Package ‘qsea’
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Description qsea (quantitative sequencing enrichment analysis) was developed as the successor of the MEDIPS package for analyzing data derived from methylated DNA immunoprecipitation (MeDIP) experiments followed by sequencing (MeDIP-seq). However, qsea provides several functionalities for the analysis of other kinds of quantitative sequencing data (e.g. ChIP-seq, MBD-seq, CMS-seq and others) including calculation of differential enrichment between groups of samples.

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R topics documented:

qsea-package ...................................................... 2
addCNV ............................................................ 3
addContrast .......................................................... 4
addCoverage .......................................................... 5
addEnrichmentParameters ........................................ 6
addLibraryFactors .................................................. 7
QSEA: Quantitative sequencing enrichment analysis and visualization

Description

QSEA (quantitative sequencing enrichment analysis) was developed as the successor of the MEDIPS package for analyzing data derived from methylated DNA immunoprecipitation (MeDIP) experiments followed by sequencing (MeDIP-seq). However, qsea provides functionality for the analysis of other kinds of quantitative sequencing data (e.g. ChIP-seq, MBD-seq, CMS-seq and others) including calculation of differential enrichment between groups of samples.

Author(s)

Matthias Lienhard, Lukas Chavez and Ralf Herwig

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References

addCNV

estimate CNV information and add to qseaSet object

Description

This function adds information on Copy Number Variation (CNV) to the qseaSet object, which is used for normalization. Sample wise CNV information can either be provided, or estimated from input or enrichment sequencing data, by incorporating functions of the HMMcopy package.

Usage

addCNV(qs, file_name, window_size=1000000, paired=FALSE, fragment_length, cnv, mu=log2(c(1/2, 2/3, 1, 3/2, 2, 3)), normal_idx, plot_dir, MeDIP=FALSE, parallel=FALSE)

Arguments

qs the qseaSet object

cnv pre-computed CNV information for each sample. If provided, the following parameters are ignored

file_name column name of the sample table for the sequencing files, from which CNV information are computed

window_size window size for CNV analysis

paired are files in file_name column paired end

fragment_length for single end sequencing, provide the average fragment length

mu a priori CNV levels of different states, parameter passed to HMMcopy

normal_idx index of samples which are assumed to be CNV free. The median of these samples serves as "normal" CNV reference level, and CNV are computed relative to this reference level. By default, QSEA looks for samples with "normal" or "control" in its name.

plot_dir If provided, detail CNV plots for each chromosome and each sample are created in the provided directory

MeDIP If set TRUE, QSEA assumes that provided files are methylation enriched sequencing data. In this case, only fragments without CpG dinucleotides are considered. This option allows QSEA to infer CNV information from MeDIP or MDB seq experiments directly

parallel Switch for parallel computing, using BiocParallel

Value

The qseaSet object, extended by the CNV information

Author(s)

Mathias Lienhard
See Also

HMMsegment

Examples

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

bam_hESCs_1 = system.file("extdata", 
  "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")
bam_hESCs_2 = system.file("extdata", 
  "hESCs.MeDIP.Rep2.chr22.bam", package="MEDIPSData")
sample_table=data.frame(sample_name=paste0("hESCs_", 1:2), 
  file_name=c(bam_hESCs_1,bam_hESCs_2),
  group=rep("hESC",2), stringsAsFactors=FALSE)
qseaSet=createQseaSet(sampleTable=sample_table, 
  BSgenome="BSgenome.Hsapiens.UCSC.hg19", 
  chr.select="chr22", 
  window_size=500)

# this is an example for computing CNVs from MeDIP data. A very limited example 
# however, since the samples do not contain CNVs.
qseaSet=addCNV(qseaSet, fragment_length=300, file_name="file_name", MeDIP=TRUE, 
  window_size=1000000)
```

---

### addContrast

**fit GLMs to reduced model and test for significance**

**Description**

This function fits negative binomial GLMs to reduced models defined either by the "contrast" parameter, or by one or several model coefficients (specified by "coef" parameter) set to zero. Subsequently, a likelihood ratio test is applied, to identify windows significantly dependent on the tested coefficient.

**Usage**

```r
addContrast(qs,glm,contrast,coef,name,verbose=TRUE )
```

**Arguments**

- **qs**: a qseaSet object
- **glm**: a qseaGLM object
- **contrast**: numeric vector specifying a contrast of the model coefficients. This contrast can for example be defined using limma::makeContrasts()
- **coef**: alternatively defines the contrast by coefficient(s) of the model tested to be equal to zero.
- **name**: short descriptive name for the contrast (as "TvsN"), used for examples in columns of result tables
- **verbose**: more messages that document the process
Value

This function returns the qseaGLM object, extended by the fitted coefficients of the reduced GLMs, as well as the test statistics. Note that one qseaGLM object can contain several contrasts.

Author(s)

Mathias Lienhard

See Also

limma::makeContrasts(), fitNBglm(), isSignificant()

Examples

qs=getExampleQseaSet()
design=model.matrix(~group, getSampleTable(qs))
TvN_glm=fitNBglm(qs, design, norm_method="beta")
TvN_glm=addContrast(qs,TvN_glm, coef=2, name="TvN")

addCoverage

Import sequencing data

Description

This function imports the alignment files (in sam/bam format) and counts the reads per genomic window or directly imports coverage files (in wiggle/bigwiggle format)

Usage

addCoverage(qs, fragment_length, uniquePos=TRUE, minMapQual=1, paired=FALSE, parallel=FALSE)

Arguments

qs qseaSet object, e.g. produced by the createQseaSet() function
fragment_length For single end data, provide the expected fragment length
paired If set to TRUE, data is considered to be paired end sequencing, and the actual fragments size is used.
uniquePos If set to TRUE, fragments with same position and orientation are considered to be PCR duplicates and replaced by one representative.
minMapQual The minimal mapping quality for reads to be considered. Note that the definition of mapping quality depends on the alignment tool.
parallel Switch for parallel computing, using BiocParallel

Details

The coverage is imported from the files specified in the file_name column of the sample table, provided for the createQseaSet() function. In case of alignment files, the reads are counted for the window at the center of the sequencing fragment. For single end data, Filetypes is detected automatically from the file suffix.
addEnrichmentParameters

Value

The function returns the qseaSet object, extended by the number of reads per window for all samples

Author(s)

Mathias Lienhard

See Also

crateQseaSet

Examples

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

bam_hESCs_1 = system.file("extdata", 
    "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")
bam_hESCs_2 = system.file("extdata", 
    "hESCs.MeDIP.Rep2.chr22.bam", package="MEDIPSData")
sample_table=data.frame(sample_name=paste0("hESCs_", 1:2),
    file_name=c(bam_hESCs_1,bam_hESCs_2),
    group=rep("hESC",2), stringsAsFactors=FALSE)
qseaSet=createQseaSet(sampleTable=sample_table, 
    BSgenome="BSgenome.Hsapiens.UCSC.hg19", 
    chr.select="chr22", 
    window_size=500)
qseaSet=addCoverage(qseaSet, fragment_length=300)
```

addEnrichmentParameters

Enrichment analysis

Description

This function analyses the dependency of enrichment on a sequence pattern, based on a subset of windows for which the signal is known.

Usage

```r
addEnrichmentParameters(qs, pattern_name="CpG", signal, windowIdx, 
    min_wd=5,bins=seq(.5,40.5,1))
```

Arguments

- **qs**: The qseaSet object
- **pattern_name**: The name of the pattern, on which the enrichment depends on (usually CpG for methylation analysis). This name must correspond to the name specified in addPatternDensity()
- **windowIdx**: vector of window indices, for which "true" values are known (or can be estimated)
**addLibraryFactors**

Estimate effective library size

**Description**

Normalization factors for effective library size are computed using the trimmed mean of m-values approach (TMM).

**Usage**

```r
addLibraryFactors(qs, factors,...)
```

**Arguments**

- `qs` The qseaSet object
- `factors` In case normalization factors have been pre-computed by the user, they can be passed with this parameter. In this case QSEA adds this factors to the qseaSet object and does not compute normalization factors.
- `...` Further parameters used for the TMM normalization (see details)

**Value**

The function returns the qseaSet object, extended by the parameters of the enrichment profiles for all samples.

**Author(s)**

Mathias Lienhard

**See Also**

plotEnrichmentProfile, addPatternDensity

**Examples**

```r
qs=getExampleQseaSet(enrichmentAnalysis=FALSE)
#this procedure assumes that regions with low CpG density is 80% methylated
#on average, and regions within CpG islands are 25% methylated on average.
wd=which(getRegions(qs)$CpG_density>1 &
            getRegions(qs)$CpG_density<15)
signal=(15-getRegions(qs)$CpG_density[wd])*.55/15+.25
signal=matrix(signal,nrow=length(signal),ncol=length(getSampleNames(qs)))
qs=addEnrichmentParameters(qs, pattern_name="CpG",
                            windowIdx=wd, signal=signal)
```
The user can specify the TMM normalization by setting the following additional parameters, which are passed to the internal functions.

- \texttt{trimA} [default: c(0.5, 0.99)] lower and upper quantiles for trimming of A values
- \texttt{trimM} [default: c(0.1, 0.9)] lower and upper quantiles for trimming of M values
- \texttt{doWeighting} [default: TRUE] computes a weighted TMM
- \texttt{ref} [default: 1] the index of the reference sample
- \texttt{plot} [default: FALSE] if set to TRUE, MvsA plots depicting the TMM normalization are created.
- \texttt{numReg} [default: 500000] Number of regions to be analyzed for normalization. Regions are drawn uniformly over the whole genome.

This function returns the \texttt{qseaSet} object, containing effective library size normalization factors.

**Author(s)**

Mathias Lienhard

**See Also**

\texttt{edgeR::calcNormFactors}

**Examples**

```r
qs = getExampleQseaSet(expSamplingDepth = 500 * 10^((1:5), repl=5)
# in this case, the first sample has only view reads, so it is important to set
# the reference sample
qs = addLibraryFactors(qs, plot = TRUE, ref = "Sim5N")
```

---

### Description

This function sets the background reads offset parameters for the \texttt{qseaSet} object, either by estimating offset reads, or by setting user provided values.

**Usage**

```r
addOffset(qs, enrichmentPattern, maxPatternDensity=0.01, offset)
```

**Arguments**

- \texttt{qs} the \texttt{qseaSet} object
- \texttt{enrichmentPattern} name of the enrichment pattern, as specified in \texttt{addPatternDensity}
- \texttt{maxPatternDensity} Maximum pattern density, at which the window is treated as pattern free.
- \texttt{offset} This parameter alternatively allows to specify the amount of background reads for each sample manually. In this case, please provide average background reads for CNV free windows in rpk scale.
addPatternDensity

Value

The function returns the qseaSet object, extended by the estimated amount of background reads for
all samples

Author(s)

Mathias Lienhard

See Also

addPatternDensity, getOffset

Examples

# simulate data with varying background fractions
qs = getExampleQseaSet(expSamplingDepth=5e4, repl=5, bgfraction=seq(0, .8, .2))
# estimate the background in simulated data
addOffset(qs, "CpG", maxPatternDensity=0.7)
# return the background on different scales
getOffset(qs, scale="fraction") # estimated fraction of total reads
getOffset(qs, scale="rpw") # average background reads per CNV free window

addPatternDensity  Infer sequence pattern density values and add to qseaSet object

Description

This function estimates the average occurrences of a sequence pattern (such as CpG dinucleotides)
within the overlapping sequencing fragments for each genomic window

Usage

addPatternDensity(qs, pattern, name, fragment_length, fragment_sd,
patternDensity, fixed=TRUE)

Arguments

qs  a qseaSet object
pattern actual sequence of the pattern (e.g. "CG"),
name  a name for the sequence pattern (e.g. "CpG"),
fragment_length  the average fragment length to be assumed for pattern density estimation. If
omitted, this parameter is taken from the qseaSet object.
fragment_sd  the standard deviation of fragment length to be assumed for pattern density esti-
               mation. If omitted, this parameter is taken from the qseaSet object.
patternDensity  this parameter alternatively allows to specify the pattern density manually. In
                this case, please provide a numerical vector, containing a value (greater than 0)
                for each genomic window.
fixed  if FALSE, an IUPAC ambiguity code in the pattern can match any letter in the
reference genome that is associated with the code, and vice versa.
Value

The function returns the qseaSet object, extended by the pattern density for all genomic windows.

Author(s)

Mathias Lienhard

Examples

```r
library("BSgenome.Hsapiens.UCSC.hg19")
bam_hESCs_1 = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")
bam_hESCs_2 = system.file("extdata", "hESCs.MeDIP.Rep2.chr22.bam", package="MEDIPSData")
sample_table=data.frame(sample_name=paste0("hESCs_", 1:2),
                        file_name=c(bam_hESCs_1,bam_hESCs_2),
group=rep("hESC",2), stringsAsFactors=FALSE)
qseaSet=createQseaSet(sampleTable=sample_table,
                       BSgenome="BSgenome.Hsapiens.UCSC.hg19",
                       chr.select="chr22",
                       window_size=500)
qseaSet=addPatternDensity(qseaSet, "CG", name="CpG", fragment_length=300)
```

addSeqPref  Add sequence preference to qseaSet object

Description

This function allows to add window specific sequencing preference, that can be used by the normalization procedure. This preference can be defined by the user, or estimated from sequencing of input libraries.

Usage

```r
addSeqPref(qs, seqPref, file_name, fragment_length, paired=FALSE, uniquePos=TRUE, alpha=0.05, pseudocount=5, cut=3)
```

Arguments

- `qs`: a qseaSet object
- `seqPref`: A vector with predefined sequencing preference for each window. Values are interpreted as log2 ratios relative to normal/average sequencing preference.
- `file_name`: alternatively, the sequencing preference can be estimated from input sequencing. In this case, provide the column of the sample table that contains the file names for input sequencing alignment or coverage files.
- `fragment_length`: for single end data, provide the expected fragment length
- `paired`: if set to TRUE, data is considered to be paired end sequencing, and the actual fragments size is used.
createQseaSet

uniquePos if set to TRUE, fragments with same position and orientation are considered to be PCR duplicates and replaced by one representative.

alpha currently ignored

pseudocount this value is added to the coverage of each window, to smooth the estimates.

cut absolute log2 value threshold for windows to be excluded from later analysis due to extreme preference values.

Value

the function returns the qseaSet object, extended by the sequencing preference for all genomic windows.

Author(s)

Mathias Lienhard

createQseaSet Prepares a qseaSet Object

Description

This method prepares the qseaSet object, and prepares genome wide bins. Coverage and normalization parameters are added in succeeding functions.

Usage

createQseaSet(sampleTable, BSgenome, chr.select, Regions, window_size=250 )

Arguments

BSgenome name of BSgenome package

Regions GRanges object. If specified, only selected regions are processed

chr.select If specified, only selected chromosomes are processed

sampleTable data.frame, containing at least 3 columns: the sample names (sample_name), paths to alignment or coverage file in sam/bam/wiggle/bigwig format (file_name), and one or more test condition(s) (group).

Optional it may contain a column with alignment or coverage files for CNV analysis, and further information in the samples that are of interest for the analysis.

window_size size for the genome wide bins in base pairs

Value

An object of class qseaSet, containing the sample and genome information.

Author(s)

Mathias Lienhard
Examples

```r
library("BSgenome.Hsapiens.UCSC.hg19")
bam_hESCs_1 = system.file("extdata", "hESCs.MeDIP.Rep1.chr22.bam", package="MEDIPSData")
bam_hESCs_2 = system.file("extdata", "hESCs.MeDIP.Rep2.chr22.bam", package="MEDIPSData")
samplesTable = data.frame(sample_name=paste0("hESCs_", 1:2),
file_name=c(bam_hESCs_1, bam_hESCs_2),
group=rep("hESC", 2), stringsAsFactors=FALSE)
qs = createQseaSet(samplesTable, BSgenome="BSgenome.Hsapiens.UCSC.hg19",
chr.select="chr22", window_size=500)
```

### fitNBglm

**Fit GLM for each window**

**Description**

This function fits a negative binomial GLM for each genomic window, according to the design matrix.

**Usage**

```r
fitNBglm(qs, design, link="log", keep, disp_method="region_wise", 
norm_method="rpkm", init_disp=0.5, verbose=TRUE, minRowSum=10, pseudocount=0 )
```

**Arguments**

- `qs`: a qseaSet object
- `design`: the design matrix for the GLMs
- `link`: name of the link function. Currently, only the canonical dQuotelog link function is implemented.
- `keep`: indices of windows to be included in the analysis.
- `disp_method`: method to estimate dispersion parameters. Allowed values are dQuoteregion_wise for independent window wise estimates, dQuotecommon for a single estimate for all windows, dQuoteCutAtQuantiles for window wise estimates trimmed at the 25% and 75% quantiles, or dQuoteInitial for using the dispersion parameters provided with the init_disp parameter.
- `norm_method`: normalization method, as defined by normMethod() function
- `init_disp`: initial estimate for dispersion parameter. Either a single parameter for all regions, or a vector with window wise parameters. 
- `verbose`: more messages that document the process
- `minRowSum`: filter out windows with less than minRowSum reads over all samples
- `pseudocount`: this value is added to the read counts

**Value**

This function returns a qseaGLM object, containing the fitted coefficients of the GLMs.
**getExampleQseaSet**

**Author(s)**
Mathias Lienhard

**See Also**
addContrast()

**Examples**

```r
#tbd
gs=getExampleQseaSet()
design=model.matrix(~group, getSampleTable(gs))
TvN glm=fitNBglm(qs, design, norm_method="beta")
```

---

**getExampleQseaSet**  Simulation of MeDIP seq QSEA set

**Description**

Creates a example qseaSet object by sampling reads for simulated Tumor and Normal samples. Number of replicates, sequencing depth and fraction of background reads can be specified.

**Usage**

```r
getExampleQseaSet(CpG=TRUE,CNV=TRUE,repl=2,
doSampling=TRUE,enrichmentAnalysis=TRUE, expSamplingDepth=50000, bgfraction=.1)
```

**Arguments**

- **CpG**: if TRUE CpG density is added to the object
- **CNV**: if TRUE CNV are emulated for the tumor samples
- **repl**: number of replicates for tumor and normal samples
- **doSampling**: if TRUE, read counts are sampled and added to the object
- **enrichmentAnalysis**: if TRUE, parameters for enrichment profiles are added
- **expSamplingDepth**: expected value of sequencing depth
- **bgfraction**: fraction of background reads

**Details**

The function creates an example and test qseaSet object for an toy example genome (one chromosome, 50kb) with 500 bases windows.

**Value**

The qseaSet object
getPCA

Author(s)
Mathias Lienhard

See Also
createQseaSet()

Examples
qs=getExampleQseaSet()

---

getPCA  Principle Component Analysis (PCA) in QSea

Description
The getPCA() function performs a Principle Component Analysis (PCA) of the coverage profiles from a qsea object for exploratory data analysis.

Usage
getcA(qs, chr = getChrNames(qs), ROIs, minRowSum = 20, keep , norm_method = normMethod(logRPM = c("log", "library_size", "cnv", "preference", "psC10")), topVar = 1000, samples = getSampleNames(qs))

Arguments
- **qs**: DIPSset (mandatory)
- **chr**: chromosomes to consider
- **ROIs**: If specified, only windows overlapping ROIs are considered.
- **minRowSum**: minimal number of total read counts per window over all samples
- **keep**: windows to consider
- **norm_method**: name of predefined normalization (e.g. "beta"), or user defined normalization by calling normMethod() function
- **topVar**: only the top variable windows are considered
- **samples**: names of samples to be considered

Details
The principle component analysis is calculated using the singular value decomposition (svd).

Value
getcA() returns a list object, containing the svd and information on the selected windows.

Author(s)
Mathias Lienhard
isSignificant

See Also
plotPCA

Examples

qs=getExampleQseaSet( repl=5)
pca=getPCA(qs, norm_method="beta")
colors=c(rep("red", 5), rep("green", 5))
plotPCA(pca, bgColor=colors)
#plotPCAfactors is more interesting, if ROIs have been specified in getPCA
plotPCAfactors(pca)

desc

isSignificant

Finds Significant Regions

Description
This function looks for regions, where the test statistic is below the defined thresholds.

Usage

isSignificant(glm, contrast = NULL, fdr_th = NULL, pval_th = NULL,
absLogFC_th = NULL, direction = "both")

Arguments

- glm: A qseaGLM object (mandatory)
- contrast: name of contrast to be used
- fdr_th: a threshold for the false discovery rate
- pval_th: a p value threshold
- absLogFC_th: the threshold for the absolute value of logFC
- direction: direction of change: either "both", "loss", or "gain"

Details
If a threshold is NULL, it is ignored.
For the direction parameter, the following synonyms are valid:
"loss" == "less" == "hypo"
"gain" == "more" == "hyper"

Value
A vector with indices of significant windows, which can be passed to keep parameter of makeTable() function

Author(s)
Mathias Lienhard
See Also
makeTable

Examples

qs=getExampleQseaSet()
design=model.matrix(~group, getSampleTable(qs))
TvN_glm=fitNBglm(qs, design, norm_method="beta")
TvN_glm=addContrast(qs,TvN_glm, coef=2, name="TvN")
sig=isSignificant(TvN_glm, fdr_th=0.01)

makeTable
Create a Results Table

Description
This function creates a table from the qsea objects qseaSet and qseaTvN_glm

Usage

makeTable(qs,glm,norm_methods="counts",samples,groupMeans, keep, ROIs,
annotation, minPvalSummarize, CNV=FALSE, verbose=TRUE, minEnrichment=3,
chunksize=1e5)

Arguments

qs a qseaSet object (mandatory)
glm a list of one or more qseaGLM objects (optional)
norm_methods either a character vector of pre-defined normalization combinations, or a list
defining normalization combinations. This affects both individual and mean
values.
samples The indices of the samples for which individual values are to be written out in
the specified order

groupMeans a named list of indices vectors, defining groups for which mean values are to be
written out

keep a vector of indices of the windows that are considered (as created by isSignifi-
cant)

ROIs A GRanges object, containing regions of interest (ROIs). Only windows over-
lapping ROIs are considered.

annotation a named list of GRange objects, containing annotations (e.g. genes, CpG islands,
...) that are added to the table.

minPvalSummarize If ROIs are given, you can specify a QseaTvN_glm object. For each ROI the
window with the most significant differential coverage is written out

CNV If set TRUE, the CNV logFC for the samples specified by samples are written out.

verbose verbosity level
For transformation to absolute methylation level, you can specify the minimal number of expected reads for a fully methylated window. This avoids inaccurate estimates, due to low enrichment.

For efficient memory usage, the table is built up in chunks. With this parameter, the maximum number of windows processed in one chunk is specified.

Note that, if overlapping ROIs are specified, windows might emerge in the table several times.

A result table containing the specified normalized values for the selected windows and samples/groups

Mathias Lienhard

isSignificant

# create example set
qs=getExampleQseaSet()
design=model.matrix(~group, getSampleTable(qs))
TvN_glm=fitNBglm(qs, design, norm_method="beta")
TvN_glm=addContrast(qs,TvN_glm, coef=2, name="TvN")
sig=isSignificant(TvN_glm, fdr_th=0.01)

## Table containing all significant windows
## additional CNV logFC for the selected samples
## explicit selection of normalization:
## counts (i.e. no normalization, only counts)
## counts AND %methylation values for individual samples and group means
normMethod

Definition of normalization procedure

Description
This function allows to define normalization methods by specifying components.

Usage
normMethod(methods, ...)

Arguments
methods
names of predefined normalization methods (for a list of predefined methods, see details)

... sets of normalization components, that can be combined to user defined normalization methods

Details
Predefined normalization methods:
“counts”: no normalization, simply raw count values
“reads”: same as counts
“rpm”: reads per million mappable reads
“nrpm”: CNV normalized reads per million mappable reads
“beta”: transformation to % methylation, posterior mean point estimator
“logitbeta”: logit transformed beta values
“betaLB”: 2.5 lower bound for the point estimator
“betaUB”: 97.5 upper bound for the point estimator

Allowed components for user defined normalization methods:
“library_size”: scale by effective library size
“region_length”: scale by window size
“preference”: scale by positional sequencing preference
“cnv”: scale by CNV ratio
“enrichment”: use enrichment profiles for transformation to absolute methylation level
“qXY”: quantile estimator for transformation to absolute methylation level. XY must be replaced by the quantile (see example with self defined lower and upper bound)
“offset”: consider background reads

WARNING: not all combinations are allowed (eg qXY requires enrichment) and not all allowed combinations are meaningful. Inexperienced users should stick to predefined normalization methods.

Value
a list object, containing the components for the specified normalization procedure
plotCNV

Plots a Heatmap-like Overview of the CNVs

Description

This function plots the Copy Number Variations (CNVs) of the samples in a heatmap like representation. Amplified regions are depicted in red, whereas deletions are depicted green, and CNV free regions blue. The samples are ordered by an hierarchical clustering.

Usage

```r
plotCNV(qs, dist = c("euclid", "cor")[1], clust_method = "complete", chr = getChrNames(qs), samples = getSampleNames(qs), cex = 1, labels = c(TRUE, TRUE, TRUE, TRUE), naColor = "darkgrey", indicateLogFC = TRUE )
```

Arguments

- **qs**: a qseaSet object (mandatory)
- **dist**: distance measure for clustering. dQuoteuclidian or dQuotecorrelation based (1-cor)
- **clust_method**: method to be passed to hclust
- **chr**: vector of chromosomes to be depicted
- **samples**: samples for which CNVs are depicted
- **cex**: font size of labels
- **labels**: Boolean vector of length four (bottom, left, top, right), specifying the sides of the map to be labeled
- **naColor**: Color for regions without CNV information
- **indicateLogFC**: indicate the CNV logFC values in the legend
plotCoverage

Value

This function returns the pairwise distances of the CNV profiles, on which the clustering is based on.

Author(s)

Mathias Lienhard

Examples

```r
qs=getExampleQseaSet()
plotCNV(qs, labels=c(FALSE, TRUE, TRUE, FALSE))
```

plotCoverage

Plots a genome-browser-like image of a region

Description

This function plots the normalized coverage of specified samples in a specified region, together with annotations, in a genome-browser-like fashion.

Usage

```r
plotCoverage(qs, test_results, chr, start, end, samples, samples2,
norm_method="nrpkm", yoffset, xlab="Position",
ylab="MeDIP seq", col="black", main, reorder="non",
distfun=dist, clustmethod="complete", scale=TRUE, steps=TRUE, space=0.05,
baselines=TRUE, scale_val, scale_unit=NULL, logFC_pc=.1, cex=1, smooth_width,
smooth_function=mean, regions, regions_lwd=1, regions_col,
regions_offset, regions_names, regions_dash=0.1)
```

Arguments

- **qs**: a qseaSet object
- **chr**: the chromosome of the region to be depicted
- **start**: the start position of the region to be depicted
- **end**: the end position of the region to be depicted
- **samples**: the indices of the samples to be depicted
- **samples2**: if specified, used to calculate logFC (samples/samples2) profiles, must be of same length as samples
- **logFC_pc**: if samples2 is specified and logFC are calculated, this parameter specifies the pseudocount to avoid division by zero
- **norm_method**: a vector of normalization methods to be combined
- **yoffset**: horizontal offset, used to adjust the space between the profiles
- **xlab**: title for the x axis
- **ylab**: title for the y axis
- **main**: an overall title for the plot
plotCoverage

col color vector for the samples (is recycled)
reorder indicate whether, and if yes how, the samples are reordered. Valid values are "non", "clust", "max", "minP", or a genomic position within the range that is depicted
test_results a qseaGLM object, used to find the region with minimal p value (only if reorder="minP")
indicate_reorder indicate the window that has been used for reordering by an arrow.
distfun if reorder="clust": for hierarchical clustering for reordering
clustmethod if reorder="clust": for hierarchical clustering for reordering
scale if set TRUE, print a bar scale
scale_val length of the bar scale
scale_unit unit of the bar scale
steps plot the coverage as step function (steps=TRUE), or as lines
space fraction of the plot set aside for sample names etc.
baselines depict the baselines (zero) of the coverage profiles
cex font size
smooth_width number of windows to be considered for sliding window smoothing
smooth_function function to be applied on the sliding windows for smoothing
regions named list of GenomicRanges objects, containing annotation (eg exons) to be depicted below the coverage profiles
regions_lwd vector of line width for the
regions_col vector of colors for the regions
regions_offset offset value, defining the space between the regions
regions_names vector of column names, that store the names of the regions
regions_dash vector, specifying the length of the end dashes of the regions

Value
list containing a table containing the plotted coverage values, the position that has been used for ordering, and the image coordinates

Author(s)
Mathias Lienhard

Examples
qs=getExampleQseaSet(repl=5)
colors=c(rep("red", 5), rep("green", 5))
plot()
plotCoverage(qs,samples=getSampleNames(qs),
    chr="chr1", start=1960001, end=1970001, col=colors,
    norm_method="beta", yoffset=1, space=.2, reorder=1964500)
plotCoverage(qs,samples=getSampleNames(qs),
    chr="chr1", start=1960001, end=1970001, col=colors,
    norm_method="beta", yoffset=1, space=.2, reorder="clust")
plotEnrichmentProfile  

Plotting functions for enrichment profiles

Description
Plots the estimated sequence pattern dependent enrichment profile for one or several samples as a matrix of plots.

Usage
plotEnrichmentProfile(qs, sample, sPoints=seq(0,30,1),
  fitPar=list(lty=2, col="green"), cfPar=list(lty=1), densityPar, meanPar,...
) plotEPmatrix(qs, sa=getSampleNames(qs), nrow=ceiling(sqrt(length(sa))),
  ncol=ceiling(length(sa)/nrow), ...)

Arguments
qs The qseaSet object
sample The index of the sample for which the enrichment profile should be depicted
sPoints The values at which the enrichment profile function is evaluated
fitPar List of parameters for depiction of the fitted enrichment profile function (see details)
cfPar List of parameters for depiction of the empirical enrichment profile (see details)
densityPar List of parameters for depiction high density scatterplot of coverage and pattern density (see details)
meanPar List of parameters for depiction of the mean coverage per pattern density bin (see details)
sa vector of samples to be depicted in matrix plot
nrow number of rows in matrix plot
ncol number of columns in matrix plot
... Further graphical parameters may also be supplied

Details
Parameter lists for lines in the plot (e.g. fitPar, cfPar and meanPar) are passed to graphics::lines(), densityPar are passed to graphics::smoothScatter() function.

Value
plotEnrichmentProfile returns the coordinates of the enrichment profile. plotEPmatrix returns enrichment profile coordinates for all depicted samples.

Author(s)
Mathias Lienhard

See Also
addEnrichmentParameters
plotPCA

Examples

# create example object with different sequencing depth
qs = getExampleQseaSet(expSamplingDepth = 50 + 10 * (1:4), repl = 4)
# enrichment profile for one sample
plotEnrichmentProfile(qs, "Sim4T")
# enrichment profile for all samples
plotEPmatrix(qs)

plotPCA  

Plots for Principle Component Analysis (PCA) in QSEA

Description

The principle components can be depicted using the plotting methods plotPCA and plotPCAfactors

Usage

## S4 method for signature 'qseaPCA'
plotPCA(object, plotComponents = c(1, 2), fgColor = "black",
        bgColor = "white", legend, plotLabels = TRUE, radius = 5,
        labelOffset = .5, labelPos = 1, labelAdj, labelColor = "black",
        cex = 1, ...)

## S4 method for signature 'qseaPCA'
plotPCAfactors(object, plotComponents = c(1, 2),
                fgColor = "black", bgColor = "white", plotTopLabels = 100,
                labelsOfInterest, radius = 1, labelOffset = .5,
                labelPos = 1, labelColor = "black", cex = 1, ...)

Arguments

object the qseaPCA object, resulting from the getPCA function
plotComponents vector of the two components of the PCA
fgColor vector of foreground colors for the circles
bgColor vector of background colors for the circles
legend add a legend to the plot
plotLabels if set TRUE, the labels of the samples are written in the plot
radius defines the size of the plotted circles
labelOffset defines the offset of the labels to the circles
labelPos specify position of the labels in the plot (see graphics::text)
labelAdj alternative way to specify position of the labels in the plot (see graphics::text)
labelColor a vector of colors for the labels
cex font size of the labels
plotTopLabels labels of factors with strongest contribution to plotted components are shown
labelsOfInterest vector of factor names that are highlighted and labeled in the plot
... further graphical parameters
Value

The functions return a list with the coordinates of the depicted components.

Author(s)

Mathias Lienhard

See Also

plotPCA

Examples

```r
qs <- getExampleQseaSet(repl=5)
pca <- getPCA(qs, norm_method="beta")
colors <- c(rep("red", 5), rep("green", 5))
plotPCA(pca, bgColor=colors)
#plotPCAfactors is more interesting, if ROIs have been specified in getPCA
plotPCAfactors(pca)
```

Description

The qseaGLM class is used in qsea to store fitted coefficients of the GLM.

Slots

- `fullModelDesign`: design matrix of full model
- `fullModel`: list containing parameters and fitted coefficients of full model
- `parameters`: list of parameters used to create the object
- `contrast`: list of lists containing parameters and the fitted model coefficients of the reduced models
- `windows`: vector of window indices, for which GLMs have been fitted

Author(s)

Mathias Lienhard

Examples

```r
showClass("qseaGLM")
```
qseaPCA-class

qsea PCA class and its methods

Description

The qseaPCA class is used in qsea to store results of the principle component analysis.

Slots

- svd: singular value decomposition
- sample_names: names of the samples
- factor_names: names of the genomic windows involved

Author(s)

Matthias Lienhard

Examples

showClass("qseaPCA")

qseaSet-class

qsea Set class and its methods

Description

The qseaSet class is used in qsea to store information about the coverage, the dependent organism, the chromosomes included in the input file, the length of the included chromosomes (automatically loaded), the number of regions, and optionally CNV information.

Slots

- sampleTable: Object of class "data.frame": the sample table
- count_matrix: Object of class "matrix": matrix containing the coverage for all samples
- zygosity: Object of class "matrix": matrix containing the zygosity for all chromosomes and all samples
- regions: Object of class "GenomicRanges": the genomic regions for the coverage matrix
- parameters: Object of class "list": the parameter list used to create this object
- cnv: Object of class "GenomicRanges": CNV ranges and logFCs
- enrichment: Object of class "list": parameters of the sequence pattern enrichment analysis
- libraries: Object of class "matrix": parameters of the sequencing libraries
Methods

**getSampleTable** signature(object = "qseaSet"): extracts the sample table of a qsea set

**getSampleNames** signature(object = "qseaSet"): extracts the sample names of a qsea set

**getSampleGroups** signature(object = "qseaSet"): extracts the sample groups of a qsea set

**getChrNames** signature(object = "qseaSet"): returns the analysed chromosomes

**getCounts** signature(object = "qseaSet"): extracts the count matrix a qsea set

**getRegions** signature(object = "qseaSet"): extracts the regions object of a qsea set

**getParameters** signature(object = "qseaSet"): extracts the parameter list of a qsea set

**getLibSize** signature(object = "qseaSet"): extracts the library size (eg the total number of read counts per sample)

**getNormFactors** signature(object = "qseaSet"): extracts the list with the different normalization factors

**hasCNV** signature(object = "qseaSet"): TRUE if CNV information is present, FALSE otherwise

**getCNV** signature(object = "qseaSet"): extracts the CNV regions and logFCs

**getOffset** signature(object = "qseaSet"): extracts offset of rpkm scaled background reads

**getWindowSize** signature(object = "qseaSet"): returns the window size of the object

**getZyosity** signature(object = "qseaSet"): returns the zygosity matrix of the object

**setZyosity** signature(object = "qseaSet", zygosityMatrix): sets the zygosity matrix, and resets CNV

Author(s)

Matthias Lienhard

Examples

```r
showClass("qseaSet")
```

---

**regionStats**

*Counts the Windows in Regions of Interest*

Description

This function takes a list of window indices and a list of ROIs and counts the number of overlapping windows

Usage

```r
regionStats(qs, subsets = list(covered = which(rowSums(getCounts(qs)) >= 20)), ROIs = list(), minoverlap = 1, maxgap = 0)
```

Arguments

- **qs**: A qsea Set object
- **subsets**: A list of window indices
- **ROIs**: A list of Regions of Interest
- **minoverlap**: Passed to findOverlaps
- **maxgap**: Passed to findOverlaps
regionStats

Value

a matrix, containing the total number of windows overlapping the ROIs and the numbers of windows from the subset list overlapping ROIs

Author(s)

Mathias Lienhard

See Also

findOverlaps

Examples

qs=getExampleQseaSet()
# as an example, we analyze the fraction of reads covered by at least 10
# or at least 20 reads, for bins of CpG density
ROIs=list()
regs=getRegions(qs)
cpg=getRegions(qs)$CpG_density
bins=seq(0,30,5)
for(i in 1:(length(bins)-1)){
  n=paste0(bins[i],"-",bins[i+1]," CpGs")
  ROIs[[n]]=regs[which(cpg>=bins[i] & cpg < bins[i+1])]
}
subsets = list(
  ">10" = which(rowSums(getCounts(qs)) >= 10),
  ">20" = which(rowSums(getCounts(qs)) >= 20))
coverage_stats=regionStats(qs, subsets, ROIs)
coverage_stats_rel=coverage_stats[,-1]/coverage_stats[,1]
x=barplot(t(coverage_stats_rel)*100,ylab="fraction of windows[%]",
  beside=TRUE, legend=TRUE, las=2, args.legend=list(x="topleft"),
  main="Covered Windows")
Index

*Topic classes
  qseaGLM-class, 24
  qseaPCA-class, 25
  qseaSet-class, 25

*Topic package
  qsea-package, 2

addCNV, 3
addContrast, 4
addCoverage, 5
addEnrichmentParameters, 6
addLibraryFactors, 7
addOffset, 8
addPatternDensity, 9
addSeqPref, 10
createQseaSet, 11

fitNBglm, 12

getChrNames (qseaSet-class), 25
getchrNames, qseaSet-method (qseaSet-class), 25
getCNV (qseaSet-class), 25
getCNV, qseaSet-method (qseaSet-class), 25
getCounts (qseaSet-class), 25
getCounts, qseaSet-method (qseaSet-class), 25
getAddressQseaSet, 13
getLibSize (qseaSet-class), 25
getLibSize, qseaSet-method (qseaSet-class), 25
getNormFactors (qseaSet-class), 25
getNormFactors, qseaSet-method (qseaSet-class), 25
getOffset (qseaSet-class), 25
getoffset, qseaSet-method (qseaSet-class), 25
getParameters (qseaSet-class), 25
getParameters, qseaGLM-method (qseaGLM-class), 24
getParameters, qseaSet-method (qseaSet-class), 25

getPCA, 14
getRegions (qseaSet-class), 25
getRegions, qseaSet-method (qseaSet-class), 25
getSampleGroups (qseaSet-class), 25
getSampleGroups, qseaSet-method (qseaSet-class), 25
getSampleNames (qseaSet-class), 25
getSampleNames, qseaGLM-method (qseaGLM-class), 24
getSampleNames, qseaPCA-method (qseaPCA-class), 25
getSampleNames, qseaSet-method (qseaSet-class), 25
getSampleTable (qseaSet-class), 25
getSampleTable, qseaSet-method (qseaSet-class), 25
getWindowSize (qseaSet-class), 25
ggetWindowSize, qseaSet-method (qseaSet-class), 25
getZygosity (qseaSet-class), 25
getZygosity, qseaSet-method (qseaSet-class), 25

hasCNV (qseaSet-class), 25
hasCNV, qseaSet-method (qseaSet-class), 25

isSignificant, 15

makeTable, 16

normMethod, 18

plotCNV, 19
plotCoverage, 20
plotEnrichmentProfile, 22
plotEPmatrix (plotEnrichmentProfile), 22
plotPCA, 23
plotPCA, qseaPCA-method (plotPCA), 23
plotPCAfactors (plotPCA), 23
plotPCAfactors, qseaPCA-method (plotPCA), 23

QSEA (qsea-package), 2
INDEX

qsea (qsea-package), 2
qsea-package, 2
qseaGLM (qseaGLM-class), 24
qseaGLM-class, 24
qseaPCA (qseaPCA-class), 25
qseaPCA-class, 25
qseaSet (qseaSet-class), 25
qseaSet-class, 25

regionStats, 26

setZygosity (qseaSet-class), 25
setZygosity, qseaSet-method
  (qseaSet-class), 25
show, qseaGLM-method (qseaGLM-class), 24
show, qseaPCA-method (qseaPCA-class), 25
show, qseaSet-method (qseaSet-class), 25