

Package ‘OUTRIDER’

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BugRepos <https://github.com/gagneurlab/OUTRIDER/issues>

Description Identification of aberrant gene expression in RNA-seq data.

Read count expectations are modeled by an autoencoder to control for confounders in the data. Given these expectations, the RNA-seq read counts are assumed to follow a negative binomial distribution with a gene-specific dispersion. Outliers are then identified as read counts that significantly deviate from this distribution. Furthermore, OUTRIDER provides useful plotting functions to analyze and visualize the results.

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inputCheckerFunctions.R helperFunctions.R getNSetterFuns.R
getNSetterFunsInternal.R autoencoder.R fitNB.R ZscoreMatrix.R
method-evaluation.R method-counts.R method-gridSearch.R
method-plot.R method-results.R pValMatrix.R filterExpression.R
OUTRIDER.R sizeFactor.R RcppExports.R updateE.R updateD.R
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| | |
|----------|----------------------------------|
| aberrant | <i>Number of aberrant events</i> |
|----------|----------------------------------|

Description

Identifies the aberrant events and returns the number of aberrant counts per gene or sample or returns a matrix indicating aberrant events.

Usage

```
aberrant(object, ...)  
  
## S4 method for signature 'OutriderDataSet'  
aberrant(  
  object,  
  padjCutoff = 0.05,  
  zScoreCutoff = 0,  
  by = c("none", "sample", "gene")  
)
```

Arguments

| | |
|--------------|--|
| object | An OutriderDataSet object |
| ... | Currently not in use. |
| padjCutoff | The padjust cutoff |
| zScoreCutoff | The absolute Z-score cutoff, if NA or NULL no Z-score cutoff is used |
| by | if the results should be summarized by 'sample', 'gene' or not at all (default). |

Value

The number of aberrant events by gene or sample or a TRUE/FALSE matrix of the size sample x gene of aberrant events.

Examples

```
ods <- makeExampleOutriderDataSet()  
ods <- OTRIDER(ods, implementation='pca')  
  
aberrant(ods)[1:10,1:10]  
tail(sort(aberrant(ods, by="sample")))  
tail(sort(aberrant(ods, by="gene")))
```

| | |
|-------------------|--|
| computeGeneLength | <i>Extracting the gene length from annotations</i> |
|-------------------|--|

Description

Computes the length for each gene based on the given GTF file or annotation. Here the length of a gene is defined by the total number of bases covered by exons.

Usage

```
computeGeneLength(ods, gtfFile, format = "gtf", mapping = NULL, ...)
```

Arguments

| | |
|---------|---|
| ods | An OutriderDataSet for which the gene length should be computed. |
| gtfFile | Can be a GTF file or an txDb object with annotation. |
| format | The format parameter from makeTxDbFromGFF |
| mapping | If set, it is used to map gene names between the GFT and the ods object. This should be a 2 column data.frame: 1. column GTF names and 2. column ods names. |
| ... | further arguments to makeTxDbFromGFF |

Value

An OutriderDataSet containing a basepairs column with the calculated gene length. Accessible through `mcols(ods)['basepairs']`

Examples

```
ods <- makeExampleOutriderDataSet(dataset="GTExSkinSmall")
annotationFile <- system.file("extdata", "gencode.v19.genes.small.gtf.gz",
  package="OUTRIDER")
ods <- computeGeneLength(ods, annotationFile)

mcols(ods)['basepairs']
fpkm(ods)[1:10,1:10]
```

computeLatentSpace *Extracting the latent space*

Description

Extracts the latent space from the OutriderDataSet object determined by the autoencoder.

Usage

```
computeLatentSpace(ods)
```

Arguments

ods An OutriderDataSet

Value

A matrix containing the latent space determined by the autoencoder.

Examples

```
ods <- makeExampleOutriderDataSet()

ods <- estimateSizeFactors(ods)
ods <- controlForConfounders(ods, implementation="pca")
computeLatentSpace(ods)[,1:6]
```

computePvalues *Calculate P-values*

Description

This function computes the P-values based on the fitted negative binomial model. It computes two matrices with the same dimension as the count matrix (samples x genes), which contain the corresponding P-values and adjusted P-values of every count.

Usage

```
computePvalues(object, ...)

## S4 method for signature 'OutriderDataSet'
computePvalues(
  object,
  alternative = c("two.sided", "greater", "less"),
  method = "BY",
  BPPARAM = bpparam()
)
```

Arguments

| | |
|-------------|--|
| object | An OutriderDataSet |
| ... | additional params, currently not used. |
| alternative | Can be one of "two.sided", "greater" or "less" to specify the alternative hypothesis used to calculate the P-values, defaults to "two.sided" |
| method | Method used for multiple testing |
| BPPARAM | Can be used to parallelize the computation, defaults to bpparam() |

Value

An OutriderDataSet object with computed nominal and adjusted P-values

See Also

p.adjust

Examples

```
ods <- makeExampleOutriderDataSet()

ods <- estimateSizeFactors(ods)
ods <- fit(ods)

ods <- computePvalues(ods)

assays(ods)[['pValue']][1:10,1:10]
```

| | |
|----------------|----------------------------|
| computeZscores | <i>Z score computation</i> |
|----------------|----------------------------|

Description

Computes the z scores for every count in the matrix. The z score is defined in the log2 space as follows:

$$z_{ij} = \frac{l_{ij} - \mu_j^l}{\sigma_j^l}$$

, where l is the log2 transformed normalized count and μ and σ the mean and standard deviation for gene j , respectively.

Usage

```
computeZscores(ods, ...)

## S4 method for signature 'OutriderDataSet'
computeZscores(ods, peerResiduals = FALSE, ...)
```

Arguments

ods OutriderDataSet
 ... Further arguments passed on to ZscoreMatrix.
 peerResiduals If TRUE, PEER residuals are used to compute Z scores

Value

An OutriderDataSet containing the Z score matrix "zScore" and the log2 fold changes "l2fc" as asasys.

Examples

```
ods <- makeExampleOutriderDataSet()
ods <- estimateSizeFactors(ods)

ods <- controlForConfounders(ods, implementation="pca")
ods <- computeZscores(ods)

zScore(ods)[1:10,1:10]
assay(ods, "l2fc")[1:10,1:10]
```

controlForConfounders *Autoencoder function to correct for confounders.*

Description

This is the wrapper function for the autoencoder implementation. It can be used to call the standard R implementation or the experimental Python implementation.

Usage

```
controlForConfounders(
  ods,
  q,
  implementation = c("autoencoder", "pca"),
  BPPARAM = bpparam(),
  ...
)
```

Arguments

ods An OutriderDataSet object
 q The encoding dimensions
 implementation "autoencoder", the default, will use the autoencoder implementation. Also 'pca' and 'peer' can be used to control for confounding effects
 BPPARAM A [BiocParallelParam](#) instance to be used for parallel computing.
 ... Further arguments passed on to the specific implementation method.

Value

An ods object including the control factors

Examples

```
ods <- makeExampleOutriderDataSet()
implementation <- 'autoencoder'

ods <- estimateSizeFactors(ods)
ods <- controlForConfounders(ods, implementation=implementation)

plotCountCorHeatmap(ods, normalized=FALSE)
plotCountCorHeatmap(ods, normalized=TRUE)
```

counts

Accessors for the 'counts' slot of an OutriderDataSet object.

Description

The counts slot holds the count data as a matrix of non-negative integer count values, one row for each observational unit (eg.: gene), and one column for each sample.

Usage

```
## S4 method for signature 'OutriderDataSet'
counts(object, normalized = FALSE, minE = 0.5, ...)

## S4 replacement method for signature 'OutriderDataSet,matrix'
counts(object, ...) <- value
```

Arguments

| | |
|------------|--|
| object | An OutriderDataSet object |
| normalized | TRUE/FALSE whether counts should be normalized |
| minE | The minimal expected count, defaults to 0.5, to be used in computing the expected log geom mean. |
| ... | Further arguments are passed on to the underlying assay function |
| value | An integer matrix containing the counts |

Details

By default this function returns the raw counts. If control factors are computed or provided the normalized counts can be returned using `normalized = TRUE`. The `offset` parameter can be used to add a pseudocount to the count before dividing by the normalization. This can be useful when the `log(counts)` are computed and in case the control values are in the same order of magnitude as the counts.

Value

A matrix containing the counts

See Also

[sizeFactors](#), [normalizationFactors](#)

Examples

```
ods <- makeExampleOutriderDataSet()
counts(ods)[1:10,1:10]

ods <- estimateSizeFactors(ods)
counts(ods, normalized=TRUE)[1:10,1:10]
```

| | |
|---------------|-------------------------------------|
| estimateBestQ | <i>Estimation of Q</i> |
|---------------|-------------------------------------|

Description

Estimating the best q for the given data set

Usage

```
estimateBestQ(ods)
```

Arguments

ods An OutriderDataSet object

Value

The estimated dimension of hidden confounders

Examples

```
ods <- makeExampleOutriderDataSet()

estimateBestQ(ods)
```

| | |
|------------------|--------------------------|
| filterExpression | <i>Filter expression</i> |
|------------------|--------------------------|

Description

To filter out non expressed genes this method uses the FPKM values to get a comparable value over genes. For each gene, if the pth- percentile is greater than the fpkmCutoff value, it passes the filter. To calculate the FPKM values the user needs to provide a GTF file or the basepair parameter as described in [fpkm](#).

Usage

```
filterExpression(object, ...)

## S4 method for signature 'OutriderDataSet'
filterExpression(
  object,
  gtfFile,
  fpkmCutoff = 1,
  percentile = 0.95,
  filterGenes = TRUE,
  savefpkm = FALSE,
  minCounts = FALSE,
  addExpressedGenes = TRUE,
  ...
)
```

Arguments

| | |
|-------------------|--|
| object | An OutriderDataSet object |
| ... | Additional arguments passed to computeGeneLength |
| gtfFile | A txDb object or a GTF/GFF file to be used as annotation |
| fpkmCutoff | The threshold for filtering based on the FPKM value |
| percentile | a numeric indicating the percentile FPKM value to compare against the fpkmCutoff |
| filterGenes | If TRUE, the default, the object is subsetted. |
| savefpkm | If TRUE, the FPKM values are saved as assay |
| minCounts | If TRUE, only genes with 0 counts in all samples are filtered |
| addExpressedGenes | If TRUE (default), adds 5 columns to the colData with information regarding the number of expressed genes per sample |

Value

An OutriderDataSet containing the passedFilter column, which indicates if the given gene passed the filtering threshold. If filterGenes is TRUE the object is already subsetted.

Examples

```
ods <- makeExampleOutriderDataSet(dataset="GTExSkinSmall")
annotationFile <- system.file("extdata",
  "genecode.v19.genes.small.gtf.gz", package="OUTRIDER")
ods <- filterExpression(ods, annotationFile, filterGenes=FALSE)

mcols(ods)['passedFilter']
fpkm(ods)[1:10,1:10]
dim(ods)

ods <- ods[mcols(ods)[['passedFilter']]
dim(ods)
```

findEncodingDim

Find the optimal encoding dimension

Description

Finds the optimal encoding dimension for a given data set by running a grid search based on the provided parameter set.

Usage

```
findEncodingDim(
  ods,
  params = seq(2, min(100, ncol(ods) - 1, nrow(ods) - 1), 2),
  freq = 0.01,
  zScore = 3,
  sdlog = log(1.6),
  lnorm = TRUE,
  inj = "both",
  ...,
  BPPARAM = bpparam()
)

findInjectZscore(
  ods,
  freq = 0.01,
  zScoreParams = c(seq(1.5, 4, 0.5), "lnorm"),
  encDimParams = c(seq(3, 40, 3), seq(45, 70, 5), 100, 130, 160),
  inj = "both",
  ...,
  BPPARAM = bpparam()
)
```

Arguments

| | |
|----------------------|--|
| ods | An OutriderDataSet |
| params, encDimParams | Set of possible q values. |
| freq | Frequency of outlier, defaults to 1E-2 |
| zScore, zScoreParams | Set of possible injection Z-score, defaults to 3. |
| sdlog | Standard deviation of the sitribution on the log scale. |
| lnorm | If TRUE, the default, Z-scores are drawn from a log normal distribution with a mean of log(zScore) in log-scale. |
| inj | Injection strategy, by default 'both'. |
| ... | Further arguments passed on to the controlForConfounders function. |
| BPPARAM | BPPARAM object by default bpparam(). |

Value

The optimal encoding dimension

Examples

```
ods <- makeExampleOutriderDataSet()
encDimSearchParams <- c(5, 8, 10, 12, 15)
zScoreParams <- c(2, 3, 5, 'lnorm')
implementation <- 'autoencoder'
register(MulticoreParam(4))

ods1 <- findEncodingDim(ods, params=encDimSearchParams,
  implementation=implementation)
plotEncDimSearch(ods1)

ods2 <- findInjectZscore(ods, zScoreParams=zScoreParams,
  encDimParams=encDimSearchParams, implementation=implementation)
plotEncDimSearch(ods2)
```

 fit

Fit the negative binomial distribution

Description

Fit a negative binomial (NB) distribution to the counts per gene over all samples using, if available, the precomputed control factors. If no normalization factors are provided only the sizeFactors are used.

Usage

```
## S3 method for class 'OutriderDataSet'
fit(object, BPPARAM = bpparam(), ...)
```

Arguments

| | |
|---------|----------------------|
| object | An OutriderDataSet |
| BPPARAM | by default bpparam() |
| ... | Currently not used. |

Value

An OutriderDataSet object with the fitted model. Accessible through: `mcols(ods)[,c('mu', 'theta')]`.

Examples

```
ods <- makeExampleOutriderDataSet()
ods <- estimateSizeFactors(ods)
ods <- fit(ods)

mcols(ods)[1:10,c('mu', 'theta')]
```

fpkm

Calculate FPM and FPKM values

Description

This is the `fpm` and `fpkm` function from DESeq2. For more details see: [fpkm](#) and [fpm](#)

See Also

[fpkm](#) [fpm](#)

Examples

```
ods <- makeExampleOutriderDataSet()
mcols(ods)['basepairs'] <- round(rnorm(nrow(ods), 1000, 500))

mcols(ods)['basepairs']
fpkm(ods)[1:10,1:10]
fpm(ods)[1:10,1:10]
```

| | |
|----------|--------------------------------|
| getBestQ | <i>Getter/Setter functions</i> |
|----------|--------------------------------|

Description

This is a collection of small accessor/setter functions for easy access to the values within the OUTRIDER model.

Usage

```
getBestQ(ods)

zScore(ods)

pValue(ods)

padj(ods)

## S4 method for signature 'OutriderDataSet'
dispersions(object, ...)

theta(ods)
```

Arguments

| | |
|-------------|---|
| ods, object | An OutriderDataSet object. |
| ... | Further arguments passed on to the underlying assay function. |

Value

A matrix or vector dependent on the type of data retrieved.

See Also

[estimateDispersions](#)

Examples

```
ods <- makeExampleOutriderDataSet(10, 10)
ods <- OUTRIDER(ods)

zScore(ods)
pValue(ods)
padj(ods)
theta(ods)
theta(ods) == 1/dispersions(ods)
getBestQ(ods)
```

`makeExampleOutriderDataSet`*Create example data sets for OTRIDER*

Description

Creates an example data set from a file or simulates a data set based on random counts following a negative binomial distribution with injected outliers with a fixed z score away from the mean of the gene.

Usage

```
makeExampleOutriderDataSet(  
  n = 200,  
  m = 80,  
  q = 10,  
  freq = 0.001,  
  zScore = 6,  
  inj = c("both", "low", "high"),  
  sf = rnorm(m, mean = 1, sd = 0.1),  
  dataset = c("none", "GTEXSkinSmall", "KremerNBaderSmall")  
)
```

Arguments

| | |
|----------------------|--|
| <code>n</code> | Number of simulated genes |
| <code>m</code> | Number of simulated samples |
| <code>q</code> | number of simulated latent variables. |
| <code>freq</code> | Frequency of in-silico outliers |
| <code>zScore</code> | Absolute z score of in-silico outliers (default 6). |
| <code>inj</code> | Determines whether counts are injected with the strategy ('both', 'low', 'high'), default is 'both'. |
| <code>sf</code> | Artificial Size Factors |
| <code>dataset</code> | If "none", the default, an example data set is simulated. One can also use example data set included in the package by specifying 'GTEXSkinSmall' or 'KremerNBaderSmall' |

Value

An `OutriderDataSet` containing an example dataset. Depending on the parameters it is based on a real data set or it is simulated

Examples

```
# A generic dataset
ods1 <- makeExampleOutriderDataSet()
ods1

# A generic dataset with specified sample size and injection method
ods2 <- makeExampleOutriderDataSet(n=200, m=50, inj='low')
ods2

# A subset of a real world dataset from GTEX
ods3 <- makeExampleOutriderDataSet(dataset="GTEXSkinSmall")
ods3
```

normalizationFactors *Accessor functions for the normalization factors in an Outrider-DataSet object.*

Description

To normalize raw count data normalization factors can be provided as a matrix. When running [controlForConfounders](#) the normalization factors are stored within the OutriderDataset object. This normalization factors are then used to compute the normalized counts.

Usage

```
## S4 method for signature 'OutriderDataSet'
normalizationFactors(object, ...)

## S4 replacement method for signature 'OutriderDataSet,matrix'
normalizationFactors(object, minE = 0.5, ...) <- value

## S4 replacement method for signature 'OutriderDataSet,DataFrame'
normalizationFactors(object, minE = 0.5, ...) <- value

## S4 replacement method for signature 'OutriderDataSet,data.frame'
normalizationFactors(object, minE = 0.5, ...) <- value

## S4 replacement method for signature 'OutriderDataSet,`NULL`'
normalizationFactors(object) <- value
```

Arguments

| | |
|--------|--|
| object | An OutriderDataSet object |
| ... | Further arguments are passed on to the underlying assay function |
| minE | The minimal expected count, defaults to 0.5, to be used in computing the expected log geom mean. |
| value | The matrix of normalization factors |

Value

A numeric matrix containing the normalization factors or the `OutriderDataSet` object with an updated `normalizationFactors` assay.

See Also

[sizeFactors](#) [normalizationFactors](#)

Examples

```
ods <- makeExampleOutriderDataSet()

normFactors <- matrix(runif(nrow(ods)*ncol(ods),0.5,1.5),
  ncol=ncol(ods),nrow=nrow(ods))

# the normalization factors matrix should not have 0's in it
# it should have geometric mean near 1 for each row
normFactorsRM <- normFactors / exp(rowMeans(log(normFactors)))
normalizationFactors(ods) <- normFactorsRM
normalizationFactors(ods)[1:10,1:10]

normalizationFactors(ods) <- NULL
ods <- estimateSizeFactors(ods)
normalizationFactors(ods) <- normFactors
all(normalizationFactors(ods) == t(sizeFactors(ods) * t(normFactors)))
```

Description

The `OUTRIDER` function runs the default `OUTRIDER` pipeline combining the fit, the computation of Z scores and P-values. All computed values are returned as an `OutriderDataSet` object.

To have more control over each analysis step, one can call each function separately.

1. [estimateSizeFactors](#) to calculate the `sizeFactors`
2. [controlForConfounders](#) to control for confounding effects
3. [fit](#) to fit the negative binomial model (only needed if the autoencoder is not used)
4. [computePvalues](#) to calculate the nominal and adjusted P-values
5. [computeZscores](#) to calculate the Z scores

Usage

```
OUTRIDER(
  ods,
  q,
  controlData = TRUE,
  implementation = "autoencoder",
  BPPARAM = bpparam(),
  ...
)
```

Arguments

| | |
|----------------|---|
| ods | An <code>OutriderDataSet</code> object |
| q | The encoding dimensions |
| controlData | If TRUE, the default, the raw counts are controlled for confounders by the autoencoder |
| implementation | "autoencoder", the default, will use the autoencoder implementation. Also 'pca' and 'peer' can be used to control for confounding effects |
| BPPARAM | A BiocParallelParam instance to be used for parallel computing. |
| ... | Further arguments passed on to <code>controlForConfounders</code> |

Value

`OutriderDataSet` with all the computed values. The values are stored as assays and can be accessed by: `assay(ods, 'value')`. To get a full list of calculated values run: `assayNames(ods)`

Examples

```
ods <- makeExampleOutriderDataSet()
implementation <- 'autoencoder'

ods <- OUTRIDER(ods, implementation=implementation)

pValue(ods)[1:10,1:10]
res <- results(ods, all=TRUE)
res

plotAberrantPerSample(ods)
plotVolcano(ods, 1)
```

OutriderDataSet-class *OutriderDataSet class and constructors*

Description

The `OutriderDataSet` class is designed to store the whole OUTRIDER data set needed for an analysis. It is a subclass of `RangedSummarizedExperiment`. All calculated values and results are stored as assays or as annotation in the `mcols` structure provided by the `RangedSummarizedExperiment` class.

Usage

```
OutriderDataSet(se, countData, colData, ...)
```

Arguments

| | |
|------------------------|---|
| <code>se</code> | A <code>RangedSummarizedExperiment</code> object or any object which inherits from it and contains a count matrix as the first element in the assay list. |
| <code>countData</code> | A simple count matrix. If dim names are provided, they have to be unique. This is only used if no <code>se</code> object is provided. |
| <code>colData</code> | Additional to the count data a <code>DataFrame</code> can be provided to annotate the samples. |
| <code>...</code> | Further arguments can be passed to <code>DESeqDataSet</code> , which is used to parse the user input and create the initial <code>RangedSummarizedExperiment</code> object. |

Value

An `OutriderDataSet` object.

Author(s)

Christian Mertes <mertes@in.tum.de>, Felix Brechtmann <brechtma@in.tum.de>

Examples

```
ods <- makeExampleOutriderDataSet()
ods

ods <- makeExampleOutriderDataSet(dataset="Kremer")
ods
```

plotAberrantPerSample *Visualization functions for OTRIDER*

Description

The OTRIDER package provides multiple functions to visualize the data and the results of a full data set analysis.

This is the list of all plotting function provided by OTRIDER:

- plotAberrantPerSample()
- plotVolcano()
- plotExpressionRank()
- plotQQ()
- plotExpectedVsObservedCounts()
- plotCountCorHeatmap()
- plotCountGeneSampleHeatmap()
- plotSizeFactors()
- plotFPKM()
- plotExpressedGenes()
- plotDispEsts()
- plotPowerAnalysis()
- plotEncDimSearch()

For a detailed description of each plot function please see the details. Most of the functions share the same parameters.

Usage

```
plotAberrantPerSample(object, ...)  
  
plotCountCorHeatmap(object, ...)  
  
plotEncDimSearch(object, ...)  
  
plotQQ(object, ...)  
  
plotVolcano(object, ...)  
  
## S4 method for signature 'OutriderDataSet'  
plotVolcano(  
  object,  
  sampleID,  
  main,
```

```
    padjCutoff = 0.05,
    zScoreCutoff = 0,
    pch = 16,
    basePlot = FALSE,
    col = c("gray", "firebrick")
)

## S4 method for signature 'OutriderDataSet'
plotQQ(
  object,
  geneID,
  main,
  global = FALSE,
  padjCutoff = 0.05,
  zScoreCutoff = 0,
  samplePoints = TRUE,
  legendPos = "topleft",
  outlierRatio = 0.001,
  conf.alpha = 0.05,
  pch = 16,
  xlim = NULL,
  ylim = NULL,
  col = NULL
)

plotExpectedVsObservedCounts(
  ods,
  geneID,
  main,
  basePlot = FALSE,
  log = TRUE,
  groups = c(),
  groupColSet = "Set1",
  ...
)

plotExpressionRank(
  ods,
  geneID,
  main,
  padjCutoff = 0.05,
  zScoreCutoff = 0,
  normalized = TRUE,
  basePlot = FALSE,
  log = TRUE,
  col = c("gray", "firebrick"),
  groups = c(),
  groupColSet = "Accent"
```

```
)  
  
## S4 method for signature 'OutriderDataSet'  
plotCountCorHeatmap(  
  object,  
  normalized = TRUE,  
  rowCentered = TRUE,  
  rowGroups = NA,  
  rowColSet = NA,  
  colGroups = NA,  
  colColSet = NA,  
  nRowCluster = 4,  
  nColCluster = 4,  
  main = "Count correlation heatmap",  
  basePlot = TRUE,  
  nBreaks = 50,  
  show_names = c("none", "row", "col", "both"),  
  ...  
)  
  
plotCountGeneSampleHeatmap(  
  ods,  
  normalized = TRUE,  
  rowCentered = TRUE,  
  rowGroups = NA,  
  rowColSet = NA,  
  colGroups = NA,  
  colColSet = NA,  
  nRowCluster = 4,  
  nColCluster = 4,  
  main = "Count Gene vs Sample Heatmap",  
  bcvQuantile = 0.9,  
  show_names = c("none", "col", "row", "both"),  
  nGenes = 500,  
  nBreaks = 50,  
  ...  
)  
  
## S4 method for signature 'OutriderDataSet'  
plotAberrantPerSample(  
  object,  
  main = "Aberrant Genes per Sample",  
  outlierRatio = 0.001,  
  col = "Dark2",  
  yadjust = 1.2,  
  ylab = "#Aberrantly expressed genes",  
  ...  
)
```

```

plotFPKM(ods, bins = 100)

## S4 method for signature 'OutriderDataSet'
plotDispEsts(
  object,
  compareDisp,
  xlim,
  ylim,
  main = "Dispersion estimates versus mean expression",
  ...
)

plotPowerAnalysis(ods)

## S4 method for signature 'OutriderDataSet'
plotEncDimSearch(object)

plotExpressedGenes(ods, main = "Statistics of expressed genes")

plotSizeFactors(ods, basePlot = TRUE)

```

Arguments

| | |
|--------------------------|--|
| ... | Additional parameters passed to plot() or plot_ly() if not stated otherwise in the details for each plot function |
| sampleID, geneID | A sample or gene ID, which should be plotted. Can also be a vector. Integers are treated as indices. |
| main | Title for the plot, if missing a default title will be used. |
| padjCutoff, zScoreCutoff | Significance or Z-score cutoff to mark outliers |
| pch | Integer or character to be used for plotting the points |
| basePlot | if TRUE, use the R base plot version, else use the plotly framework, which is the default |
| col | Set color for the points. If set, it must be a character vector of length 2. (1. normal point; 2. outlier point) or a single character referring to a color palette from RColorBrewer. |
| global | Flag to plot a global Q-Q plot, default FALSE |
| samplePoints | Sample points for Q-Q plot, defaults to max 30k points |
| legendPos | Set legendpos, by default topleft. |
| outlierRatio | The fraction to be used for the outlier sample filtering |
| conf.alpha | If set, a confidence interval is plotted, defaults to 0.05 |
| xlim, ylim | The x/y limits for the plot or NULL to use the full data range |
| ods, object | An OutriderDataSet object. |

| | |
|--------------------------|--|
| log | If TRUE, the default, counts are plotted in log10. |
| groups | A character vector containing either group assignments of samples or sample IDs. Is empty by default. If group assignments are given, the vector must have the same length as the number of samples. If sample IDs are provided the assignment will result in a binary group assignment. |
| groupColSet | A color set from RColorBrewer or a manual vector of colors, which length must match the number of categories from groups. |
| normalized | If TRUE, the normalized counts are used, the default, otherwise the raw counts |
| rowCentered | If TRUE, the counts are row-wise (gene-wise) centered |
| rowGroups, colGroups | A vector of co-factors (colnames of colData) for color coding the rows. It also accepts a data.frame of dim = (#samples, #groups). Must have more than 2 groups. |
| rowColSet, colColSet | A color set from RColorBrewer/colorRampPalette |
| nRowCluster, nColCluster | Number of clusters to show in the row and column dendrograms. If this argument is set the resulting cluster assignments are added to the OutriderDataSet. |
| nBreaks | number of breaks for the heatmap color scheme. Default to 50. |
| show_names | character string indicating whether to show 'none', 'row', 'col', or 'both' names on the heatmap axes. |
| bcvQuantile | quantile for choosing the cutoff for the biological coefficient of variation (BCV) |
| nGenes | upper limit of number of genes (defaults to 500). Subsets the top n genes based on the BCV. |
| yadjust | Option to adjust position of Median and 90 percentile labels. |
| ylab | The y axis label |
| bins | Number of bins used in the histogram. Defaults to 100. |
| compareDisp | If TRUE, the default, and if the autoCorrect normalization was used it computes the dispersion without autoCorrect and plots it for comparison. |

Details

plotAberrantPerSample: The number of aberrant events per sample are plotted sorted by rank. The ... parameters are passed on to the [aberrant](#) function.

plotVolcano: the volcano plot is sample-centric. It plots for a given sample the negative log10 nominal P-values against the Z-scores for all genes.

plotExpressionRank: This function plots for a given gene the expression level against the expression rank for all samples. This can be used with normalized and unnormalized expression values.

plotQQ: the quantile-quantile plot for a given gene or if global is set to TRUE over the full data set. Here the observed P-values are plotted against the expected ones in the negative log10 space.

plotExpectedVsObservedCounts: A scatter plot of the observed counts against the predicted expression for a given gene.

plotCountCorHeatmap: The correlation heatmap of the count data of the full data set. Default the values are log transformed and row centered. This function returns an OutriderDataSet with annotated clusters if requested. The ... arguments are passed to the [pheatmap](#) function.

plotCountGeneSampleHeatmap: A gene x sample heatmap of the raw or normalized counts. By default they are log transformed and row centered. Only the top 500 viable genes based on the BCV (biological coefficient of variation) is used by default.

plotSizeFactors: The sizefactor distribution within the dataset.

plotFPKM: The distribution of FPKM values. If the OutriderDataSet object contains the passedFilter column, it will plot both FPKM distributions for the expressed genes and for the filtered genes.

plotExpressedGenes: A summary statistic plot on the number of genes expressed within this dataset. It plots the sample rank (based on the number of expressed genes) against the accumulated statistics up to the given sample.

plotDispEsts: Plots the dispersion of the OutriderDataSet model against the normalized mean count. If autoCorrect is used it will also estimate the dispersion without normalization for comparison.

plotPowerAnalysis: The power analysis plot should give the user a ruff estimate of the events one can be detected with OTRIDER. Based on the dispersion of the provided OTRIDER data set the theoretical P-value over the mean expression is plotted. This is done for different expression levels. The curves are smooths to make the reading of the plot easier.

plotEncDimSearch: Visualization of the hyperparameter optimization. It plots the encoding dimension against the achieved loss (area under the precision-recall curve). From this plot the optimum should be choosen for the q in fitting process.

Value

If base R graphics are used nothing is returned else the plotly or the gplot object is returned.

Examples

```
ods <- makeExampleOutriderDataSet(dataset="Kremer")
implementation <- 'autoencoder'

mcols(ods)$basepairs <- 300 # assign pseudo gene length for filtering
ods <- filterExpression(ods)
ods <- OTRIDER(ods, implementation=implementation)

plotAberrantPerSample(ods)

plotVolcano(ods, 49)
plotVolcano(ods, 'MUC1365', basePlot=TRUE)

plotExpressionRank(ods, 35)
plotExpressionRank(ods, "NDUFS5", normalized=FALSE,
  log=FALSE, main="Over expression outlier", basePlot=TRUE)

plotQQ(ods, 149)
plotQQ(ods, global=TRUE, outlierRatio=0.001)
```

```

plotExpectedVsObservedCounts(ods, 149)
plotExpectedVsObservedCounts(ods, "ATAD3C", basePlot=TRUE)

plotExpressedGenes(ods)

sex <- sample(c("female", "male"), dim(ods)[2], replace=TRUE)
colData(ods)$Sex <- sex
ods <- plotCountCorHeatmap(ods, nColCluster=4, normalized=FALSE)
ods <- plotCountCorHeatmap(ods, colGroup="Sex", colColSet="Set1")
table(colData(ods)$clusterNumber_4)

plotCountGeneSampleHeatmap(ods, normalized=FALSE)
plotCountGeneSampleHeatmap(ods, rowGroups="theta",
  rowColSet=list(c("white", "darkgreen")))

plotSizeFactors(ods)

mcols(ods)$basepairs <- 1
mcols(ods)$passedFilter <- rowMeans(counts(ods)) > 10
plotFPKM(ods)

plotDispEsts(ods, compareDisp=FALSE)

plotPowerAnalysis(ods)

## Not run:
# for speed reasons we only search for 5 different dimensions
ods <- findEncodingDim(ods, params=c(3, 10, 20, 35, 50),
  implementation=implementation)
plotEncDimSearch(ods)

## End(Not run)

```

results

Accessor function for the 'results' object in an OutriderDataSet object.

Description

This function assembles a results table of significant outlier events based on the given filter criteria. The table contains various information accumulated over the analysis pipeline.

Usage

```

results(object, ...)

## S4 method for signature 'OutriderDataSet'
results(
  object,

```

```

    padjCutoff = 0.05,
    zScoreCutoff = 0,
    round = 2,
    all = FALSE,
    ...
)

```

Arguments

| | |
|--------------|---|
| object | An OutriderDataSet object |
| ... | Additional arguments, currently not used |
| padjCutoff | The significant threshold to be applied |
| zScoreCutoff | If provided additionally a z score threshold is applied |
| round | Can be TRUE, defaults to 2, or an integer used for rounding with round to make the output more user friendly |
| all | By default FALSE, only significant read counts are listed in the results. If TRUE all results are assembled resulting in a data.table of length samples x genes |

Value

A data.table where each row is an outlier event and the columns contain additional information about this event. Eg padj, l2fc

Examples

```

ods <- makeExampleOutriderDataSet()

ods <- OUTRIDER(ods)

res <- results(ods, all=TRUE)
res

```

sampleExclusionMask *Sample exclusion*

Description

To exclude a sample from the fit process, one can use this function to mask specific samples. This can be used if replicates are present.

Usage

```

sampleExclusionMask(ods, aeMatrix = FALSE)

sampleExclusionMask(ods) <- value

```

Arguments

| | |
|----------|---|
| ods | An OutriderDataSet object |
| aeMatrix | If TRUE, it returns a 0/1 matrix for the internal autoencoder functions in the form of feature x sample |
| value | A logical vector of the length of the samples. If TRUE, the corresponding sample will be excluded from the autoencoder fit. |

Value

The exclusion vector/matrix.

Examples

```
ods <- makeExampleOutriderDataSet()
sampleExclusionMask(ods) <- sample(c(FALSE, TRUE), ncol(ods), replace=TRUE)

sampleExclusionMask(ods)
```

sizeFactors

SizeFactors accessor and estimation function

Description

Accessor functions for the 'sizeFactors' information in a OutriderDataSet object.

Usage

```
## S4 method for signature 'OutriderDataSet'
sizeFactors(object)

## S4 replacement method for signature 'OutriderDataSet,numeric'
sizeFactors(object) <- value

## S4 replacement method for signature 'OutriderDataSet,`NULL`'
sizeFactors(object) <- value

## S4 method for signature 'OutriderDataSet'
estimateSizeFactors(object)
```

Arguments

| | |
|--------|---------------------------------|
| object | OutriderDataSet |
| value | A numeric vector of sizeFactors |

Details

The estimation of the size factors can also make use of the existing log geometric means in the object. Those can be loaded from an existing model.

Value

An `OutriderDataSet` with the estimated `sizeFactors`, or with the getter function it returns a numeric vector containing the `sizeFactors`.

See Also

[estimateSizeFactors](#)

Examples

```
ods <- makeExampleOutriderDataSet()
ods <- estimateSizeFactors(ods)
head(sizeFactors(ods))

sizeFactors(ods) <- runif(dim(ods)[2], 0.5, 1.5)
sizeFactors(ods)
counts(ods, normalized=TRUE)[1:10,1:10]
```

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