Package ‘methylMnM’
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Title detect different methylation level (DMR)
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Author Yan Zhou, Bo Zhang, Nan Lin, BaoXue Zhang and Ting Wang
Maintainer Yan Zhou<zhouy1016@163.com>
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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R topics documented:

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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**

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**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

Amends of TMM normalization for our method.

**Usage**

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

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 )
m<-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 Call C programs to R.

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.
calculatecount1

Call C programs to R.

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

Usage

```r
calculatecount1(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
calculatecountneg

Examples

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])))

calculatecountneg Call C programs to R.

Description

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

Usage

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

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])))
**CNVnormal**

*Normalize copy number variation (CNV).*

**Description**

The function is used to normalize copy number variation.

**Usage**

```r
CNVnormal(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, nrow=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
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**

```r
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**

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

**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)
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(0,60000,1000)
winbin[,3]<-winbin[,2]+100
count<-matrix(rpois(600, lambda=5), nrow=100)
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

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

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, ncol=100, 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)
```r
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(Files=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,mkadde=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)
- `a` Cut-off for recalculating p-value with multinomial distribution when normal p-values smaller than a and the sum of observations smaller than top.
- `cut` Cut-off for recalculating p-value with multinomial distribution when the sum of observations smaller than cut.
- `top` Cut-off for recalculating p-value with multinomial distribution when normal p-values smaller than a and the sum of observations smaller than top.
Value

The output file "writefile" will own eleven columns, that is, "chr", "chrSt", "chrEnd", "Medip1", "Medip2", "MRE1", "MRE2", "cg", "mrecg", "pvalue" and "plus-minus". We also output a report file which will include parameters "s1/s2", "s3/s4", "N1", "N2", "N3", "N4", "c1", "c2", "Number of windows" and "Spend time".

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)
wbin<-CpGsite[1:100,1:4]
wbin[,2]<-seq(0,49500,500)
wbin[,3]<wbin[,2]+500
wbin[,4]<rpois(100, lambda=5)
winbinfile1<-paste(setwd(getwd()), "/winbinfile1.bed", sep="")
write.table(wbin, winbinfile1,sep="\t", quote=FALSE, row.names =FALSE)
wbin<winbin
winbinfile2<-paste(setwd(getwd()), "/winbinfile2.bed", sep="")
write.table(wbin1, winbinfile2,sep="\t", quote=FALSE, row.names =FALSE)
datafile<-c(winbinfile1,winbinfile2)
cpg<-winbin
cpg[,4]<rpois(100, lambda=12)
cpgfile<-paste(setwd(getwd()), "/cpgfile.bed", sep="")
write.table(cpg, cpgfile, sep="\t", quote=FALSE, row.names =FALSE)
f<-MnM.test(file.dataset=datafile,file.cpgbin=cpgfile)

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

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

statistic of a bin.

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)
**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.

**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)))
```
qvalue.rank

Description
The function is used to rank values.

Usage
qvalue.rank(x)

Arguments
x Value.

Value
Ranked values.

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

Examples
x<-c(4,2,50,42,80,9)
qvalue.rank(x)

removeblacklist

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.

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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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