` The path of all cpg bin file. For computing the number of sequence tag of each window, we use the file as a normalization window position. (If we do not use the file, there will be defaulted as NULL).
- `writefile` The path of output results. (If writefile=NULL, there will return the results back to main program.)
- `reportfile` The path of output results of bin length, the number of bin, total reads before processing and total reads after processing.
- `binlength` The length of each window.(Defaulted length is 500).

Value

The CpG site should include at least three columns "chromosome", "start position" and "end position". The output file is include four columns, that is "chromosome", "start position", "end position" and "CpG count". Also, the function output a report for some parameters.

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

```r
datafile<system.file("extdata", package = "methylMnM")
filepath<datafile[1]
file.cpgsite<-paste(filepath,"/all_CpGsite_chr18.txt",sep="")
f<-countcpgbin(file.cpgsite, binlength=5000)
```
countMeDIPbin

Compute the total MeDIP-seq number of each bin.

Description
The function is used to compute the total MeDIP-seq number of each bin.

Usage
countMeDIPbin (file.Medipsite, file.blacklist=NULL, file.bin=NULL,
file.CNV=NULL, writefile=NULL, reportfile=NULL, binlength=500)

Arguments
- file.Medipsite: The path of MeDIP-seq site file or sequence tag file.
- file.blacklist: The path of blacklist file. (If we do not use the file, there will be defaulted as NULL).
- file.bin: The path of all bins file. For computing the number of sequence tag of each window, we use the file as a normalization window position. (If we do not use the file, there will be defaulted as NULL).
- file.CNV: If need, we should input CNV file to normalize count of each bin.
- writefile: The path of output results. (If writefile=NULL, there will return the results back to main program.)
- reportfile: The path of output results of bin length, the number of bin, total reads before processing and total reads after processing.
- binlength: The length of each window. (Defaulted length is 500).

Value
The MeDIP-seq site should include at least three columns “chromosome”, “start position” and “end position”. The output file is include four columns, that is “chromosome”, “start position”, “end position” and “MeDIP-seq count”. Also, the function output a report for some parameters.

Author(s)
Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples
datafile<-system.file("extdata", package = "methylMnM")
filepath<-datafile[1]
file.Medipsite<-paste(filepath,"/all_CpGsite_chr18.txt",sep="")
f<-countMeDIPbin(file.Medipsite, binlength=500)
**countMREbin**

*Compute the total MRE-seq number of each bin.*

**Description**

The function is used to compute the total MRE-seq number of each bin.

**Usage**

```r
countMREbin(file.MREsite, file.blacklist=NULL, file.bin=NULL, file.CNV=NULL, cutoff=0, writefile=NULL, reportfile=NULL, binlength=500)
```

**Arguments**

- `file.MREsite`: The path of MRE-seq sites file.
- `file.blacklist`: The path of blacklist file (If we do not use the file, there will be defaulted as NULL).
- `file.bin`: The path of all bin file. For computing the number of sequence tag of each window, we use the file as a normalization window position. (If we do not use the file, there will be defaulted as NULL).
- `file.CNV`: If need, we should input CNV file to normalize count of each bin.
- `cutoff`: The critical value of PCR. (If we do not use the critical value, there will be defaulted as 0.)
- `writefile`: The path of output results. (If writefile=NULL, there will return the results back to main program.)
- `reportfile`: The path of output results of bin length, the number of bin, total reads before processing and total reads after processing.
- `binlength`: The length of each window. (Defaulted length is 500).

**Value**

The MRE-seq sites should include at least three columns "chromosome", "start position" and "end position". The output file is include four columns, that is "chromosome", "start position", "end position" and "MRE-seq count". Also, the function output a report for some parameters.

**Author(s)**

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

**Examples**

```r
datafile<-system.file("extdata", package = "methylMnM")
filepath<-datafile[1]
file.MREsite<-paste(filepath,"/all_CpGsite_chr18.txt",sep="")
f<-countMREbin(file.MREsite, binlength=5000)
```
countMREcpgbin

Compute the MRE CpG number of each bin with MRE CpG sites.

Description

The function is used to compute the MRE CpG number of each bin with MRE CpG sites. MRE CpG is some specific CpGs in genome-wide, such as "CCGG", "GCGC" and "CCGC". The specific CpG number is directly bound up with each experiment.

Usage

countMREcpgbin(mrecpg.site,file.allcpgsite,file.bin=NULL, writeFile=NULL, binlength=500)

Arguments

mrecpg.site The data of mreCpG site.
file.allcpgsite The path of all cpg site file or sequence tag file.
file.bin The path of all bins file. For computing the number of sequence tag of each window, we use the file as a normalize window position. (If we do not use the file, there will be defaulted as NULL).
writefile The path of output result. (If writeFile=NULL, there will return the results back to main program)
binlength The length of each window. (Defaulted length is 500)

Value

The output file is include four columns, that is "chromosome", "start position", "end position" and "MRE CpG count".

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

datafile<-system.file("extdata", package = "methylMnM")
filepath<-datafile[1]
file<-'-paste(filepath,"/three_Mre_CpGsite_chr18.txt",sep="")
file1<-'-paste(filepath,"/all_CpGsite_chr18.txt",sep="")
five_Mre_CpGsite<-read.table(file, header=FALSE, as.is=TRUE)
f<-countMREcpgbin(mrecpg.site=five_Mre_CpGsite[1:1000,], file.allcpgsite=file1, binlength=5000)
cpgcount  

Call C programs to R.

Description

Call C programs to R for calculate which CpG are contained in MRE-CpG.

Usage

cpgcount(data2, data3, cpg2, cpg3, datalength, cpglength, count=rep(0,cpglength))

Arguments

data2  Start position of each MRE-CpG.
data3  End position of each MRE-CpG.
cpg2  Start position of each CpG.
cpg3  End position of each CpG.
datalength  The number of MRE-CpG.
cpglength  The number of MRE-CpG.
count  MRE-CpG count of each CpG.

Value

MRE-CpG count of each CpG.

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

cpg<-matrix( 1:800, nrow=400 )
cpg[,2]<-cpg[,1]+2
data<-cpg[3:100,]
data[,1]<-data[,1]-1
data[,2]<-data[,2]+1
f <- cpgcount(data[,1], data[,2], cpg[,1], cpg[,2], length(data[,1]),
length(cpg[,2]), count=rep(0,length(cpg[,2])))
MnM.qvalue

Estimate the q-values for a given set of p-values

Description

The function is used to estimate the q-values for a given set of p-values. The q-value of a test measures the proportion of false positives incurred (called the false discovery rate) when that particular test is called significant.

Usage

MnM.qvalue(datafile, writefile=NULL, reportfile=NULL)

Arguments

datafile   Input data of p-values file (Including all input)
writefile  The file path of output result. (If writefile=NULL, there will return the results back to main program)
reportfile The path of output results of bin length, the number of bin, total reads before processing and total reads after processing.

Value

The output file is just add a q-value column to the input file.

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

datafile<-system.file("extdata", package = "methylMnM")
filepath<-datafile[1]
file1<-paste(filepath,"/all_CpGsite_chr18.txt",sep="")
CpGsite<-read.table(file1, header=FALSE, skip=0, nrow=200, as.is=TRUE)
winbin<-CpGsite[,1:100,1:4]
winbin[,2]<-seq(0,49500,500)
winbin[,3]<-winbin[,2]+500
count<-matrix(rpois(600, lambda=5), nrow=100)
count[,6]<-count[,5]
pvalue<-runif(100, min=0, max=1)
ts<-rnorm(100, mean=0, sd=1)
cpgpq<-cbind(winbin[,1:3], count, pvalue, ts)
colnames(cpgpq)=c("chr", "chrSt", "chrEnd", "Medip1", "Medip2", "MRE1", "MRE2", "cg", "mrecg", "pvalue", "Ts")
pvaluefile<-paste(setwd(getwd()), "/pvalue.bed", sep = "")
write.table(cpgpq, pvaluefile, sep="\t", quote=FALSE, row.names =FALSE)
f<-MnM.qvalue(datafile=pvaluefile)
**MnM.selectDMR**  
*Select significants of each comparation.*

**Description**

The function is used to select significants of each comparation.

**Usage**

```r
MnM.selectDMR(frames = NULL, up = 1.45, down = 1/1.45, p.value.MM = 0.01,  
p.value.SAGE = 0.01, q.value = 0.01, cutoff = "q-value", quant = 0.6)
```

**Arguments**

- `frames`: The input dataset and q-values of each bin.
- `up`: The ratio of Medip1/Medip2 should be larger than "up" value if we call it significant.
- `down`: The ratio of Medip1/Medip2 should be smaller than "down" value if we call it significant.
- `p.value.MM`: The p-value of the bin which use MM test should be smaller than "p.value.MM" if we call it significant.
- `p.value.SAGE`: The p-value of the bin which use SAGE test should be smaller than "p.value.SAGE" if we call it significant.
- `q.value`: The q-value of the bin should be smaller than "q.value" if we call it significant.
- `cutoff`: We should use p-value or q-value cutoff to detect DMRs (If cutoff="q-value", then we use q-value to detect DMRs, else we use p-value).
- `quant`: The rank of absolute value of (Medip1-Medip2) should be larger than "quant" value if we call it significant.

**Value**

The DMRs of the comparation.

**Author(s)**

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

**Examples**

```r
datafile<-system.file("extdata", package = "methylMnM")
filepath<-datafile[1]
file1<-paste(filepath,"/all_CpGsite_chr18.txt",sep="")
CpGsite<-read.table(file1, header=FALSE,skip=0, nrows=200, as.is=TRUE)
winbin<-CpGsite[1:100,1:4]
winbin[,2]<-seq(0,49500,500)
```
MnM.test

winbin[,3]<-winbin[,2]+500
count<-matrix(rpois(600, lambda=5), nrow=100)
count[,6]<-count[,5]
pvalue<-runif(100, min=0, max=1)
ts<-rnorm(100, mean=0, sd=1)
cpgpq<-cbind(winbin[,1:3],count,pvalue,ts)
colnames(cpgpq)=c("chr", "chrSt","chrEnd","Medip1","Medip2","MRE1", "MRE2","cg","mrecg","pvalue","Ts")
f<-MnM.selectDMR(frames=cpgpq, p.value.MM = 0.1, p.value.SAGE = 0.1, cutoff="p-value")

MnM.test
Compute p-value of each bin.

Description
The function is used to compute p-value of each bin.

Usage
MnM.test(file.dataset=NULL,chrstring=NULL,file.cpgbin=NULL, file.mrecpgbin=NULL,writefile=NULL,reportfile=NULL, mreratio=3/7,method="XXYY", psd=2,mkadded=1,a=1e-16, cut=100,top=500)

Arguments

file.dataset The files path of sample. (datafile should be c(datafile1,datafile2,datafile3,datafile4), where datafile1 and datafile2 are path of Medip-seq data, datafile3 and datafile4 are path of MRE-seq data).
chrstring The chromosome should be test.
file.cpgbin The file path of all CpG number of each bin.
file.mrecpgbin The file path of MRE-CpG number of each window (If NULL, mrecpgfile will equal to cpgfile).
writefile The file path of output result. (If writefile=NULL, there will return the results back to main program )
reportfile The path of output results of bin length, the number of bin, total reads before processing and total reads after processing.
mreratio The ratio of total unmethylation level with total methylation level (Defaulted mreratio is 3/7).
method Option different data for the test.
psd The parameters of pseudo count, which pseudo count added to Medip-seq and MRE-seq count.
mkadded Added to all CpG and MRE CpG (We set psd=2 and mkadded=1 as defaulted for robust)
normpdf

Compute p-value with normal distribution.

Description

The function is used to compute p-value with normal distribution.
Usage

`normpdf(t, n, p, c1, c2)`

Arguments

- `t`: Statistic.
- `n`: The sum of MeDIP-seq count and MRE-seq count of each bin of two samples.
- `p`: The probability in multinomial distribution.
- `c1`: A constant to balance MeDIP-seq of sample 1 and sample 2.
- `c2`: A constant to balance MRE-seq of sample 1 and sample 2.

Value

p-values.

Author(s)

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

```
t<-0.1
n<-200
p<-c(0.25,0.25,0.25,0.25)
c1<-1
c2<-1
f<-normpdf(t,n,p,c1,c2)
```

Description

The function is used to compute p-value with normal distribution.

Usage

`normpdf1(t, n, p, c1, c2)`

Arguments

- `t`: Statistic.
- `n`: The sum of MeDIP-seq count and MRE-seq count of each bin of two samples.
- `p`: The probability in multinomial distribution.
- `c1`: A constant to balance MeDIP-seq of sample 1 and sample 2.
- `c2`: A constant to balance MRE-seq of sample 1 and sample 2.
**pmultinom**

**Value**

Statistic of a bin.

**Author(s)**

Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

**Examples**

```r
t<-0.1
n<-200
p<-c(0.25,0.25,0.25,0.25)
c1<-1
c2<-1
f<-normpdf(t,n,p,c1,c2)
```

---

**pmultinom**  
*Call C programs to R.*

---

**Description**

Call C programs to R for calculate p-value of each bin with multinomial distribution.

**Usage**

```r
pmultinom(T, SIZE, length, P1, P2, P3, P4, C1, C2, 
pvalue=rep(0,length(T)))
```

**Arguments**

- **T** Statistic.
- **SIZE** The sum of MeDIP-seq count and MRE-seq count of each bin of two samples.
- **length** The number of bins.
- **P1** The probability of MeDIP-seq of sample 1 in multinomial distribution.
- **P2** The probability of MeDIP-seq of sample 2 in multinomial distribution.
- **P3** The probability of MRE-seq of sample 1 in multinomial distribution.
- **P4** The probability of MRE-seq of sample 2 in multinomial distribution.
- **C1** A constant to balance MeDIP-seq of sample 1 and sample 2.
- **C2** A constant to balance MRE-seq of sample 1 and sample 2.
- **pvalue** p-values of windows.

**Value**

p-value.
qvalue.rank

Author(s)
Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

```r
T<-4
SIZE<-200
p<-c(0.25,0.25,0.25,0.25)
c1<-1
c2<-1
length<-1
f<-pmultinom(T, SIZE, length, p[1], p[2], p[3], p[4], c1, c2, pvalue=rep(0,length(T)))
```

Description
The function is used to rank values.

Usage

```r
qvalue.rank(x)
```

Arguments

- `x`: Value.

Value

Ranked values.

Author(s)
Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples

```r
x<-c(4,2,50,42,80,9)
qvalue.rank(x)
```
removeblacklist  Remove blacklist.

Description
The function is used to remove blacklist which we are not interest.

Usage
removeblacklist(file2,cpg)

Arguments
file2 The path of blacklist site file.
cpg All bins.

Value
All bins except blacklist region.

Author(s)
Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang

Examples
```
datafile<-system.file("extdata", package = "methylMnM")
filepath<-datafile[1]
file1<-paste(filepath,"/all_CpGsite_chr18.txt",sep="")
CpGsite<-read.table(file1, header=FALSE,skip=0, nrows=200, as.is=TRUE)
winbin<-CpGsite[1:100,1:4]
winbin[,2]<-seq(0,49500,500)
winbin[,3]<-winbin[,2]+500
winbin[,4]<-rpois(100, lambda=5)
blacklist<-winbin[1:5,]
blacklist[,2]<-c(0,10000,20000,30000,40000)
blacklist[,3]<-blacklist[,2]+1000
blacklistfile<-paste(setwd(getwd()), ",/blacklist.bed", sep = "")
write.table(blacklist, blacklistfile, quote=FALSE, row.names =FALSE,col.names =FALSE)
f<-removeblacklist(blacklistfile,winbin)
```
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