

Package ‘MEDIPS’

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Type Package

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Description MEDIPS was developed for analyzing data derived from methylated DNA immunoprecipitation (MeDIP) experiments followed by sequencing (MeDIP-seq). However, MEDIPS provides functionalities for the analysis of any kind of quantitative sequencing data (e.g. ChIP-seq, MBD-seq, CMS-seq and others) including calculation of differential coverage between groups of samples and saturation and correlation analysis.

License GPL (>=2)

LazyLoad yes

Depends R (>= 3.0), BSgenome, Rsamtools

Imports GenomicRanges, Biostrings, graphics, gtools, IRanges, methods, stats, utils, edgeR, DNACopy, biomaRt, rtracklayer, preprocessCore

Suggests BSgenome.Hsapiens.UCSC.hg19, MEDIPSData, BiocStyle

biocViews DNAMethylation, CpGIsland, DifferentialExpression, Sequencing, ChIPSeq, Preprocessing, QualityControl, Visualization, Microarray, Genetics, Coverage, GenomeAnnotation, CopyNumberVariation, SequenceMatching

NeedsCompilation no

R topics documented:

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MEDIPS-package *(MeD)IP-seq data analysis*

Description

MEDIPS was developed for analyzing data derived from methylated DNA immunoprecipitation (MeDIP) experiments followed by sequencing (MeDIP-seq). Nevertheless, several functionalities may be applied to other types of sequencing data (e.g. differential coverage or testing the saturation of ChIP-seq data). MEDIPS addresses several aspects in the context of MeDIP-seq data analysis including basic data processing, several quality controls, normalization, and identification of differential coverage.

Details

Package: MEDIPS
 Type: Package
 License: GPL (>=2)
 LazyLoad: yes

Author(s)

Lukas Chavez, Matthias Lienhard, Joern Dietrich

Maintainer: Lukas Chavez <lchavez@liai.org>

References

Chavez L, Jozefczuk J, Grimm C, Dietrich J, Timmermann B, Lehrach H, Herwig R, Adjaye J., Computational analysis of genome-wide DNA methylation during the differentiation of human embryonic stem cells along the endodermal lineage, *Genome Res.* 2010 Oct;20(10):1441-50. Epub 2010 Aug 27.

COUPLINGset-class *COUPLINGset class and internal functions*

Description

COUPLINGset class is used in the MEDIPS library to store and extract information generated during the creation of a coupling vector.

Objects from the Class

Objects of the classes contain information about sequence pattern information, included chromosomes, and further parameter settings. A COUPLING SET object is created by the MEDIPS.couplingVector() function. According slots will be filled during the workflow.

Slots

genome_name: Object of class "character" : the reference genome

window_size: Object of class "numeric" : the window size for the genome vector

chr_names: Object of class "character" : the names of the chromosomes included within the MEDIPS/COUPLING SET

chr_lengths: Object of class "numeric" : the lengths of the chromosomes included within the MEDIPS/COUPLING SET

seq_pattern: Object of class "character" : the sequence pattern (e.g. CG)

genome_CF: Object of class "numeric" : the coupling factor at the genomic bins

number_pattern: Object of class "numeric" : the total number of sequence pattern

Methods

genome_name signature(object = "COUPLINGset"): extracts the reference genome of the COUPLING SET

window_size signature(object = "COUPLINGset"): extracts the window size from the window_size slot COUPLING SET

chr_names signature(object = "COUPLINGset"): extracts the names of the chromosomes included within the COUPLING SET

chr_lengths signature(object = "COUPLINGset"): extracts the length of the chromosomes included within the COUPLING SET

seq_pattern signature(object = "COUPLINGset"): extracts the sequence pattern (e.g. CpG)

genome_CF signature(object = "COUPLINGset"): extracts the coupling factor at the genomic bins

number_pattern signature(object = "COUPLINGset"): extracts the total number of sequence pattern

show signature(object = "COUPLINGset"): prints a summary of the COUPLING SET object content

Author(s)

Lukas Chavez, Matthias Lienhard, Joern Dietrich

Examples

```
showClass("COUPLINGset")
```

MEDIPS.addCNV

Function to run a copy number variation analysis.

Description

Function calculates a CNV analysis based on two INPUT SETs by employing the DNACopy package. The results are attached to a provided result table.

Usage

```
MEDIPS.addCNV(ISet1, ISet2, results, cnv.Frame=1000)
```

Arguments

ISet1	First group of INPUT SETs
ISet2	Second group of INPUT SETs
results	result table as returned by the MEDIPS.meth function
cnv.Frame	window size used for calculating CNVs. Can be of different size than the result table.

Value

The result table with an additional column containing DNACopy's log-ratio.

Author(s)

Joern Dietrich

Examples

```

library(MEDIPSData)
library("BSgenome.Hsapiens.UCSC.hg19")

bam.file.hESCs.Input = system.file("extdata", "hESCs.Input.chr22.bam", package="MEDIPSData")
bam.file.DE.Input = system.file("extdata", "DE.Input.chr22.bam", package="MEDIPSData")

hESCs.Input = MEDIPS.createSet(file=bam.file.hESCs.Input, BSgenome="BSgenome.Hsapiens.UCSC.hg19", extend=250, shift=0)
DE.Input = MEDIPS.createSet(file=bam.file.DE.Input, BSgenome="BSgenome.Hsapiens.UCSC.hg19", extend=250, shift=0)

data(resultTable)

resultTable = MEDIPS.addCNV(cnv.Frame=10000, ISet1=hESCs.Input, ISet2=DE.Input, results=resultTable)

```

MEDIPS.correlation	<i>Calculates pairwise Pearson correlations between provided MEDIPS SETs</i>
--------------------	--

Description

The function calculates genome wide Pearson correlations between all pairs of provided MEDIPS SETs.

Usage

```
MEDIPS.correlation(MSets=NULL, plot = T, method="pearson")
```

Arguments

MSets	a concatenated set of MEDIPS SETs
plot	if specified, the correlation will be depicted as a scatter plot
method	default: pearson; alternatives: kendall, spearman

Value

a correlation matrix

Author(s)

Lukas Chavez

Examples

```
library(MEDIPSData)
data(hESCs_MeDIP)
data(DE_MeDIP)

correlation = MEDIPS.correlation(MSets=c(hESCs_MeDIP[[1]], DE_MeDIP[[1]]), plot = FALSE)
```

MEDIPS.couplingVector *Calculates the sequence pattern densities at genome wide windows.*

Description

The function calculates the local densities of a defined sequence pattern (e.g. CpGs) and returns a COUPLING SET object which is necessary for normalizing MeDIP data.

Usage

```
MEDIPS.couplingVector(pattern="CG", refObj=NULL)
```

Arguments

pattern	defines the sequence pattern, e.g. CG for CpGs.
refObj	a MEDIPS Set or MEDIPS ROI Set that serves as reference for the genome and window parameters.

Value

A COUPLING SET object.

Author(s)

Lukas Chavez

Examples

```
library("MEDIPSData")
library("BSgenome.Hsapiens.UCSC.hg19")

data(hESCs_MeDIP)
CS = MEDIPS.couplingVector(pattern="CG", refObj=hESCs_MeDIP)
```

MEDIPS.CpGenrich	<i>Calculates CpG enrichment of provided short reads compared to the reference genome.</i>
------------------	--

Description

As a quality check for the enrichment of CpG rich DNA fragments obtained by the immunoprecipitation step of a MeDIP experiment, this function provides the functionality to calculate CpG enrichment values. The main idea is to check, how strong the regions are enriched for CpGs compared to the reference genome. For this, the function counts the number of Cs, the number of Gs, the number CpGs, and the total number of bases within the stated reference genome. Subsequently, the function calculates the relative frequency of CpGs and the observed/expected ratio of CpGs present in the reference genome. Additionally, the function calculates the same for the DNA sequences underlying the given regions. The final enrichment values result by dividing the relative frequency of CpGs (or the observed/expected value, respectively) of the regions by the relative frequency of CpGs (or the observed/expected value, respectively) of the reference genome.

Usage

```
MEDIPS.CpGenrich(file=NULL, BSgenome=NULL, extend=0, shift=0, uniq=TRUE, chr.select=NULL, paired=F)
```

Arguments

file	Path and file name of the input data
BSgenome	The reference genome name as defined by BSgenome
extend	defines the number of bases by which the region will be extended before the genome vector is calculated. Regions will be extended along the plus or the minus strand as defined by their provided strand information.
shift	As an alternative to the extend parameter, the shift parameter can be specified. Here, the reads are not extended but shifted by the specified number of nucleotides with respect to the given strand information. One of the two parameters extend or shift has to be 0.
uniq	MEDIPS will replace all reads which map to exactly the same start and end positions by only one representative, if uniq=TRUE
chr.select	only data at the specified chromosomes will be processed.
paired	option for paired end reads

Value

regions.CG	the number of CpGs within the regions
regions.C	the number of Cs within the regions
regions.G	the number of Gs within the regions
regions.relH	the relative frequency of CpGs within the regions
regions.GoGe	the observed/expected ratio of CpGs within the regions

genome.CG	the numbe of CpGs within the reference genome
genome.C	the number of Cs within the reference genome
genome.G	the number of Gs within the reference genome
genome.relH	the relative frequency of CpGs within the reference genome
genome.GoGe	the observed/expected ratio of CpGs within the reference genome
enrichment.score.relH	regions.relH/genome.relH
enrichment.score.GoGe	regions.GoGe/genome.GoGe

Author(s)

Joern Dietrich and Matthias Lienhard

Examples

```
library(MEDIPSData)
library("BSgenome.Hsapiens.UCSC.hg19")
bam.file.hESCs.Rep1.MeDIP = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")

#er=MEDIPS.CpGenrich(file=bam.file.hESCs.Rep1.MeDIP, BSgenome="BSgenome.Hsapiens.UCSC.hg19", chr.select="chr22")
```

MEDIPS.createROIset *Creates a MEDIPS ROI SET by reading a suitable input file*

Description

Reads the input file and calculates the short read coverage (counts) for the specified regions of interest(ROI). After reading of the input file, the MEDIPS ROI SET contains information about the input file name, the dependent organism, the chromosomes included in the input file, the length of the included chromosomes (automatically loaded), the number of regions, and a GRange object of the ROIs.

Usage

```
MEDIPS.createROIset(file=NULL, ROI=NULL, extend=0, shift=0, bn=1, BSgenome=NULL, uniq=TRUE, chr.select="chr22")
```

Arguments

file	Path and file name of the input data
ROI	Data.frame with columns "chr", "start", "end" and "name" of regions of interest
extend	defines the number of bases by which the region will be extended before the genome vector is calculated. Regions will be extended along the plus or the minus strand as defined by their provided strand information.

shift	As an alternative to the extend parameter, the shift parameter can be specified. Here, the reads are not extended but shifted by the specified number of nucleotides with respect to the given strand information. One of the two parameters extend or shift has to be 0.
bn	Number of bins per ROI
BSgenome	The reference genome name as defined by BSgenome
uniq	MEDIPS will replace all reads which map to exactly the same start and end positions by only one representative, if uniq=TRUE
chr.select	only data at the specified chromosomes will be processed.
paired	option for paired end reads
sample_name	name of the sample to be stored with the MEDIPSRoi SET.
bwa	Indicates, if the alignment file has been generated by bwa (default=FALSE). Enabling bwa allows that the first mate of pairs can be the 'left' or the 'right' mate.

Value

An object of class MEDIPSRoiSet.

Author(s)

Lukas Chavez and Matthias Lienhard

Examples

```
library("BSgenome.Hsapiens.UCSC.hg19")
bam.file.hESCs.Rep1.MeDIP = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")
rois=data.frame(chr=c("chr22","chr22"), start=c(19136001, 19753401), stop=c(19136200, 19753500), ID=c("ID_1", "ID_2"))
MSet=MEDIPS.createROIset(file=bam.file.hESCs.Rep1.MeDIP,ROI=rois, BSgenome="BSgenome.Hsapiens.UCSC.hg19", extend=0)
```

MEDIPS.createSet

Creates a MEDIPS SET by reading a suitable input file

Description

Reads the input file and calculates genome wide short read coverage (counts) at the specified window size. After reading of the input file, the MEDIPS SET contains information about the input file name, the dependent organism, the chromosomes included in the input file, the length of the included chromosomes (automatically loaded), and the number of regions.

Usage

```
MEDIPS.createSet(file=NULL, extend=0, shift=0, window_size=300, BSgenome=NULL, uniq=TRUE, chr.select=)
```

Arguments

file	Path and file name of the input data
BSgenome	The reference genome name as defined by BSgenome
extend	defines the number of bases by which the region will be extended before the genome vector is calculated. Regions will be extended along the plus or the minus strand as defined by their provided strand information.
shift	As an alternative to the extend parameter, the shift parameter can be specified. Here, the reads are not extended but shifted by the specified number of nucleotides with respect to the given strand information. One of the two parameters extend or shift has to be 0.
uniq	MEDIPS will replace all reads which map to exactly the same start and end positions by only one representative, if uniq=TRUE
chr.select	only data at the specified chromosomes will be processed.
window_size	defines the genomic resolution by which short read coverage is calculated.
paired	option for paired end reads
sample_name	name of the sample to be stored with the MEDIPS SET.
bwa	Indicates, if the alignment file has been generated by bwa (default=FALSE). Enabling bwa allows that the first mate of pairs can be the 'left' or the 'right' mate.

Value

An object of class MEDIPSset.

Author(s)

Lukas Chavez and Mathias Lienhard

Examples

```
library("BSgenome.Hsapiens.UCSC.hg19")
bam.file.hESCs.Rep1.MeDIP = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")

MSet=MEDIPS.createSet(file=bam.file.hESCs.Rep1.MeDIP, BSgenome="BSgenome.Hsapiens.UCSC.hg19", chr.select="chr22")
```

MEDIPS.exportWIG *Exports count, rpkm, or sequence pattern densities into a wiggle file.*

Description

The function allows for exporting the calculated methylation values (counts or rpkm) or sequence pattern densities from a MEDIPS or COUPLING SET into a wiggle (WIG) file. The wiggle file will contain values for all genomic windows of the genome/coupling vector and can be used for data visualization using appropriate genome browsers. Either a MEDIPS SET (parameter MSet) or a COUPLING SET (parameter CSet) has to be given.

Usage

```
MEDIPS.exportWIG(Set=NULL, CSet=NULL, file=NULL, format="rpkm", descr="")
```

Arguments

Set	has to be a MEDIPS SET object. Required when the parameter 'format' is 'count', 'rpkm', or 'rms'.
CSet	has to be a COUPLING SET object. Required when the parameter 'format' is 'pdensity' or 'rms'.
file	defines the name of the exported file
format	if set to "count", there must be a MEDIPS SET at 'Set'. The number of overlapping (extended) short reads per window will be exported. if set to "rpkm", there must be a MEDIPS SET at 'Set'. The rpkm values will be exported (default). If set to "pdensity", there must be a COUPLING SET at 'CSet'. The pattern densities (counts per window) will be exported (parameter Set will be ignored). If set to 'rms', there must be a MEDIPS SET at 'Set' and a corresponding COUPLING SET at 'CSet'. The CpG density normalized methylatin estimates will be exported.
descr	the exported wiggle file will include a track name and description that will be visualized by the utilized genome browser. Both, track name and description will be the same as defined here.

Value

the funtion exports the specified data from the MEDIPS or COUPLING SET into the stated file

Author(s)

Lukas Chavez

Examples

```
library("BSgenome.Hsapiens.UCSC.hg19")

bam.file.hESCs.Rep1.MeDIP = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPData")
MSet=MEDIPS.createSet(file=bam.file.hESCs.Rep1.MeDIP, BSgenome="BSgenome.Hsapiens.UCSC.hg19", chr.select="chr22")

MEDIPS.exportWIG(Set=MSet, file="hESCs.Rep1.wig", format="rpkm", descr="hESCs.Rep1")
```

MEDIPS.getAnnotation *Function to fetch annotations from biomaRt.*

Description

The function receives predefined annotations from ensembl biomaRt for subsequent annotation of a result table.

Usage

```
MEDIPS.getAnnotation(host="www.biomart.org", dataset=c("hsapiens_gene_ensembl", "mmusculus_gene_ensembl"))
```

Arguments

host	BioMart database host you want to connect to. For current ensembl version, use "www.biomart.org". For other versions, set to the respective archive host, e.g. "may2012.archive.ensembl.org" for Ensembl 67 Please ensure that the ensembl version is compatible to the used genome version.
dataset	The dataset you want to use. To see the different datasets available within a biomaRt you can e.g. do: <code>mart = useMart('ensembl')</code> , followed by <code>listDatasets(mart)</code> .
annotation	Type of annotation you want to retrieve. You can select "EXON" for exonic regions, "GENE" for gene regions including introns and "TSS" for regions at the transcripion start site.
tssSz	Defines the TSS region: start and end position relative to the strand and position of the transcript.
chr	Chromosome names for which the annotations should be filtered.

Value

The MEDIPS.getAnnotation function returns a list of annotation tables where each table consists of

id	the id of the annotation
chr	the chromosome of the annotation
start	the start position (5') of the annotation
end	the end position (3') of the annotation

Author(s)

Joern Dietrich and Matthias Lienhard

Examples

```
#homo sapiens, current ensembl version
#annotation_hs = MEDIPS.getAnnotation(dataset="hsapiens_gene_ensembl", annotation="TSS", chr=c("chr22"), tssSz=c(
#mus musculus, ensembl version 58 (mm9)
annotation_mm9 = MEDIPS.getAnnotation(host="may2010.archive.ensembl.org", dataset="mmusculus_gene_ensembl", anno
```

MEDIPS.mergeFrames	<i>Merges genomic coordinates of neighboring windows into one super-sized window</i>
--------------------	--

Description

After having filtered the result table returned by the MEDIPS.meth function using the MEDIPS.selectSig function, there might be neighboring significant frames. For these cases it is worthwhile to merge neighbouring regions into one supersized frame.

Usage

```
MEDIPS.mergeFrames(frames=NULL, distance=1)
```

Arguments

frames	is a filtered result table received by the MEDIPS.selectSig function.
distance	allows an according number of bases as a gap between neighboring significant windows to be merged. The default value is 1 in order to merge adjacent windows.

Value

The remaining distinct frames are represented only by their genomic coordinates within the returned results table

chromosome	the chromosome of the merged frame
start	the start position of the merged frame
stop	the stop position of the merged frame
ID	a numbered ID of the merged frame

The result table does not contain any merged significant values.

Author(s)

Lukas Chavez

Examples

```
regions=as.data.frame(list(chr=c("chr22", "chr22"), start=c(1001, 1501), stop=c(1500,1750)))
regions.merged=MEDIPS.mergeFrames(regions)

regions.merged
```

MEDIPS.mergeSets *Creates one merged MEDIPS SET out of two.*

Description

A MEDIPS SET contains a genome vector which is the count coverage at genome wide windows. Moreover, the MEDIPS SET stores the total number of reads given for calculating the genome vector. Two MEDIPS SETs can be merged whenever they have been constructed based on the same reference genome, the same set of chromosomes and for the same window size. The returned MEDIPS SET will contain a genome vector where at each window the counts of both given MEDIPS SETs are added. In addition, the total number of reads will be the sum of both given MEDIPS SETs. Please note, several other attributes like the extend or shift value can be different in both of the given MEDIPS SETs and will be empty in the merged MEDIPS SET. The merged MEDIPS SET will not contain any path to a concrete input file anymore and therefore, cannot be used for the MEDIPS.addCNV function anymore.

Usage

```
MEDIPS.mergeSets(MSet1=NULL, MSet2=NULL, name="Merged Set")
```

Arguments

MSet1	A MEDIPS SET object as created by the MEDIPS.createSet function
MSet2	A MEDIPS SET object as created by the MEDIPS.createSet function
name	The new sample name of the merged MEDIPS SET

Author(s)

Lukas Chavez

Examples

```
library(MEDIPSData)
data(hESCs_Input)
data(DE_Input)

merged_Set = MEDIPS.mergeSets(hESCs_Input, DE_Input, name="Merged_input")

merged_Set
```

MEDIPS.meth	<i>Function summarizes coverage profiles for given MEDIPS SETs and allows to calculate differential coverage and copy number variation, if applicable.</i>
-------------	--

Description

The function summarizes coverage profiles (counts, rpkm) for given MEDIPS SETs at the slots MSet1, MSet2, ISet1, and ISet2. In case the parameter MeDIP is set to TRUE and a COUPLING SET was provided at the slot CSet, the function will calculate CpG density normalized methylation profiles (relative methylation score, rms) for the MEDIPS SETs at the slots MSet1 and MSet2. In case two groups of MEDIPS SETs have been provided at MSet1 and MSet2, the function will calculate differential coverage. In case two groups of MEDIPS SETs have been provided at ISet1 and ISet2 and the parameter CNV was set to TRUE, the function will calculate copy number variation. The function allows for processing a variable number of provided MEDIPS SETs and therefore, the returned matrix is of variable length.

Usage

```
MEDIPS.meth(MSet1 = NULL, MSet2 = NULL, CSet = NULL, ISet1 = NULL, ISet2 = NULL, chr = NULL, p.adj="bonferroni")
```

Arguments

MSet1	has to be one or a concatenated list of MEDIPS SET objects (the control replicates)
MSet2	has to be one or a concatenated list of MEDIPS SET objects (the treatment data) or empty
CSet	has to be a COUPLING SET object (must fit the given MEDIPS SET objects with respect to reference genome and represented chromosomes)
ISet1	has to be one or a concatenated list of Input derived MEDIPS SET objects (general Input data or Inputs from the control replicates) or empty
ISet2	has to be one or a concatenated list of Input derived MEDIPS SET objects (Inputs from the treatment replicates) or empty
chr	specify one or several chromosomes (e.g. c("chr1", "chr2")), if only a subset of available chromosomes have to be processed.
p.adj	in order to correct p.values derived from the differential coverage analysis for multiple testing, MEDIPS uses Rs' p.adjust function. Therefore, the following methods are available: holm, hochberg, hommel, bonferroni (default), BH, BY, fdr, none.
diff.method	method for calculating differential coverage. Available methods: ttest (default) and edgeR.
CNV	In case there are INPUT SETs provided at both Input slots (i.e. ISet1 and ISet2), copy number variation will be tested by applying the package DNACopy to the window-wise log-ratios calculated based on the the means per group. By setting

	CNV=F this function will be disabled (default: CNV=TRUE). Please note, there is the function MEDIPS.addCNV which allows to run the CNV analysis on two groups of INPUT SETs using another (typically increased) window size.
MeDIP	This parameter determines, whether for the MEDIPS SETs given at the slots MSet1 and MSet2, CpG density dependent normalization values (rms and prob) will be calculated (default: MeDIP=TRUE).
type	In case diff.method has been set to ttest, this parameter specifies, if differential coverage is calculated based on the rpkm (default) or rms values. This parameter is ignored in case the edgeR method is selected as the underlying model requires count data.
minRowSum	threshold for the sum of counts in a window for the staistical test (default=10).
quantile	This parameter enables quantile normalization on the count matrix prior to calculating differential coverage (default=FALSE).

Value

Chr	the chromosome of the ROI
Start	the start position of the ROI
Stop	the stop position of the ROI
CF	the number of CpGs in the window
*counts	a variable number of columns (according to the number of provided MEDIPS SETs) containing for each set the number of (extended/shifted) reads that overlap with the window.
*rpkm	a variable number of columns (according to the number of provided MEDIPS SETs) containing for each set the rpkm value of the window.
*rms	optional (if MeDIP=TRUE): a variable number of columns (according to the number of provided MEDIPS SETs) containing for each set the rms value of the window.
*counts	optional (if INPUT SETs given): a variable number of columns (according to the number of provided INPUT SETs) containing for each set the number of (extended/shifted) reads that overlap with the window.
*rpkm	optional (if INPUT SETs given): a variable number of columns (according to the number of provided INPUT SETs) containing for each set the rpkm value of the window.
MSets1.counts.mean	optional (if more than one MEDIPS SET given): the mean count over all MEDIPS SETs at MSet1.
MSets1.rpkm.mean	optional (if more than one MEDIPS SET given): the mean rpkm value over all MEDIPS SETs at MSet1.
MSets1.rms.mean	optional (if more than one MEDIPS SET given): the mean rms value over all MEDIPS SETs at MSet1.
MSets2.counts.mean	optional (if more than one MEDIPS SET given): the mean count over all MEDIPS SETs at MSet2.

MSet2.rpkm.mean	optional (if more than one MEDIPS SET given): the mean rpkm value over all MEDIPS SETs at MSet2.
MSet2.rms.mean	optional (if more than one MEDIPS SET given): the mean rms value over all MEDIPS SETs at MSet2.
ISet1.counts.mean	optional (if more than one INPUT SET given): the mean count over all INPUT SETs at ISet1.
ISet1.rpkm.mean	optional (if more than one INPUT SET given): the mean rpkm value over all INPUT SETs at ISet1.
ISet2.counts.mean	optional (if more than one INPUT SET given): the mean count over all INPUT SETs at ISet2.
ISet2.rpkm.mean	optional (if more than one INPUT SET given): the mean rpkm value over all INPUT SETs at ISet2.
edgeR.logFC	optional (if diff.method=edgeR): log fold change between MSet1 and MSet2 as returned by edgeR.
edgeR.logCPM	optional (if diff.method=edgeR): logCPM between MSet1 and MSet2 as returned by edgeR.
edgeR.p.value	optional (if diff.method=edgeR): p.value as returned by edgeR.
edgeR.adj.p.value	optional (if diff.method=edgeR): adjusted p.value as calculated by the p.adjust function using edgeR's p.values as input.
score.log2.ratio	optional (if diff.method=ttest): log2 fold change between the means of the groups MSet1 and MSet2.
score.p.value	optional (if diff.method=ttest): p.value as returned by the t.test function.
score.adj.p.value	optional (if diff.method=ttest): adjusted p.value as calculated by the p.adjust function using the ttest p.values as input.
score	optional (if diff.method=ttest): $\text{score} = (-\log_{10}(\text{score.p.value}) * 10) * \log(\text{score.log2.ratio})$
CNV.log2.ratio	optional (if two INPUT SETs given and CNV=TRUE): the log2 ratio for segments as calculated by the DNACopy package.

Author(s)

Lukas Chavez, Matthias Lienhard, Joern Dietrich

Examples

```
library(MEDIPSData)
data(hESCs_MeDIP)
data(DE_MeDIP)
data(hESCs_Input)
```

```
data(DE_Input)
data(CS)
```

```
resultTable = MEDIPS.meth(MSet1 = hESCs_MeDIP, MSet2 = DE_MeDIP, CSet = CS, ISet1 = hESCs_Input, ISet2 = DE_Input)
```

```
MEDIPS.plotCalibrationPlot
```

Creates the calibration plot.

Description

Visualizes the dependency between MeDIP-seq signals and CpG densities together with the calibration curve and the results of the linear modelling.

Usage

```
MEDIPS.plotCalibrationPlot(MSet=NULL, ISet=NULL, CSet=NULL, plot_chr="all", rpkm=T, main="Calibration")
```

Arguments

MSet	a MEDIPS SET object
ISet	an INPUT SET (i.e. a MEDIPS SET created from Input sequencing data)
CSet	an according COUPLING SET object
plot_chr	default="all". It is recommended to call a graphics device (e.g. png("calibrationPlot.png")) before calling the plot command, because R might not be able to plot the full amount of data in reasonable time.
rpkm	can be either TRUE or FALSE. If set to TRUE, the methylation signals will be transformed into rpkm before plotted. The coupling values remain untouched. It is necessary to set rpkm=T, if both, a MSet and an ISet are given and plotted at the same time.
main	The title of the calibration plot.
xrange	The signal range of the calibration curve typically falls into a low signal range. By setting the xrange parameter to TRUE, the calibration plot will visualize the low signal range only.

Value

The calibration plot will be visualized.

Author(s)

Lukas Chavez, Matthias Lienhard

Examples

```
library(MEDIPSData)
data(hESCs_MeDIP)
data(CS)
```

```
MEDIPS.plotCalibrationPlot(CSet=CS, main="Calibration Plot", MSet=hESCs_MeDIP[[1]], plot_chr="chr22", rpkm=TRUE)
```

MEDIPS.plotSaturation *Function plots the results of the MEDIPS.saturationAnalysis function.*

Description

The results of the saturation analysis will be visualized by the function.

Usage

```
MEDIPS.plotSaturation(saturationObj = NULL, main="Saturation analysis")
```

Arguments

saturationObj	The saturation results object returned by the MEDIPS.saturationAnalysis function
main	The title of the coverage plot.

Value

The coverage plot will be visualized.

Author(s)

Lukas Chavez

Examples

```
library(MEDIPSData)
library(BSgenome.Hsapiens.UCSC.hg19)
bam.file.hESCs.Rep1.MeDIP = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")
```

```
sr=MEDIPS.saturation(file=bam.file.hESCs.Rep1.MeDIP, BSgenome="BSgenome.Hsapiens.UCSC.hg19", uniq=TRUE, extend=TRUE)
MEDIPS.plotSaturation(saturationObj = sr, main="Saturation analysis")
```

MEDIPS.plotSeqCoverage

Function plots the results of the MEDIPS.seqCoverage function.

Description

The results of the sequence pattern coverage analysis will be visualized in two possible ways.

Usage

```
MEDIPS.plotSeqCoverage(seqCoverageObj=NULL, main=NULL, type="pie", cov.level = c(0,1,2,3,4,5), t="In
```

Arguments

seqCoverageObj	The coverage results object returned by the MEDIPS.seqCoverage function.
main	The title of the coverage plot.
type	there are two types of visualization. The pie chart (default) illustrates the fraction of CpGs covered by the given reads at different coverage level (see also the parameter cov.level). As an alternative, a histogram over all coverage level can be plotted ("hist").
cov.level	The pie chart illustrates the fraction of CpGs covered by the given reads according to their coverage level. The visualized coverage levels can be adjusted by the cov.level parameter.
t	specifies the maximal coverage depth to be plotted, if type="hist"

Value

The sequence pattern coverage plot will be visualized.

Author(s)

Lukas Chavez

Examples

```
library(MEDIPSData)
library(BSgenome.Hsapiens.UCSC.hg19)
bam.file.hESCs.Rep1.MeDIP = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")

cr=MEDIPS.seqCoverage(file=bam.file.hESCs.Rep1.MeDIP, pattern="CG", BSgenome="BSgenome.Hsapiens.UCSC.hg19", chr

MEDIPS.plotSeqCoverage(seqCoverageObj=cr, main="Sequence pattern coverage", type="pie", cov.level = c(0,1,2,3,4,
```

MEDIPS.saturation	<i>Function calculates the saturation/reproducibility of the provided IP-Seq data.</i>
-------------------	--

Description

The saturation analysis addresses the question, whether the number of short reads is sufficient to generate a saturated and reproducible coverage profile of the reference genome. The main idea is that an insufficient number of short reads will not result in a saturated methylation profile. Only if there is a sufficient number of short reads, the resulting genome wide coverage profile will be reproducible by another independent set of a similar number of short reads.

Usage

```
MEDIPS.saturation(file=NULL, BSgenome=NULL, nit=10, nrnt=1, empty_bins=TRUE, rank=FALSE, extend=0, sh
```

Arguments

file	Path and file name of the IP data
BSgenome	The reference genome name as defined by BSgenome
nit	defines the number of subsets created from the full sets of available regions (default=10)
nrnt	methods which randomly select data entries may be processed several times in order to obtain more stable results. By specifying the nrnt parameter (default=1) it is possible to run the saturation analysis several times. The final results returned to the saturation results object are the averaged results of each random iteration step.
empty_bins	can be either TRUE or FALSE (default TRUE). This parameter effects the way of calculating correlations between the resulting genome vectors. A genome vector consists of concatenated vectors for each included chromosome. The size of the vectors is defined by the bin_size parameter. If there occur genomic bins which contain no overlapping regions, neither from the subsets of A nor from the subsets of B, these bins will be neglected when the parameter is set to FALSE.
rank	can be either TRUE or FALSE (default FALSE). This parameter also effects the way of calculating correlations between the resulting genome vectors. If rank is set to TRUE, the correlation will be calculated for the ranks of the windows instead of considering the counts (Spearman correlation). Setting this parameter to TRUE is a more robust approach that reduces the effect of possible occurring outliers (these are windows with a very high number of overlapping regions) to the correlation.
extend	defines the number of bases by which the region will be extended before the genome vector is calculated. Regions will be extended along the plus or the minus strand as defined by their provided strand information. Please note, the extend and shift parameter are mutual exclusive.

shift	defines the number of bases by which the region will be shifted before the genome vector is calculated. Regions will be shifted along the plus or the minus strand as defined by their provided strand information. Please note, the extend and shift parameter are mutual exclusive.
window_size	defines the size of genome wide windows and therefore, the size of the genome vector.
uniq	if uniq=TRUE (default), all reads with exactly the same start and end positions will be replaced by one representative before the saturation analysis is performed.
chr.select	specify a subset of chromosomes for which the saturation analysis is performed.
paired	option for paired end reads

Value

distinctSets	Contains the results of each iteration step (row-wise) of the saturation analysis. The first column is the number of considered regions in each set, the second column is the resulting pearson correlation coefficient when comparing the two independent genome vectors.
estimation	Contains the results of each iteration step (row-wise) of the estimated saturation analysis. The first column is the number of considered regions in each set, the second column is the resulting pearson correlation coefficient when comparing the two independent genome vectors.
distinctSets	the total number of available regions
maxEstCor	contains the best pearson correlation (second column) obtained by considering the artificially doubled set of reads (first column)
distinctSets	contains the best pearson correlation (second column) obtained by considering the total set of reads (first column)

Author(s)

Lukas Chavez

Examples

```
library(MEDIPSData)
library(BSgenome.Hsapiens.UCSC.hg19)
bam.file.hESCs.Rep1.MeDIP = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")

sr=MEDIPS.saturation(file=bam.file.hESCs.Rep1.MeDIP, BSgenome="BSgenome.Hsapiens.UCSC.hg19", uniq=TRUE, extend=
```

MEDIPS.selectROIs	<i>Selects row-wise subsets of a result table as returned by the MEDIPS.meth function.</i>
-------------------	--

Description

MEDIPS provides the functionality to select subsets of the result matrix returned by the MEDIPS.meth function according to any given set of regions of interest (ROIs).

Usage

```
MEDIPS.selectROIs(results=NULL, rois=NULL, columns=NULL, summarize=NULL)
```

Arguments

results	a result table as returned by the function MEDIPS.meth
rois	A matrix containing genomic coordinates of any regions of interest.
columns	Only selected columns will be returned as determined by the columns parameter. It is possible to specify one or more concrete column names, please see an example below.
summarize	By setting summarize=NULL (default) all windows included in the genomic ranges of the given ROIs will be returned. As an alternative, it is possible to calculate mean values over the individual windows included in each ROI (summarize = "avg"), or to select only the most significant window within each given ROI (summarize="minP").

Author(s)

Lukas Chavez, Matthias Lienhard

Examples

```
library(MEDIPSData)
data(resultTable)

rois=data.frame(chr=c("chr22","chr22"), start=c(19136001, 19753401), stop=c(19136200, 19753500), ID=c("ID_1", "ID_2"))
columns=names(resultTable)[grep("counts|rpkm|logFC",names(resultTable))]
s = MEDIPS.selectROIs(results=resultTable, rois=rois, columns=columns, summarize=NULL)
```

MEDIPS.selectSig	<i>Selects windows which show significant differential coverage between two MEDIPS SETs from a resultTable (as returned by the function MEDIPS.meth).</i>
------------------	---

Description

Based on the results table returned by the MEDIPS.meth function, the function selects windows which show significant differential coverage between the two groups of MEDIPS SETs. Selection of significant windows follows according to the specification of the available parameters.

Usage

```
MEDIPS.selectSig(results=NULL, p.value=0.01, adj=T, ratio=NULL, bg.counts=NULL, CNV=F)
```

Arguments

results	specifies the result table derived from the MEDIPS.meth function.
p.value	this is the p.value threshold as calculated either by the ttest or edgeR method
adj	this parameter specifies whether the p.value or the adjusted p.values is considered
ratio	this parameter sets an additional threshold for the ratio where the ratio is either score.log2.ratio or edgeR.logFC depending on the previously selected method. Please note, the specified value will be transformed into log2 internally.
bg.counts	as an additional filter parameter, it is possible to require a minimal number of reads per window in at least one of the MEDIPS SET groups. For this, the mean of counts per group is considered. The parameter bg.counts can either be a concrete integer or an appropriate column name of the result table. By specifying a column name, the 0.95 quantile of the according genome wide count distribution is determined and used as a minimal background threshold (please note, only count columns are reasonable).
CNV	The information on CNVs present in the samples of interest can be used for correcting differential coverage observed in the corresponding IP data (e.g. MeDIP or ChIP data). In case Input data has been provided for both conditions, MEDIPS is capable of calculating genome wide CNV ratios by employing the package DNACopy. In case the parameter CNV is set to TRUE, MEDIPS will subtract the CNV ratio from the IP ratio. Subsequently, only genomic windows having a CNV corrected IP ratio higher than the specified ratio threshold (specification of the ratio parameter is required in this case) will be considered as windows with sufficient differential IP coverage.

Author(s)

Lukas Chavez, Matthias Lienhard

Examples

```
library(MEDIPSData)
data(resultTable)

sig = MEDIPS.selectSig(results=resultTable, p.value=0.05, adj=TRUE, ratio=NULL, bg.counts=NULL, CNV=FALSE)
```

MEDIPS.seqCoverage *The function identifies the number of CpGs (or any other predefined sequence pattern) covered by the given short reads.*

Description

The main idea of the sequence pattern coverage analysis is to test the number of CpGs (or any other predefined sequence pattern) covered by the given short reads and to test the depth of coverage.

Usage

```
MEDIPS.seqCoverage(file = NULL, BSgenome = NULL, pattern = "CG", extend = 0, shift = 0, uniq = TRUE, chr.select = NULL, paired = FALSE)
```

Arguments

file	Path and file name of the input data
BSgenome	The reference genome name as defined by BSgenome
pattern	defines the sequence pattern, e.g. CG for CpGs.
extend	defines the number of bases by which the region will be extended before the genome vector is calculated. Regions will be extended along the plus or the minus strand as defined by their provided strand information. Please note, the extend and shift parameter are mutual exclusive.
shift	defines the number of bases by which the region will be shifted before the genome vector is calculated. Regions will be shifted along the plus or the minus strand as defined by their provided strand information. Please note, the extend and shift parameter are mutual exclusive.
uniq	if uniq=TRUE (default), all reads with exactly the same start and end positions will be replaced by one representative before the saturation analysis is performed.
chr.select	specify a subset of chromosomes for which the saturation analysis is performed.
paired	option for paired end reads

Author(s)

Lukas Chavez

Examples

```
library(MEDIPSData)
library(BSgenome.Hsapiens.UCSC.hg19)
bam.file.hESCs.Rep1.MeDIP = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")

cr = MEDIPS.seqCoverage(file=bam.file.hESCs.Rep1.MeDIP, BSgenome="BSgenome.Hsapiens.UCSC.hg19", pattern="CG", e
```

MEDIPS.setAnnotation *Function to annotate a matrix of genomic coordinates (i.e. a result table) by a given annotation object.*

Description

The function appends any annotation IDs included in the given annotation object to the given regions object. An annotation object can be retrieved by the MEDIPS.getAnnotation function and the regions object is typically a (filtered) result table as returned by the MEDIPS.meth function. An annotation ID is appended to a genomic region if their genomic coordinates overlap by at least one base. There will be as many columns added to the regions object as overlapping annotations exist in the annotation object.

Usage

```
MEDIPS.setAnnotation(regions, annotation, cnv=F)
```

Arguments

regions	a matrix that contains row-wise genomic regions, e.g. as a result of the MEDIPS.meth function.
annotation	the annotation data object contains the genomic coordinates of annotations. An annotation object can be e.g. retrieved by the MEDIPS.getAnnotation function.
cnv	the MEDIPS.setAnnotation function is also internally used by the MEDIPS.addCNV function which automatically sets this parameter to TRUE. Otherwise cnv should be set to FALSE.

Value

The provided result object with added columns containing overlapping annotations.

Author(s)

Joern Dietrich, Matthias Lienhard

Examples

```

library(MEDIPsData)
data(resultTable)

sig = MEDIPs.selectSig(results=resultTable, p.value=0.05, adj=TRUE, ratio=NULL, bg.counts=NULL, CNV=FALSE)
sig = MEDIPs.mergeFrames(frames=sig, distance=1)
ens_gene = MEDIPs.getAnnotation( annotation="GENE",chr="chr22")
sig = MEDIPs.setAnnotation(regions=sig, annotation=ens_gene)

```

MEDIPsroiSet-class *MEDIPsroiSet class and internal functions*

Description

MEDIPsroiSet class is used in the MEDIPs library in order to store and extract objects and information of the specified regions of interest (ROI) from the input file as well as parameter settings specified during the workflow.

Objects from the Class

Objects of the classes contain information about the provided short reads, MeDIP raw/count signals, and further parameter settings. A MEDIPs ROI SET object is created by the MEDIPs.createROIset() function. According slots will be filled during the workflow.

Slots

genome_name: Object of class "character" : the reference genome
chr_names: Object of class "character" : the names of the chromosomes included within the MEDIPs ROI SET
chr_lengths: Object of class "numeric" : the lengths of the chromosomes included within the MEDIPs ROI SET
sample_name: Object of class "character" : the name of the input file
path_name: Object of class "character" : the path to the input file
number_regions: Object of class "numeric" : the total number of included regions
genome_count: Object of class "numeric" : the raw MeDIP-seq signals at the bins
extend: Object of class "numeric" : the length of the reads after extension
shifted: Object of class "numeric" : the number of bases by which the reads are shifted along the sequencing direction
uniq: Object of class "logical" : determines if reads mapping to exactly the same genomic position should be replaced by only on representative
ROI: Object of class "GRanges": the genomic positions of the regions of interest
bin_number: Object of class "numeric": the number of bins per region

Methods

- genome_name** signature(object = "MEDIPsroiSet"): extracts the reference genome of the MEDIPS ROI SET
- bin_number** signature(object = "MEDIPsroiSet"): extracts the number of bins per ROI the bin_number slot of the MEDIPS ROI SET
- chr_names** signature(object = "MEDIPsroiSet"): extracts the names of the chromosomes included within the MEDIPS ROI SET
- chr_lengths** signature(object = "MEDIPsroiSet"): extracts the length of the chromosomes included within the MEDIPS ROI SET
- sample_name** signature(object = "MEDIPsroiSet"): extracts the name of the input file
- path_name** signature(object = "MEDIPsroiSet"): extracts the path to the input file
- number_regions** signature(object = "MEDIPsroiSet"): extracts the total number of included regions
- genome_count** signature(object = "MEDIPsroiSet"): extracts the raw MeDIP-Seq signals at the genomic bins
- extend** signature(object = "MEDIPsroiSet"): extracts the number of bases by which the regions are extended
- show** signature(object = "MEDIPsroiSet"): prints a summary of the MEDIPS SET object content
- shifted** signature(object = "MEDIPsroiSet"): extracts the number of bases by which the regions are shifted
- uniq** signature(object = "MEDIPsroiSet"): extracts the specified value for the uniq parameter
- rois** signature(object = "MEDIPsroiSet"): extracts the GRange object containing the regions of interest
- MEDIPS.calibrationCurve** signature(MSet = "MEDIPsroiSet", CSet="COUPLINGset"): internal function for calculating the calibration curve
- MEDIPS.negBin** signature(MSet="MEDIPsroiSet", CSet="COUPLINGset"): internal function for calculating methylation probabilities with respect to CpG density dependent negative binomial distributions
- MEDIPS.pois** signature(MSet="MEDIPsroiSet", CSet="COUPLINGset"): internal function for calculating methylation probabilities with respect to CpG density dependent poisson distributions
- MEDIPS.rms** signature(MSet="MEDIPsroiSet", CSet="COUPLINGset"): internal function for calculating relative methylation scores

Author(s)

Lukas Chavez, Joern Dietrich

Examples

```
showClass("MEDIPsroiSet")
```

MEDIPSset-class *MEDIPSset class and internal functions*

Description

MEDIPSset class is used in the MEDIPS library in order to store and extract objects and information from the input file as well as parameter settings specified during the workflow.

Objects from the Class

Objects of the classes contain information about the provided short reads, MeDIP raw/count signals, and further parameter settings. A MEDIPS SET object is created by the MEDIPS.genomeVector() function. According slots will be filled during the workflow.

Slots

genome_name: Object of class "character" : the refernce genome
 window_size: Object of class "numeric" : the window size for the genome vector
 chr_names: Object of class "character" : the names of the chromosomes included within the MEDIPS/COUPLING SET
 chr_lengths: Object of class "numeric" : the lengths of the chromosomes included within the MEDIPS/COUPLING SET
 sample_name: Object of class "character" : the name of the input file
 path_name: Object of class "character" : the path to the input file
 number_regions: Object of class "numeric" : the total number of included regions
 genome_count: Object of class "numeric" : the raw MeDIP-seq signals at the genomic bins
 extend: Object of class "numeric" : the length of the regions after extension
 shifted: Object of class "numeric" : the number of bases by which the reads are shifted along the sequencing direction
 uniq: Object of class "logical" : determines if reads mapping to exactly the same genomic position should be replaced by only one representative

Methods

genome_name signature(object = "MEDIPSset"): extracts the reference genome of the MEDIPS SET
window_size signature(object = "MEDIPSset"): extracts the window size from the bin_size slot of the MEDIPS SET
chr_names signature(object = "MEDIPSset"): extracts the names of the chromosomes included within the MEDIPS SET
chr_lengths signature(object = "MEDIPSset"): extracts the length of the chromosomes included within the MEDIPS SET

fragmentLength signature(object = "MEDIPSset"): extracts the estimated fragment length of the DNA fragments

sample_name signature(object = "MEDIPSset"): extracts the name of the input file

path_name signature(object = "MEDIPSset"): extracts the path to the input file

number_regions signature(object = "MEDIPSset"): extracts the total number of included regions

genome_count signature(object = "MEDIPSset"): extracts the raw MeDIP-Seq signals at the genomic bins

extend signature(object = "MEDIPSset"): extracts the number of bases by which the regions are extended

show signature(object = "MEDIPSset"): prints a summary of the MEDIPS SET object content

shifted signature(object = "MEDIPSset"): extracts the number of bases by which the regions are shifted

uniq signature(object = "MEDIPSset"): extracts the specified value for the uniq parameter

MEDIPS.GenomicCoordinates signature(object = "MEDIPSset"): internal function for calculating coordinates for the genomic bins

MEDIPS.readRegionsFile signature(object = "MEDIPSset"): internal function for reading short read information

MEDIPS.calibrationCurve signature(object = "MEDIPSset"): internal function for calculating the calibration curve

MEDIPS.cnv signature(object = "MEDIPSset"): internal function for calculating CNVs in case two groups of INPUT SETs have been provided to the MEDIPS.meth function

MEDIPS.diffMeth signature(object = "MEDIPSset"): internal function for calculating differential coverage in case two groups of MEDIPS SETs have been provided to the MEDIPS.meth function

MEDIPS.getPositions signature(object = "MEDIPSset"): internal function for receiving genomic coordinates of a given sequence pattern (e.g. CG)

MEDIPS.rms signature(object = "MEDIPSset"): internal function for calculating relative methylation scores

matNnotNA signature(object = "MEDIPSset"): internal function for vectorized calculation of the t.test

matMin signature(object = "MEDIPSset"): internal function for vectorized calculation of the t.test

matDiff signature(object = "MEDIPSset"): internal function for vectorized calculation of the t.test

matMax signature(object = "MEDIPSset"): internal function for vectorized calculation of the t.test

matMean signature(object = "MEDIPSset"): internal function for vectorized calculation of the t.test

matSd signature(object = "MEDIPSset"): internal function for vectorized calculation of the t.test

matTtest signature(object = "MEDIPSset"): internal function for vectorized calculation of the t.test

Author(s)

Lukas Chavez, Joern Dietrich

Examples

```
showClass("MEDIPSset")
```

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