Package ‘metagenomeSeq’

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Title  Statistical analysis for sparse high-throughput sequencing
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Description metagenomeSeq is designed to determine features (be it Operational Taxanomic Unit (OTU), species, etc.) that are differentially abundant between two or more groups of multiple samples. metagenomeSeq is designed to address the effects of both normalization and under-sampling of microbial communities on disease association detection and the testing of feature correlations.

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Depends R(>= 3.0), Biobase, limma, glmnet, methods, RColorBrewer
Suggests annotate, BiocGenerics, biomformat, knitr, gss, testthat (>= 0.8), vegan, interactiveDisplay
Imports parallel, matrixStats, foreach, Matrix, gplots, graphics, grDevices, stats, utils, Wrench, IHW

VignetteBuilder knitr

URL  https://github.com/nosson/metagenomeSeq/

BugReports https://github.com/nosson/metagenomeSeq/issues

biocViews  ImmunoOncology, Classification, Clustering, GeneticVariability, DifferentialExpression, Microbiome, Metagenomics, Normalization, Visualization, MultipleComparison, Sequencing, Software

RoxygenNote  6.1.1

git_url https://git.bioconductor.org/packages/metagenomeSeq

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R topics documented:

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Description

metagenomeSeq is designed to determine features (be it Operational Taxonomic Unit (OTU), species, etc.) that are differentially abundant between two or more groups of multiple samples. metagenomeSeq is designed to address the effects of both normalization and under-sampling of microbial communities on disease association detection and the testing of feature correlations.

A user’s guide is available, and can be opened by typing vignette("metagenomeSeq")

The metagenomeSeq package implements novel normalization and statistical methodology in the following papers.
aggregateBySample

**Author(s)**

Paulson, JN <jpaulson@umiacs.umd.edu>; Pop, M; Corrada Bravo, H

**References**


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**aggregateBySample**

Aggregates a MRexperiment object or counts matrix to by a factor.

**Description**

Using the phenoData information in the MRexperiment, calling aggregateBySample on a MRexperiment and a particular phenoData column (i.e. 'diet') will aggregate counts using the aggfun function (default rowMeans). Possible aggfun alternatives include rowMeans and rowMedians.

**Usage**

```r
aggregateBySample(obj, fct, aggfun = rowMeans, out = "MRexperiment")
```

```r
aggSamp(obj, fct, aggfun = rowMeans, out = "MRexperiment")
```

**Arguments**

- `obj` A MRexperiment object or count matrix.
- `fct` phenoData column name from the MRexperiment object or if count matrix object a vector of labels.
- `aggfun` Aggregation function.
- `out` Either 'MRexperiment' or 'matrix'

**Value**

An aggregated count matrix or MRexperiment object where the new pData is a vector of 'fct' levels.

**Examples**

```r
data(mouseData)
aggregateBySample(mouseData[1:100,,], fct="diet", aggfun=rowSums)
# not run
# aggregateBySample(mouseData, fct="diet", aggfun=matrixStats::rowMedians)
# aggSamp(mouseData, fct='diet', aggfun=rowMaxs)
```
aggregateByTaxonomy

Aggregates a MRexperiment object or counts matrix to a particular level.

Description

Using the featureData information in the MRexperiment, calling aggregateByTaxonomy on a MRexperiment and a particular featureData column (i.e. 'genus') will aggregate counts to the desired level using the aggfun function (default colSums). Possible aggfun alternatives include colMeans and colMedians.

Usage

aggregateByTaxonomy(obj, lvl, alternate = FALSE, norm = FALSE, log = FALSE, aggfun = colSums, sl = 1000, featureOrder = NULL, returnFullHierarchy = TRUE, out = "MRexperiment")

aggTax(obj, lvl, alternate = FALSE, norm = FALSE, log = FALSE, aggfun = colSums, sl = 1000, featureOrder = NULL, returnFullHierarchy = TRUE, out = "MRexperiment")

Arguments

obj A MRexperiment object or count matrix.
lvl featureData column name from the MRexperiment object or if count matrix object a vector of labels.
alternate Use the rowname for undefined OTUs instead of aggregating to "no_match".
norm Whether to aggregate normalized counts or not.
log Whether or not to log2 transform the counts - if MRexperiment object.
aggfun Aggregation function.
sl scaling value, default is 1000.
featureOrder Hierarchy of levels in taxonomy as fData colnames
returnFullHierarchy Boolean value to indicate return single column of fData or all columns of hierarchy
out Either 'MRexperiment' or 'matrix'

Value

An aggregated count matrix.

Examples

data(mouseData)
aggregateByTaxonomy(mouseData[1:100,,],lvl="class",norm=TRUE,aggfun=colSums)
# not run
# aggregateByTaxonomy(mouseData,lvl="class",norm=TRUE,aggfun=colMedians)
# aggTax(mouseData,lvl="phylum",norm=FALSE,aggfun=colSums)
b biom2MRexperiment  Biom to MRexperiment objects

Description
Wrapper to convert biom files to MRexperiment objects.

Usage
biom2MRexperiment(obj)

Arguments
obj The biom object file.

Value
A MRexperiment object.

See Also
loadMeta loadPhenoData newMRexperiment loadBiom

Examples
library(biomformat)
rich_dense_file = system.file("extdata", "rich_dense_otu_table.biom", package = "biomformat")
x = biomformat::read_biom(rich_dense_file)
biom2MRexperiment(x)

calcNormFactors  Cumulative sum scaling (css) normalization factors

Description
Return a vector of the the sum up to and including a quantile.

Usage
calcNormFactors(obj, p = cumNormStatFast(obj))

Arguments
obj  An MRexperiment object or matrix.
p The pth quantile.

Value
Vector of the sum up to and including a sample’s pth quantile.
calcPosComponent

See Also

fitZig cumNormStatFast cumNorm

Examples

data(mouseData)
head(calcNormFactors(mouseData))

calcPosComponent Positive component

Description

Fit the positive (log-normal) component

Usage

calcPosComponent(mat, mod, weights)

Arguments

mat A matrix of normalized counts
mod A model matrix
weights Weight matrix for samples and counts

See Also

fitZeroLogNormal fitFeatureModel

calcShrinkParameters Calculate shrinkage parameters

Description

Calculate the shrunken variances and variance of parameters of interest across features.

Usage

calcShrinkParameters(fit, coef, mins2, exclude = NULL)

Arguments

fit A matrix of fits as outputted by calcZeroComponent or calcPosComponent
coef Coefficient of interest
mins2 minimum variance estimate
exclude Vector of features to exclude when shrinking

See Also

fitZeroLogNormal fitFeatureModel
**calcStandardError**  
*Calculate the zero-inflated log-normal statistic’s standard error*

**Description**
Calculated the se for the model. Code modified from "Adjusting for covariates in zero-inflated gamma and zero-inflated log-normal models for semicontinuous data", ED Mills

**Usage**
calcStandardError(mod, fitln, fitzero, coef = 2, exclude = NULL)

**Arguments**
- `mod`: The zero component model matrix
- `fitln`: A matrix with parameters from the log-normal fit
- `fitzero`: A matrix with parameters from the logistic fit
- `coef`: Coefficient of interest
- `exclude`: List of features to exclude

**See Also**
- `fitZeroLogNormal`
- `fitFeatureModel`

---

**calculateEffectiveSamples**  
*Estimated effective samples per feature*

**Description**
Calculates the number of estimated effective samples per feature from the output of a fitZig run. The estimated effective samples per feature is calculated as the sum_1^n (n = number of samples) 1-z_i where z_i is the posterior probability a feature belongs to the technical distribution.

**Usage**
calculateEffectiveSamples(obj)

**Arguments**
- `obj`: The output of fitZig run on a MRexperiment object.

**Value**
A list of the estimated effective samples per feature.

**See Also**
- `fitZig`
- `MRcoefs`
- `MRfulltable`
**calcZeroAdjustment**

*Calculate the zero-inflated component's adjustment factor*

**Description**

Calculate the log ratio of average marginal probabilities for each sample having a positive count. This becomes the adjustment factor for the log fold change.

**Usage**

```
calcZeroAdjustment(fitln, fitzero, mod, coef, exclude = NULL)
```

**Arguments**

- `fitln`: A matrix with parameters from the log-normal fit
- `fitzero`: A matrix with parameters from the logistic fit
- `mod`: The zero component model matrix
- `coef`: Coefficient of interest
- `exclude`: List of features to exclude

**See Also**

`fitZeroLogNormal` `fitFeatureModel`

---

**calcZeroComponent**

*Zero component*

**Description**

Fit the zero (logistic) component

**Usage**

```
calcZeroComponent(mat, mod, weights)
```

**Arguments**

- `mat`: A matrix of normalized counts
- `mod`: A model matrix
- `weights`: Weight matrix for samples and counts

**See Also**

`fitZeroLogNormal` `fitFeatureModel`
correctIndices  
*Calculate the correct indices for the output of correlationTest*

**Description**

Consider the upper triangular portion of a matrix of size nxn. Results from the correlationTest are output as the combination of two vectors, correlation statistic and p-values. The order of the output is 1vs2, 1vs3, 1vs4, etc. The correctIndices returns the correct indices to fill a correlation matrix or correlation-pvalue matrix.

**Usage**

```r
correctIndices(n)
```

**Arguments**

- `n`  
The number of features compared by correlationTest (nrow(mat)).

**Value**

A vector of the indices for an upper triangular matrix.

**See Also**

- correlationTest

**Examples**

```r
data(mouseData)
mat = MRcounts(mouseData)[55:60,]
cors = correlationTest(mat)
ind = correctIndices(nrow(mat))

cormat = as.matrix(dist(mat))
cormat[cormat>0] = 0
cormat[upper.tri(cormat)][ind] = cors[,1]
table(cormat[1,-1] - cors[1:5,1])
```

---

correlationTest  
*Correlation of each row of a matrix or MRexperiment object*

**Description**

Calculates the (pairwise) correlation statistics and associated p-values of a matrix or the correlation of each row with a vector.
correlationTest

Usage

correlationTest(obj, y = NULL, method = "pearson",
  alternative = "two.sided", norm = TRUE, log = TRUE, cores = 1,
  override = FALSE, ...)

Arguments

  obj    A MRexperiment object or count matrix.
  y      Vector of length ncol(obj) to compare to.
  method One of 'pearson','spearman', or 'kendall'.
  alternative Indicates the alternative hypothesis and must be one of 'two.sided', 'greater' (positive) or 'less'(negative). You can specify just the initial letter.
  norm   Whether to aggregate normalized counts or not - if MRexperiment object.
  log    Whether or not to log2 transform the counts - if MRexperiment object.
  cores  Number of cores to use.
  override If the number of rows to test is over a thousand the test will not commence (unless override==TRUE).
  ...    Extra parameters for mclapply.

Value

A matrix of size choose(number of rows, 2) by 2. The first column corresponds to the correlation value. The second column the p-value.

See Also

correctIndices

Examples

# Pairwise correlation of raw counts
data(mouseData)
cors = correlationTest(mouseData[1:10,],norm=FALSE,log=FALSE)
head(cors)

mat = MRcounts(mouseData)[1:10,]
corman = as.matrix(dist(mat)) # Creating a matrix
cormat[cormat>0] = 0 # Creating an empty matrix
ind = correctIndices(nrow(mat))
corman[upper.tri(corman)][ind] = cors[,1]
table(corman[1,-1] - cors[1:9,1])

# Correlation of raw counts with a vector (library size in this case)
data(mouseData)
cors = correlationTest(mouseData[1:10,],libSize(mouseData),norm=FALSE,log=FALSE)
head(cors)
**cumNorm**

*Cumulative sum scaling normalization*

**Description**

Calculates each column’s quantile and calculates the sum up to and including that quantile.

**Usage**

```
cumNorm(obj, p = cumNormStatFast(obj))
```

**Arguments**

- **obj**: An MRexperiment object.
- **p**: The pth quantile.

**Value**

Object with the normalization factors stored as a vector of the sum up to and including a sample’s pth quantile.

**See Also**

`fitZig` `cumNormStat`

**Examples**

```
data(mouseData)
mouseData <- cumNorm(mouseData)
head(normFactors(mouseData))
```

---

**cumNormMat**

*Cumulative sum scaling factors.*

**Description**

Calculates each column’s quantile and calculates the sum up to and including that quantile.

**Usage**

```
cumNormMat(obj, p = cumNormStatFast(obj), sl = 1000)
```

**Arguments**

- **obj**: A matrix or MRexperiment object.
- **p**: The pth quantile.
- **sl**: The value to scale by (default=1000).
cumNormStat

Value

Returns a matrix normalized by scaling counts up to and including the pth quantile.

See Also

fitZig cumNorm

Examples

data(mouseData)
head(cumNormMat(mouseData))

cumNormStat

Cumulative sum scaling percentile selection

Description

Calculates the percentile for which to sum counts up to and scale by. cumNormStat might be deprecated one day. Deviates from methods in Nature Methods paper by making use row means for generating reference.

Usage

cumNormStat(obj, qFlag = TRUE, pFlag = FALSE, rel = 0.1, ...)

Arguments

obj A matrix or MRexperiment object.
qFlag Flag to either calculate the proper percentile using R’s step-wise quantile function or approximate function.
pFlag Plot the relative difference of the median deviance from the reference.
rel Cutoff for the relative difference from one median difference from the reference to the next
... Applicable if pFlag == TRUE. Additional plotting parameters.

Value

Percentile for which to scale data

See Also

fitZig cumNorm cumNormStatFast

Examples

data(mouseData)
p = round(cumNormStat(mouseData,pFlag=FALSE),digits=2)
cumNormStatFast  Cumulative sum scaling percentile selection

Description
Calculates the percentile for which to sum counts up to and scale by. Faster version than available in cumNormStat. Deviates from methods described in Nature Methods by making use of ro means for reference.

Usage
cumNormStatFast(obj, pFlag = FALSE, rel = 0.1, ...)

Arguments
- obj: A matrix or MRexperiment object.
- pFlag: Plot the median difference quantiles.
- rel: Cutoff for the relative difference from one median difference from the reference to the next.
- ...: Applicable if pFlag == TRUE. Additional plotting parameters.

Value
Percentile for which to scale data

See Also
fitZig cumNorm cumNormStat

Examples
data(mouseData)
p = round(cumNormStatFast(mouseData,pFlag=FALSE),digits=2)

doCountMStep  Compute the Maximization step calculation for features still active.

Description
Maximization step is solved by weighted least squares. The function also computes counts residuals.

Usage
doCountMStep(z, y, mmCount, stillActive, fit2 = NULL, dfMethod = "modified")
doEStep

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>z</td>
<td>Matrix (m x n) of estimate responsiblities (probabilities that a count comes from a spike distribution at 0).</td>
</tr>
<tr>
<td>y</td>
<td>Matrix (m x n) of count observations.</td>
</tr>
<tr>
<td>mmCount</td>
<td>Model matrix for the count distribution.</td>
</tr>
<tr>
<td>stillActive</td>
<td>Boolean vector of size M, indicating whether a feature converged or not.</td>
</tr>
<tr>
<td>fit2</td>
<td>Previous fit of the count model.</td>
</tr>
<tr>
<td>dfMethod</td>
<td>Either ‘default’ or ‘modified’ (by responsibilities)</td>
</tr>
</tbody>
</table>

Details

Maximum-likelihood estimates are approximated using the EM algorithm where we treat mixture membership $\delta_{ij} = 1$ if $y_{ij}$ is generated from the zero point mass as latent indicator variables. The density is defined as $f_{\text{zig}}(y_{ij} = \pi_j(S_j) * f_0(y_{ij}) + (1-\pi_j(S_j)) * f_{\text{count}}(y_{ij};\mu_i,\sigma_i^2)$. The log-likelihood in this extended model is $(1-\delta_{ij}) \log f_{\text{count}}(y;\mu_i,\sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$. The responsibilities are defined as $z_{ij} = \Pr(\delta_{ij}=1 | \text{data})$.

Value

Update matrix (m x n) of estimate responsiblities (probabilities that a count comes from a spike distribution at 0).

See Also

fitZig

doEStep

Compute the Expectation step.

Description

Estimates the responsibilities $z_{ij} = \frac{\pi_j \cdot I_0(y_{ij}) \cdot \pi_j \cdot I_0(y_{ij})}{\pi_j \cdot I_0(y_{ij}) + (1-\pi_j) \cdot f_{\text{count}}(y_{ij})}$. The log-likelihood in this extended model is $(1-\delta_{ij}) \log f_{\text{count}}(y;\mu_i,\sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$. The responsibilities are defined as $z_{ij} = \Pr(\delta_{ij}=1 | \text{data})$.

Usage

doEStep(countResiduals, zeroResiduals, zeroIndices)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>countResiduals</td>
<td>Residuals from the count model.</td>
</tr>
<tr>
<td>zeroResiduals</td>
<td>Residuals from the zero model.</td>
</tr>
<tr>
<td>zeroIndices</td>
<td>Index (matrix m x n) of counts that are zero/non-zero.</td>
</tr>
</tbody>
</table>

Details

Maximum-likelihood estimates are approximated using the EM algorithm where we treat mixture membership $\delta_{ij}=1$ if $y_{ij}$ is generated from the zero point mass as latent indicator variables. The density is defined as $f_{\text{zig}}(y_{ij} = \pi_j(S_j) \cdot f_0(y_{ij}) + (1-\pi_j(S_j)) \cdot f_{\text{count}}(y_{ij};\mu_i,\sigma_i^2)$$. The log-likelihood in this extended model is $(1-\delta_{ij}) \log f_{\text{count}}(y;\mu_i,\sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$. The responsibilities are defined as $z_{ij} = \Pr(\delta_{ij}=1 | \text{data})$. 

doZeroMStep

Value

Updated matrix (m x n) of estimate responsibilities (probabilities that a count comes from a spike distribution at 0).

See Also

fitZig

Description

Performs Maximization step calculation for the mixture components. Uses least squares to fit the parameters of the mean of the logistic distribution. $$\pi_j = \sum_i^M \frac{1}{M} z_{ij}$$ Maximum-likelihood estimates are approximated using the EM algorithm where we treat mixture membership $$\delta_{ij} = 1$$ if $$y_{ij}$$ is generated from the zero point mass as latent indicator variables. The density is defined as $$f_{zig}(y_{ij} = \pi_j(S_j) \cdot f_0(y_{ij}) + (1 - \pi_j (S_j)) \cdot f_{count}(y_{ij}; \mu_i, \sigma_i^2)$$.

The log-likelihood in this extended model is $$(1-\delta_{ij}) \log f_{count}(y; \mu_i, \sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$$. The responsibilities are defined as $$z_{ij} = \text{pr}(\delta_{ij}=1 | \text{data})$$.

Usage

doZeroMStep(z, zeroIndices, mmZero)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>z</td>
<td>Matrix (m x n) of estimate responsibilities (probabilities that a count comes from a spike distribution at 0).</td>
</tr>
<tr>
<td>zeroIndices</td>
<td>Index (matrix m x n) of counts that are zero/non-zero.</td>
</tr>
<tr>
<td>mmZero</td>
<td>The zero model, the model matrix to account for the change in the number of OTUs observed as a linear effect of the depth of coverage.</td>
</tr>
</tbody>
</table>

Value

List of the zero fit (zero mean model) coefficients, variance - scale parameter (scalar), and normalized residuals of length sum(zeroIndices).

See Also

fitZig
**exportMat**  
*Export the normalized MRexperiment dataset as a matrix.*

**Description**  
This function allows the user to take a dataset of counts and output the dataset to the user’s workspace as a tab-delimited file, etc.

**Usage**  
```r  
exportMat(obj, log = TRUE, norm = TRUE, sep = "\t",  
file = "~/Desktop/matrix.tsv")  
```

**Arguments**
- **obj**: A MRexperiment object or count matrix.
- **log**: Whether or not to log transform the counts - if MRexperiment object.
- **norm**: Whether or not to normalize the counts - if MRexperiment object.
- **sep**: Separator for writing out the count matrix.
- **file**: Output file name.

**Value**
NA

**See Also**
- `cumNorm`

**Examples**
```r  
data(lungData)  
dataDirectory <- system.file("extdata", package="metagenomeSeq")  
exportMat(lungData[,1:5],file=file.path(dataDirectory,"tmp.tsv"))  
head(read.csv(file=file.path(dataDirectory,"tmp.tsv"),sep="\t"))  
```

---

**exportStats**  
*Various statistics of the count data.*

**Description**  
A matrix of values for each sample. The matrix consists of sample ids, the sample scaling factor, quantile value, the number identified features, and library size (depth of coverage).

**Usage**  
```r  
exportStats(obj, p = cumNormStat(obj),  
file = "~/Desktop/res.stats.tsv")  
```
expSummary

Arguments

  obj  A MRexperiment object with count data.
  p    Quantile value to calculate the scaling factor and quantiles for the various samples.
  file Output file name.

Value

  None.

See Also

  cumNorm quantile

Examples

data(lungData)
dataDirectory <- system.file("extdata", package="metagenomeSeq")
exportStats(lungData[,1:5], file=file.path(dataDirectory,"tmp.tsv"))
head(read.csv(file=file.path(dataDirectory,"tmp.tsv"), sep="\t"))

---

expSummary  Access MRexperiment object experiment data

Description

  The expSummary vectors represent the column (sample specific) sums of features, i.e. the total number of reads for a sample, libSize and also the normalization factors, normFactor.

Usage

  expSummary(obj)

Arguments

  obj  a MRexperiment object.

Value

  Experiment summary table

Author(s)

  Joseph N. Paulson, jpmallon@umiacs.umd.edu

Examples

data(mouseData)
expSummary(mouseData)
**extractMR**

*Extract the essentials of an MRexperiment.*

**Description**

Extract the essentials of an MRexperiment.

**Usage**

```r
eextractMR(obj)
```

**Arguments**

- `obj` MRexperiment-class object.

**Value**

A list containing:

- `counts` : Count data
- `librarySize` : The column sums / library size / sequencing depth
- `normFactors` : The normalization scaling factors
- `pheno` : phenotype table
- `feat` : feature table

**Examples**

```r
data(mouseData)
head(metagenomeSeq:::extractMR(mouseData))
```

**filterData**

*Filter datasets according to no. features present in features with at least a certain depth.*

**Description**

Filter the data based on the number of present features after filtering samples by depth of coverage. There are many ways to filter the object, this is just one way.

**Usage**

```r
filterData(obj, present = 1, depth = 1000)
```

**Arguments**

- `obj` A MRexperiment object or count matrix.
- `present` Features with at least 'present' positive samples.
- `depth` Samples with at least this much depth of coverage
Value

A MRexperiment object.

Examples

data(mouseData)
filterData(mouseData)

fitDO

Wrapper to calculate Discovery Odds Ratios on feature values.

Description

This function returns a data frame of p-values, odds ratios, lower and upper confidence limits for every row of a matrix. The discovery odds ratio is calculated as using Fisher’s exact test on actual counts. The test’s hypothesis is whether or not the discovery of counts for a feature (of all counts) is found in greater proportion in a particular group.

Usage

fitDO(obj, cl, norm = TRUE, log = TRUE, adjust.method = "fdr",
cores = 1, ...)

Arguments

obj A MRexperiment object with a count matrix, or a simple count matrix.
cl Group comparison
norm Whether or not to normalize the counts - if MRexperiment object.
log Whether or not to log2 transform the counts - if MRexperiment object.
adjust.method Method to adjust p-values by. Default is "FDR". Options include "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See p.adjust for more details.
cores Number of cores to use.
... Extra options for makeCluster

Value

Matrix of odds ratios, p-values, lower and upper confidence intervals

See Also
cumNorm fitZig fitPA fitMeta
Examples

```r
data(lungData)
k = grep("Extraction.Control", pData(lungData)$SampleType)
lungTrim = lungData[-k]
lungTrim = lungTrim[which(rowSums(MRcounts(lungTrim)>0)<20),]
res = fitDO(lungTrim, pData(lungTrim)$SmokingStatus);
head(res)
```

---

**fitFeatureModel** Computes differential abundance analysis using a zero-inflated log-normal model

**Description**

Wrapper to actually run zero-inflated log-normal model given a MRexperiment object and model matrix. User can decide to shrink parameter estimates.

**Usage**

```r
fitFeatureModel(obj, mod, coef = 2, B = 1, szero = FALSE, spos = TRUE)
```

**Arguments**

- `obj`: A MRexperiment object with count data.
- `mod`: The model for the count distribution.
- `coef`: Coefficient of interest to grab log fold-changes.
- `B`: Number of bootstraps to perform if >1. If >1 performs permutation test.
- `szero`: TRUE/FALSE, shrink zero component parameters.
- `spos`: TRUE/FALSE, shrink positive component parameters.

**Value**

A list of objects including:
- `call`: the call made to `fitFeatureModel`
- `fitZeroLogNormal`: list of parameter estimates for the zero-inflated log normal model
- `design`: model matrix
- `taxa`: taxa names
- `counts`: count matrix
- `pvalues`: calculated p-values
- `permuttedfits`: permuted z-score estimates under the null

**See Also**

`cumNorm`
Examples

```r
data(lungData)
lungData = lungData[, -which(is.na(pData(lungData)$SmokingStatus))]
lungData = filterData(lungData, present = 30, depth = 1)
lungData <- cumNorm(lungData, p = .5)
s <- normFactors(lungData)
pd <- pData(lungData)
mod <- model.matrix(~1 + SmokingStatus, data = pd)
lungres1 = fitFeatureModel(lungData, mod)
```

---

**fitFeatureModelResults-class**

Class "fitFeatureModelResults" – a formal class for storing results from a fitFeatureModel call

**Description**

This class contains all of the same information expected from a fitFeatureModel call, but it is defined in the S4 style as opposed to being stored as a list.

**Slots**

- `call` the call made to `fitFeatureModel`
- `fitZeroLogNormal` list of parameter estimates for the zero-inflated log normal model
- `design` model matrix
- `taxa` taxa names
- `counts` count matrix
- `pvalues` calculated p-values
- `permuttedFits` permuted z-score estimates under the null

---

**fitLogNormal**

*Computes a log-normal linear model and permutation based p-values.*

**Description**

Wrapper to perform the permutation test on the t-statistic. This is the original method employed by metastats (for non-sparse large samples). We include CSS normalization though (optional) and log2 transform the data. In this method the null distribution is not assumed to be a t-dist.

**Usage**

```r
fitLogNormal(obj, mod, useCSSoffset = TRUE, B = 1000, coef = 2, sl = 1000)
```
Arguments

obj A MRexperiment object with count data.
mod The model for the count distribution.
useCSSoffset Boolean, whether to include the default scaling parameters in the model or not.
B Number of permutations.
coef The coefficient of interest.
s The value to scale by (default=1000).

Value

Call made, fit object from lmFit, t-statistics and p-values for each feature.

Examples

# This is a simple demonstration
data(lungData)
k = grep("Extraction.Control",pData(lungData)$SampleType)
lungTrim = lungData[,,-k]
k = which(rowSums(MRcounts(lungTrim)>0)<3)
lungTrim = cumNorm(lungTrim)
lungTrim = lungTrim[-k,]
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
fit = fitLogNormal(obj = lungTrim,mod=mod,B=1)

fitMultipleTimeSeries Discover differentially abundant time intervals for all bacteria

Description

Calculate time intervals of significant differential abundance over all bacteria of a particularly specified level (lvl). If not lvl is specified, all OTUs are analyzed. Warning, function can take a while

Usage

fitMultipleTimeSeries(obj, lvl = NULL, B = 1, featureOrder = NULL, ...)

Arguments

obj metagenomeSeq MRexperiment-class object.
lvl Vector or name of column in featureData of MRexperiment-class object for aggregating counts (if not OTU level).
B Number of permutations to perform.
featureOrder Hierarchy of levels in taxonomy as fData colnames
... Options for fitTimeSeries, except feature.
Value

List of lists of matrices of time point intervals of interest, Difference in abundance area and p-value, fit, area permutations.
A list of lists for which each includes:

- timeIntervals - Matrix of time point intervals of interest, area of differential abundance, and pvalue.
- data - Data frame of abundance, class indicator, time, and id input.
- fit - Data frame of fitted values of the difference in abundance, standard error estimates and timepoints interpolated over.
- perm - Differential abundance area estimates for each permutation.
- call - Function call.

See Also
cumNorm fitSSTimeSeries fitTimeSeries

Examples

data(mouseData)
res = fitMultipleTimeSeries(obj=mouseData,lvl='phylum',class="status",
id="mouseID",time="relativeTime",B=1)

fitPA

Wrapper to run fisher’s test on presence/absence of a feature.

Description

This function returns a data frame of p-values, odds ratios, lower and upper confidence limits for every row of a matrix.

Usage

fitPA(obj, cl, thres = 0, adjust.method = "fdr", cores = 1, ...)

Arguments

obj A MRexperiment object with a count matrix, or a simple count matrix.
cl Group comparison
thres Threshold for defining presence/absence.
adjust.method Method to adjust p-values by. Default is "FDR". Options include "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See p.adjust for more details.
cores Number of cores to use.
... Extra parameters for makeCluster
fitSSTimeSeries

Value

Matrix of odds ratios, p-values, lower and upper confidence intervals

See Also

cumNorm fitZig fitDO fitMeta

Examples

data(lungData)
k = grep("Extraction.Control", pData(lungData)$SampleType)
lungTrim = lungData[-k]
lungTrim = lungTrim[-which(rowSums(MRcounts(lungTrim)>0)<=20),]
res = fitPA(lungTrim,pData(lungTrim)$SmokingStatus);
head(res)

Description

Calculate time intervals of interest using SS-Anova fitted models. Fitting is performed uses Smoothing Spline ANOVA (SS-Anova) to find interesting intervals of time. Given observations at different time points for two groups, fitSSTimeSeries calculates a function that models the difference in abundance between two groups across all time. Using permutations we estimate a null distribution of areas for the time intervals of interest and report significant intervals of time. Use of the function for analyses should cite: "Finding regions of interest in high throughput genomics data using smoothing splines" Talukder H, Paulson JN, Bravo HC. (In preparation)

Usage

fitSSTimeSeries(obj, formula, feature, class, time, id, lvl = NULL, include = c("class", "time:class"), C = 0, B = 1000, norm = TRUE, log = TRUE, sl = 1000, featureOrder = NULL, ...)

Arguments

obj metagenomeSeq MRexperiment-class object.
formula Formula for ssanova. Of the form: abundance ~ ... where ... includes any pData slot value.
feature Name or row of feature of interest.
class Name of column in phenoData of MRexperiment-class object for class membership.
time Name of column in phenoData of MRexperiment-class object for relative time.
id Name of column in phenoData of MRexperiment-class object for sample id.
lvl Vector or name of column in featureData of MRexperiment-class object for aggregating counts (if not OTU level).
include Parameters to include in prediction.
fitTimeSeries

Discover differentially abundant time intervals

Description

Calculate time intervals of significant differential abundance. Currently only one method is implemented (ssanova). fitSSTimeSeries is called with method="ssanova".

Usage

```r
fitTimeSeries(obj, formula, feature, class, time, id, method = c("ssanova"), lvl = NULL, include = c("class", "time:class"), C = 0, B = 1000, norm = TRUE, log = TRUE, sl = 1000, featureOrder = NULL, ...)
```
Arguments

- **obj**: metagenomeSeq MRexperiment-class object.
- **formula**: Formula for ssanova. Of the form: abundance ~ ... where ... includes any pData slot value.
- **feature**: Name or row of feature of interest.
- **class**: Name of column in phenoData of MRexperiment-class object for class membership.
- **time**: Name of column in phenoData of MRexperiment-class object for relative time.
- **id**: Name of column in phenoData of MRexperiment-class object for sample id.
- **method**: Method to estimate time intervals of differentially abundant bacteria (only ssanova method implemented currently).
- **lvl**: Vector or name of column in featureData of MRexperiment-class object for aggregating counts (if not OTU level).
- **include**: Parameters to include in prediction.
- **C**: Value for which difference function has to be larger or smaller than (default 0).
- **B**: Number of permutations to perform.
- **norm**: When aggregating counts to normalize or not.
- **log**: Log2 transform.
- **sl**: Scaling value.
- **featureOrder**: Hierarchy of levels in taxonomy as fData colnames
- **...**: Options for ssanova

Value

List of matrix of time point intervals of interest, Difference in abundance area and p-value, fit, area permutations, and call.

A list of objects including:

- **timeIntervals**: Matrix of time point intervals of interest, area of differential abundance, and p-value.
- **data**: Data frame of abundance, class indicator, time, and id input.
- **fit**: Data frame of fitted values of the difference in abundance, standard error estimates and timepoints interpolated over.
- **perm**: Differential abundance area estimates for each permutation.
- **call**: Function call.

See Also

cumNorm fitSSTimeSeries plotTimeSeries

Examples

data(mouseData)
res = fitTimeSeries(obj=mouseData, feature="Actinobacteria", class="status", id="mouseID", time="relativeTime", lvl='class', B=2)
fitZeroLogNormal  Compute the log fold-change estimates for the zero-inflated log-normal model

Description
Run the zero-inflated log-normal model given a MRexperiment object and model matrix. Not for the average user, assumes structure of the model matrix.

Usage
fitZeroLogNormal(obj, mod, coef = 2, szero = TRUE, spos = TRUE)

Arguments
- obj: A MRexperiment object with count data.
- mod: The model for the count distribution.
- coef: Coefficient of interest to grab log fold-changes.
- szero: TRUE/FALSE, shrink zero component parameters.
- spos: TRUE/FALSE, shrink positive component parameters.

Value
A list of objects including:
- logFC - the log fold-change estimates
- adjFactor - the adjustment factor based on the zero component
- se - standard error estimates
- fitln - parameters from the log-normal fit
- fitzero - parameters from the logistic fit
- zeroRidge - output from the ridge regression
- posRidge - output from the ridge regression
- tauPos - estimated tau^2 for positive component
- tauZero - estimated tau^2 for zero component
- exclude - features to exclude for various reasons, e.g. all zeros
- zeroExclude - features to exclude for various reasons, e.g. all zeros

See Also
cumNorm fitFeatureModel
fitZig

**Description**

Wrapper to actually run the Expectation-maximization algorithm and estimate $f_{\text{count}}$ fits. Maximum-likelihood estimates are approximated using the EM algorithm where we treat mixture membership $\delta_{ij} = 1$ if $y_{ij}$ is generated from the zero point mass as latent indicator variables. The density is defined as $f_{\text{zig}}(y_{ij} = \pi_j(S_j)^* f_0(y_{ij}) + (1-\pi_j(S_j))* f_{\text{count}}(y_{ij}; \mu_i, \sigma_i^2)$. The log-likelihood in this extended model is: $(1-\delta_{ij}) \log f_{\text{count}}(y;\mu_i,\sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$. The responsibilities are defined as $z_{ij} = \text{pr}(\delta_{ij}=1 | \text{data})$.

**Usage**

```r
fitZig(obj, mod, zeroMod = NULL, useCSSoffset = TRUE, control = zigControl(), useMixedModel = FALSE, ...)
```

**Arguments**

- **obj**: A MRexperiment object with count data.
- **mod**: The model for the count distribution.
- **zeroMod**: The zero model, the model to account for the change in the number of OTUs observed as a linear effect of the depth of coverage.
- **useCSSoffset**: Boolean, whether to include the default scaling parameters in the model or not.
- **control**: The settings for fitZig.
- **useMixedModel**: Estimate the correlation between duplicate features or replicates using duplicateCorrelation.
- **...**: Additional parameters for duplicateCorrelation.

**Value**

A list of objects including:

- call - the call made to fitZig
- fit - 'MLArrayLM' Limma object of the weighted fit
- countResiduals - standardized residuals of the fit
- z - matrix of the posterior probabilities
- eb - output of eBayes, moderated t-statistics, moderated F-statistics, etc
- taxa - vector of the taxa names
- counts - the original count matrix input
- zeroMod - the zero model matrix
- zeroCoef - the zero model fitted results
- stillActive - convergence
- stillActiveNLL - nll at convergence
- dupcor - correlation of duplicates
See Also

cumNorm zigControl

Examples

# This is a simple demonstration
data(lungData)
k = grep("Extraction.Control",pData(lungData)$SampleType)
lungTrim = lungData[-k]
k = which(rowSums(MRcounts(lungTrim)>0)<30)
lungTrim = cumNorm(lungTrim)
lungTrim = lungTrim[-k,]
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
# The maxit is not meant to be 1 - this is for demonstration/speed
settings = zigControl(maxit=1,verbose=FALSE)
fit = fitZig(obj = lungTrim,mod=mod,control=settings)

fitZigResults-class

Class "fitZigResults" – a formal class for storing results from a fitZig call

Description

This class contains all of the same information expected from a fitZig call, but it is defined in the S4 style as opposed to being stored as a list.

Slots

call  the call made to fitZig
fit  'MLArrayLM' Limma object of the weighted fit
countResiduals  standardized residuals of the fit
z  matrix of the posterior probabilities. It is defined as $z_{ij} = pr(\delta_{ij}=1 \mid data)$$zUsed  used in getZ
eb  output of eBayes, moderated t-statistics, moderated F-statistics, etc
taxa  vector of the taxa names
counts  the original count matrix input
zeroMod  the zero model matrix
zeroCoef  the zero model fitted results
stillActive  convergence
stillActiveNLL  nll at convergence
dupcor  correlation of duplicates
**getCountDensity**

**Description**

Compute the value of the count density function from the count model residuals.

Calculate density values from a normal: $f(x) = 1/(\sqrt{2 \pi} \sigma) e^{-(x-\mu)^2/(2\sigma^2)}$. Maximum-likelihood estimates are approximated using the EM algorithm where we treat mixture membership $\delta_{ij}$ = 1 if $y_{ij}$ is generated from the zero point mass as latent indicator variables. The density is defined as $f_z(y_{ij} = \pi_j(S_j) f_0(y_{ij}) + (1-\pi_j(S_j)) f_{count}(y_{ij};\mu_i,\sigma^2_i)$. The log-likelihood in this extended model is $\log f_{count}(y;\mu_i,\sigma^2_i) + \delta_{ij} \log \pi_j(S_j) + (1-\delta_{ij}) \log (1-\pi_j(S_j))$. The responsibilities are defined as $z_{ij} = p_r(\delta_{ij}=1 | data)$.

**Usage**

```r
countDensity(residuals, log = FALSE)
```

**Arguments**

- `residuals` Residuals from the count model.
- `log` Whether or not we are calculating from a log-normal distribution.

**Value**

Density values from the count model residuals.

**See Also**

`fitZig`

**getEpsilon**

Calculate the relative difference between iterations of the negative log-likelihoods.

Maximum-likelihood estimates are approximated using the EM algorithm where we treat mixture membership $\delta_{ij}$ = 1 if $y_{ij}$ is generated from the zero point mass as latent indicator variables. The log-likelihood in this extended model is $\log f_{count}(y;\mu_i,\sigma^2_i) + \delta_{ij} \log \pi_j(S_j) + (1-\delta_{ij}) \log (1-\pi_j(S_j))$. The responsibilities are defined as $z_{ij} = p_r(\delta_{ij}=1 | data)$.

**Usage**

```r
gammaEpsilon(nll, nllOld)
```

**Arguments**

- `nll` Vector of size M with the current negative log-likelihoods.
- `nllOld` Vector of size M with the previous iterations negative log-likelihoods.
Value

Vector of size M of the relative differences between the previous and current iteration nll.

See Also

fitZig

---

getNegativeLogLikelihoods

Calculate the negative log-likelihoods for the various features given the residuals.

Description

Maximum-likelihood estimates are approximated using the EM algorithm where we treat mixture membership \( \delta_{ij} \) = 1 if \( y_{ij} \) is generated from the zero point mass as latent indicator variables. The log-likelihood in this extended model is \( (1 - \delta_{ij}) \log f_{\text{count}}(y; \mu_i, \sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1 - \delta_{ij}) \log (1 - \pi_j(s_j)) \). The responsibilities are defined as \( z_{ij} = \text{pr}(\delta_{ij} = 1 | \text{data and current values}) \).

Usage

getNegativeLogLikelihoods(z, countResiduals, zeroResiduals)

Arguments

- **z** Matrix (m x n) of estimate responsibilities (probabilities that a count comes from a spike distribution at 0).
- **countResiduals** Residuals from the count model.
- **zeroResiduals** Residuals from the zero model.

Value

Vector of size M of the negative log-likelihoods for the various features.

See Also

fitZig
getPi

Calculate the mixture proportions from the zero model / spike mass model residuals.

Description
F(x) = 1 / (1 + exp(-(x-m)/s)) (the CDF of the logistic distribution). Provides the probability that a real-valued random variable X with a given probability distribution will be found at a value less than or equal to x. The output are the mixture proportions for the samples given the residuals from the zero model.

Usage
go getPi(residuals)

Arguments
   residuals: Residuals from the zero model.

Value
Mixture proportions for each sample.

See Also
   fitZig

getZ

Calculate the current Z estimate responsibilities (posterior probabilities)

Description
Calculate the current Z estimate responsibilities (posterior probabilities)

Usage
getZ(z, zUsed, stillActive, nll, nllUSED)

Arguments
   z: Matrix (m x n) of estimate responsibilities (probabilities that a count comes from a spike distribution at 0).
   zUsed: Matrix (m x n) of estimate responsibilities (probabilities that a count comes from a spike distribution at 0) that are actually used (following convergence).
   stillActive: A vector of size M booleans saying if a feature is still active or not.
   nll: Vector of size M with the current negative log-likelihoods.
   nllUSED: Vector of size M with the converged negative log-likelihoods.
Value
A list of updated zUsed and nllUSED.

See Also
fitZig

Usage
isItStillActive(eps, tol, stillActive, stillActiveNLL, nll)

Arguments
eps Vector of size M (features) representing the relative difference between the new nll and old nll.
tol The threshold tolerance for the difference
stillActive A vector of size M booleans saying if a feature is still active or not.
stillActiveNLL A vector of size M recording the negative log-likelihoods of the various features, updated for those still active.
nll Vector of size M with the current negative log-likelihoods.

Value
None.

See Also
fitZig
libSize

Access sample depth of coverage from MRexperiment object

Description
Access the libSize vector represents the column (sample specific) sums of features, i.e. the total number of reads for a sample or depth of coverage. It is used by fitZig.

Usage
libSize(object)

Arguments
object a MRexperiment object

Value
Library sizes

Author(s)
Joseph N. Paulson

Examples

data(lungData)
head(libSize(lungData))

libSize<- Replace the library sizes in a MRexperiment object

Description
Function to replace the scaling factors, aka the library sizes, of samples in a MRexperiment object.

Usage
## S4 replacement method for signature 'MRexperiment,numeric'
libSize(object) <- value

Arguments
object a MRexperiment object
value vector of library sizes

Value
vector library sizes
Author(s)

Joseph N. Paulson

Examples

data(lungData)
head(libSize(lungData) <- rnorm(1))

loadBiom

Load objects organized in the Biom format.

Description

Wrapper to load Biom formatted object.

Usage

loadBiom(file)

Arguments

file

The biom object filepath.

Value

A MRexperiment object.

See Also

loadMeta loadPhenoData newMRexperiment biom2MRexperiment

Examples

#library(biomformat)
rich_dense_file = system.file("extdata", "rich_dense_otu_table.biom", package = "biomformat")
x = loadBiom(rich_dense_file)
x
loadMeta

Load a count dataset associated with a study.

Description
Load a matrix of OTUs in a tab delimited format

Usage
loadMeta(file, sep = "\t")

Arguments
file Path and filename of the actual data file.
sep File delimiter.

Value
A list with objects 'counts' and 'taxa'.

See Also
loadPhenoData

Examples

dataDirectory <- system.file("extdata", package="metagenomeSeq")
lung = loadMeta(file.path(dataDirectory,"CHK_NAME.otus.count.csv"))

loadMetaQ

Load a count dataset associated with a study set up in a Qiime format.

Description
Load a matrix of OTUs in Qiime's format

Usage
loadMetaQ(file)

Arguments
file Path and filename of the actual data file.

Value
An list with 'counts' containing the count data, 'taxa' containing the otu annotation, and 'otus'.
loadPhenoData

**See Also**

loadMeta loadPhenoData

**Examples**

```r
# see vignette
```

---

**loadPhenoData**

Load a clinical/phenotypic dataset associated with a study.

---

**Description**

Load a matrix of metadata associated with a study.

**Usage**

```r
loadPhenoData(file, tran = TRUE, sep = "\t")
```

**Arguments**

- `file` Path and filename of the actual clinical file.
- `tran` Boolean. If the covariates are along the columns and samples along the rows, then `tran` should equal `TRUE`.
- `sep` The separator for the file.

**Value**

The metadata as a dataframe.

**See Also**

loadMeta

**Examples**

```r
dataDirectory <- system.file("extdata", package="metagenomeSeq")
clin = loadPhenoData(file.path(dataDirectory,"CHK_clinical.csv"),tran=TRUE)
```
lungData

OTU abundance matrix of samples from a smoker/non-smoker study

Description
This is a list with a matrix of OTU counts, otu names, taxa annotations for each OTU, and phenotypic data. Samples along the columns and OTUs along the rows.

Usage
lungData

Format
A list of OTU matrix, taxa, otus, and phenotypes

Value
MRexperiment-class object of 16S lung samples.

References

makeLabels
Function to make labels simpler

Description
Beginning to transition to better axes for plots

Usage
makeLabels(x = "samples", y = "abundance", norm, log)

Arguments
\texttt{x} \hspace{1cm} \text{string for the x-axis}
\texttt{y} \hspace{1cm} \text{string for the y-axis}
\texttt{norm} \hspace{1cm} \text{is the data normalized?}
\texttt{log} \hspace{1cm} \text{is the data logged?}

Value
vector of x,y labels

Examples
metagenomeSeq::makeLabels(norm=TRUE, log=TRUE)
mergeMRexperiments  

**Description**

This function will take two MRexperiment objects and merge them together finding common OTUs. If there are OTUs not found in one of the two MRexperiments then a message will announce this and values will be coerced to zero for the second table.

**Usage**

`mergeMRexperiments(x, y)`

**Arguments**

- `x`: MRexperiment-class object 1.
- `y`: MRexperiment-class object 2.

**Value**

Merged MRexperiment-class object.

**Examples**

```r
data(mouseData)
newobj = mergeMRexperiments(mouseData, mouseData)
newobj

# let me know if people are interested in an option to merge by keys instead of row names.
data(lungData)
newobj = mergeMRexperiments(mouseData, lungData)
newobj
```

mergeTable  

**Description**

Merge two tables

**Usage**

`mergeTable(x, y)`

**Arguments**

- `x`: Table 1.
- `y`: Table 2.

**Value**

Merged table
Depreciated functions in the `metagenomeSeq` package.

### Description
These functions may be removed completely in the next release.

### Usage
`deprecated_metagenomeSeq_function(x, value, ...)`

### Arguments
- **x**: For assignment operators, the object that will undergo a replacement (object inside parenthesis).
- **value**: For assignment operators, the value to replace with (the right side of the assignment).
- **...**: For functions other than assignment operators, parameters to be passed to the modern version of the function (see table).

### mouseData
**Description**
This is a list with a matrix of OTU counts, taxa annotations for each OTU, otu names, and vector of phenotypic data. Samples along the columns and OTUs along the rows.

**Usage**
`mouseData`

**Format**
A list of OTU matrix, taxa, otus, and phenotypes

**Value**
MRexperiment-class object of 16S mouse samples.

**References**
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2894525/
MRcoefs

Table of top-ranked features from fitZig or fitFeatureModel

Description

Extract a table of the top-ranked features from a linear model fit. This function will be updated soon to provide better flexibility similar to limma’s topTable.

Usage

MRcoefs(obj, by = 2, coef = NULL, number = 10, taxa = obj@taxa, uniqueNames = FALSE, adjustMethod = "fdr", alpha = 0.1, group = 0, eff = 0, numberEff = FALSE, counts = 0, file = NULL)

Arguments

- **obj**: Output of fitFeatureModel or fitZig.
- **by**: Column number or column name specifying which coefficient or contrast of the linear model is of interest.
- **coef**: Column number(s) or column name(s) specifying which coefficient or contrast of the linear model to display.
- **number**: The number of bacterial features to pick out.
- **taxa**: Taxa list.
- **uniqueNames**: Number the various taxa.
- **adjustMethod**: Method to adjust p-values by. Default is "FDR". Options include "IHW", "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See `p.adjust` for more details.
- **alpha**: Value for p-value significance threshold when running IHW. The default is set to 0.1
- **group**: One of five choices, 0,1,2,3,4. 0: the sort is ordered by a decreasing absolute value coefficient fit. 1: the sort is ordered by the raw coefficient fit in decreasing order. 2: the sort is ordered by the raw coefficient fit in increasing order. 3: the sort is ordered by the p-value of the coefficient fit in increasing order. 4: no sorting.
- **eff**: Filter features to have at least a "eff" quantile or number of effective samples.
- **numberEff**: Boolean, whether eff should represent quantile (default/TRUE) or number.
- **counts**: Filter features to have at least 'counts' counts.
- **file**: Name of output file, including location, to save the table.
- **IHWcov**: Character value specifying which covariate to use when adjusting pvalues using IHW. Options include: "nnz" (number of non-zero elements per feature), "median" (median abundance value per feature), "Amean" (adjusted mean, used for a fitZigResults obj)

Value

Table of the top-ranked features determined by the linear fit’s coefficient.
MRcounts

Accessor for the counts slot of a MRexperiment object

Description

The counts slot holds the raw count data representing (along the rows) the number of reads annotated for a particular feature and (along the columns) the sample.

Usage

MRcounts(obj, norm = FALSE, log = FALSE, sl = 1000)

Arguments

obj a MRexperiment object.
norm logical indicating whether or not to return normalized counts.
log TRUE/FALSE whether or not to log2 transform scale.
sl The value to scale by (default=1000).

Value

Normalized or raw counts

Author(s)

Joseph N. Paulson, j paulson@umiacs.umd.edu

Examples

data(lungData)
head(MRcounts(lungData))
Class "MRexperiment" – a modified eSet object for the data from high-throughput sequencing experiments

Description
This is the main class for metagenomeSeq.

Objects from the Class
Objects should be created with calls to newMRexperiment.

Extends
Class eSet (package 'Biobase'), directly. Class VersionedBiobase (package 'Biobase'), by class "eSet", distance 2. Class Versioned (package 'Biobase'), by class "eSet", distance 3.

Methods
Class-specific methods.

[] Subset operation, taking two arguments and indexing the sample and variable. Returns an MRexperiment object, including relevant metadata. Setting drop=TRUE generates an error. Subsetting the data, the experiment summary slot is repopulated and pData is repopulated after calling factor (removing levels not present).

Note
Note: This is a summary for reference. For an explanation of the actual usage, see the vignette.

MRexperiments are the main class in use by metagenomeSeq. The class extends eSet and provides additional slots which are populated during the analysis pipeline.

MRexperiment dataset are created with calls to newMRexperiment. MRexperiment datasets contain raw count matrices (integers) accessible through MRcounts. Similarly, normalized count matrices can be accessed (following normalization) through MRcounts by calling norm=TRUE. Following an analysis, a matrix of posterior probabilities for counts is accessible through posteriorProbs.

The normalization factors used in analysis can be recovered by normFactors, as can the library sizes of samples (depths of coverage), libSize.

Similarly to other RNASeq bioconductor packages available, the rows of the matrix correspond to a feature (be it OTU, species, gene, etc.) and each column an experimental sample. Pertinent clinical information and potential confounding factors are stored in the phenoData slot (accessed via pData).

To populate the various slots in an MRexperiment several functions are run. 1) cumNormStat calculates the proper percentile to calculate normalization factors. The cumNormStat slot is populated.

2) cumNorm calculates the actual normalization factors using p = cumNormStat.

Other functions will place subsequent matrices (normalized counts (cumNormMat), posterior probabilities (posteriorProbs))

As mentioned above, MRexperiment is derived from the virtual class, eSet and thereby has a phenoData slot which allows for sample annotation. In the phenoData data frame factors are stored. The normalization factors and library size information is stored in a slot called expSummary that is an annotated data frame and is repopulated for subsetted data.
**MRexperiment2biom**

**Examples**

```r
# See vignette
```

**Description**

Wrapper to convert MRexperiment objects to biom objects.

**Usage**

```r
MRexperiment2biom(obj, id = NULL, norm = FALSE, log = FALSE,
                    sl = 1000, qiimeVersion = TRUE)
```

**Arguments**

- `obj`: The MRexperiment object.
- `id`: Optional id for the biom matrix.
- `norm`: normalize count table
- `log`: log2 transform count table
- `sl`: scaling factor for normalized counts.
- `qiimeVersion`: Format fData according to QIIME specifications (assumes only taxonomy in fData).

**Value**

A biom object.

**See Also**

- `loadMeta`
- `loadPhenoData`
- `newMRexperiment`
- `loadBiom`
- `biom2MRexperiment`

**MRfulltable**

**Table of top microbial marker gene from linear model fit including sequence information**

**Description**

Extract a table of the top-ranked features from a linear model fit. This function will be updated soon to provide better flexibility similar to limma’s topTable. This function differs from `link{MRcoefs}` in that it provides other information about the presence or absence of features to help ensure significant features called are moderately present.

**Usage**

```r
MRfulltable(obj, by = 2, coef = NULL, number = 10, taxa = obj$taxa,
            uniqueNames = FALSE, adjustMethod = "fdr", group = 0, eff = 0,
            numberEff = FALSE, ncounts = 0, file = NULL)
```
Arguments

obj  
A list containing the linear model fit produced by lmFit through fitZig.

by  
Column number or column name specifying which coefficient or contrast of the  
linear model is of interest.

coeff  
Column number(s) or column name(s) specifying which coefficient or contrast  
of the linear model to display.

number  
The number of bacterial features to pick out.

taxa  
Taxa list.

uniqueNames  
Number the various taxa.

adjustMethod  
Method to adjust p-values by. Default is "FDR". Options include "holm",  
hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See `p.adjust`  
for more details.

group  
One of five choices: 0,1,2,3,4. 0: the sort is ordered by a decreasing absolute  
value coefficient fit. 1: the sort is ordered by the raw coefficient fit in decreasing  
order. 2: the sort is ordered by the raw coefficient fit in increasing order. 3:  
the sort is ordered by the p-value of the coefficient fit in increasing order. 4: no  
sorting.

eff  
Filter features to have at least a "eff" quantile or number of effective samples.

numberEff  
Boolean, whether eff should represent quantile (default/FALSE) or number.

ncounts  
Filter features to those with at least 'counts' counts.

file  
Name of output file, including location, to save the table.

Value

Table of the top-ranked features determined by the linear fit's coefficient.

See Also

fitZig fitFeatureModel MRcoefs MRtable fitPA

Examples

data(lungData)
k = grep("Extraction.Control",pData(lungData)$SampleType)
lungTrim = lungData[-k]
lungTrim=filterData(lungTrim,present=30)
lungTrim=cumNorm(lungTrim,p=0.5)
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
fit = fitZig(obj = lungTrim,mod=mod)
# head(MRfulltable(fit))
####

fit = fitFeatureModel(obj = lungTrim,mod=mod)
# head(MRfulltable(fit))
MRihw runs IHW within a MRcoefs() call

Description

Function used in MRcoefs() when "IHW" is set as the p value adjustment method

Usage

MRihw(obj, ...)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>obj</td>
<td>Either a fitFeatureModelResults or fitZigResults object</td>
</tr>
<tr>
<td>...</td>
<td>other parameters</td>
</tr>
</tbody>
</table>

MRihw,fitFeatureModelResults-method

MRihw runs IHW within a MRcoefs() call

Description

Function used in MRcoefs() when "IHW" is set as the p value adjustment method

Usage

## S4 method for signature 'fitFeatureModelResults'
MRihw(obj, p, adjustMethod, alpha)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>obj</td>
<td>Either a fitFeatureModelResults or fitZigResults object</td>
</tr>
<tr>
<td>p</td>
<td>a vector of pvalues extracted from obj</td>
</tr>
<tr>
<td>adjustMethod</td>
<td>Value specifying which adjustment method and which covariate to use for IHW pvalue adjustment. For obj of class fitFeatureModelResults, options are &quot;ihw-abundance&quot; (median feature count per row) and &quot;ihw-ubiquity&quot; (number of non-zero features per row). For obj of class fitZigResults, options are &quot;ihw-abundance&quot; (weighted mean per feature) and &quot;ihw-ubiquity&quot; (number of non-zero features per row).</td>
</tr>
<tr>
<td>alpha</td>
<td>pvalue significance level specified for IHW call. Default is 0.1</td>
</tr>
</tbody>
</table>
MRihw, fitZigResults-method

MRihw runs IHW within a MRcoefs() call

Description
Function used in MRcoefs() when "IHW" is set as the p value adjustment method

Usage

```r
## S4 method for signature 'fitZigResults'
MRihw(obj, p, adjustMethod, alpha)
```

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>obj</td>
<td>Either a fitFeatureModelResults or fitZigResults object</td>
</tr>
<tr>
<td>p</td>
<td>a vector of pvalues extracted from obj</td>
</tr>
<tr>
<td>adjustMethod</td>
<td>Value specifying which adjustment method and which covariate to use for IHW pvalue adjustment. For obj of class fitFeatureModelResults, options are &quot;ihw-abundance&quot; (median feature count per row) and &quot;ihw-ubiquity&quot; (number of non-zero features per row). For obj of class fitZigResults, options are &quot;ihw-abundance&quot; (weighted mean per feature) and &quot;ihw-ubiquity&quot; (number of non-zero features per row).</td>
</tr>
<tr>
<td>alpha</td>
<td>pvalue significance level specified for IHW call. Default is 0.1</td>
</tr>
</tbody>
</table>

MRtable

Table of top microbial marker gene from linear model fit including sequence information

Description
Extract a table of the top-ranked features from a linear model fit. This function will be updated soon to provide better flexibility similar to limma’s topTable. This function differs from link(MRcoefs) in that it provides other information about the presence or absence of features to help ensure significant features called are moderately present.

Usage

```r
MRtable(obj, by = 2, coef = NULL, number = 10, taxa = obj@taxa, uniqueNames = FALSE, adjustMethod = "fdr", group = 0, eff = 0, numberEff = FALSE, ncounts = 0, file = NULL)
```
Arguments

obj Output of fitFeatureModel or fitZig.
by Column number or column name specifying which coefficient or contrast of the linear model is of interest.
coef Column number(s) or column name(s) specifying which coefficient or contrast of the linear model to display.
number The number of bacterial features to pick out.
taxa Taxa list.
uniqueNames Number the various taxa.
adjustMethod Method to adjust p-values by. Default is "FDR". Options include "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See p.adjust for more details.
group One of five choices, 0,1,2,3,4. 0: the sort is ordered by a decreasing absolute value coefficient fit. 1: the sort is ordered by the raw coefficient fit in decreasing order. 2: the sort is ordered by the raw coefficient fit in increasing order. 3: the sort is ordered by the p-value of the coefficient fit in increasing order. 4: no sorting.
eff Filter features to have at least a "eff" quantile or number of effective samples.
numberEff Boolean, whether eff should represent quantile (default/FALSE) or number.
ncounts Filter features to have at least 'counts' of counts.
file Name of file, including location, to save the table.

Value

Table of the top-ranked features determined by the linear fit’s coefficient.

See Also

fitZig fitFeatureModel MRcoefs MRfulltable

Examples

data(lungData)
k = grep("Extraction.Control",pData(lungData)$SampleType)
lungTrim = lungData[,,-k]
lungTrim=filterData(lungTrim,present=30)
lungTrim=cumNorm(lungTrim,p=0.5)
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
fit = fitZig(obj = lungTrim,mod=mod)
head(MRtable(fit))

fit = fitFeatureModel(obj = lungTrim,mod=mod)
head(MRtable(fit))
newMRexperiment  

Create a MRexperiment object

Description

This function creates a MRexperiment object from a matrix or data frame of count data.

Usage

newMRexperiment(counts, phenoData = NULL, featureData = NULL, 
libSize = NULL, normFactors = NULL)

Arguments

counts  
A matrix or data frame of count data. The count data is representative of the number of reads annotated for a feature (be it gene, OTU, species, etc). Rows should correspond to features and columns to samples.

phenoData  
An AnnotatedDataFrame with pertinent sample information.

featureData  
An AnnotatedDataFrame with pertinent feature information.

libSize  
libSize, library size, is the total number of reads for a particular sample.

normFactors  
normFactors, the normalization factors used in either the model or as scaling factors of sample counts for each particular sample.

Details

See MRexperiment-class and eSet (from the Biobase package) for the meaning of the various slots.

Value

an object of class MRexperiment

Author(s)

Joseph N Paulson

Examples

cnts = matrix(abs(rnorm(1000)),nc=10)  
obj <- newMRexperiment(cnts)
**normFactors**

Access the normalization factors in a MRexperiment object

**Description**

Function to access the scaling factors, aka the normalization factors, of samples in a MRexperiment object.

**Usage**

```r
normFactors(object)
```

**Arguments**

- `object`: a MRexperiment object

**Value**

Normalization scaling factors

**Author(s)**

Joseph N. Paulson

**Examples**

```r
data(lungData)
head(normFactors(lungData))
```

**normFactors<-**

Replace the normalization factors in a MRexperiment object

**Description**

Function to replace the scaling factors, aka the normalization factors, of samples in a MRexperiment object.

**Usage**

```r
### S4 replacement method for signature 'MRexperiment,numeric'
normFactors(object) <- value
```

**Arguments**

- `object`: a MRexperiment object
- `value`: vector of normalization scaling factors
plotBubble

Value

Normalization scaling factors

Author(s)

Joseph N. Paulson

Examples

```r
data(lungData)
head(normFactors(lungData) <- rnorm(1))
```

---

plotBubble

*Basic plot of binned vectors.*

Description

This function plots takes two vectors, calculates the contingency table and plots circles sized by the contingency table value. Optional significance vectors of the values significant will shade the circles by proportion of significance.

Usage

```r
plotBubble(yvector, xvector, sigvector = NULL, nbreaks = 10,
           ybreak = quantile(yvector, p = seq(0, 1, length.out = nbreaks)),
           xbreak = quantile(xvector, p = seq(0, 1, length.out = nbreaks)),
           scale = 1, local = FALSE, ...)
```

Arguments

- `yvector`: A vector of values represented along y-axis.
- `xvector`: A vector of values represented along x-axis.
- `sigvector`: A vector of the names of significant features (names should match x/yvector).
- `nbreaks`: Number of bins to break yvector and xvector into.
- `ybreak`: The values to break the yvector at.
- `xbreak`: The values to break the xvector at.
- `scale`: Scaling of circle bin sizes.
- `local`: Boolean to shade by significant bin numbers (TRUE) or overall proportion (FALSE).
- `...`: Additional plot arguments.

Value

A matrix of features along rows, and the group membership along columns.

See Also

- `plotMRheatmap`
Examples

```r
data(mouseData)
mouseData = mouseData[which(rowSums(mouseData)>139),]
sparsity = rowMeans(MRcounts(mouseData)==0)
lor = log(fitPA(mouseData,cl=pData(mouseData)[,3])$oddsRatio)
plotBubble(lor,sparsity,main="lor ~ sparsity")
# Example 2
x = runif(100000)
y = runif(100000)
plotBubble(y,x)
```

plotClassTimeSeries

Plot abundances by class

Description

Plot the abundance of values for each class using a spline approach on the estimated full model.

Usage

```r
plotClassTimeSeries(res, formula, xlab = "Time", ylab = "Abundance", color0 = "black", color1 = "red", include = c("1", "class", "time:class"), ...)
```

Arguments

- `res` Output of `fitTimeSeries` function
- `formula` Formula for `ssanova`. Of the form: abundance ~ ... where ... includes any `pData` slot value.
- `xlab` X-label.
- `ylab` Y-label.
- `color0` Color of samples from first group.
- `color1` Color of samples from second group.
- `include` Parameters to include in prediction.
- `...` Extra plotting arguments.

Value

Plot for abundances of each class using a spline approach on estimated null model.

See Also

- `fitTimeSeries`
Examples

```
data(mouseData)
res = fitTimeSeries(obj=mouseData, feature="Actinobacteria",
                   class="status", id="mouseID", time="relativeTime", lvl='class', B=10)
plotClassTimeSeries(res, pch=21, bg=res$data$class, ylim=c(0,8))
```

---

**plotCorr**

Basic correlation plot function for normalized or unnormalized counts.

Description

This function plots a heatmap of the "n" features with greatest variance across rows.

Usage

```
plotCorr(obj, n, norm = TRUE, log = TRUE, fun = cor, ...)
```

Arguments

- `obj`: A MRexperiment object with count data.
- `n`: The number of features to plot. This chooses the "n" features with greatest variance.
- `norm`: Whether or not to normalize the counts - if MRexperiment object.
- `log`: Whether or not to log2 transform the counts - if MRexperiment object.
- `fun`: Function to calculate pair-wise relationships. Default is pearson correlation.
- `...`: Additional plot arguments.

Value

plotted correlation matrix

See Also

- `cumNormMat`

Examples

```
data(mouseData)
plotCorr(obj=mouseData, n=200, cexRow = 0.4, cexCol = 0.4, trace="none", dendrogram="none",
         col = colorRampPalette(brewer.pal(9, "RdBu"))(50))
```
plotFeature

Basic plot function of the raw or normalized data.

Description

This function plots the abundance of a particular OTU by class. The function is the typical manhattan plot of the abundances.

Usage

plotFeature(obj, otuIndex, classIndex, col = "black", sort = TRUE, sortby = NULL, norm = TRUE, log = TRUE, sl = 1000, ...)

Arguments

- **obj**: A MRexperiment object with count data.
- **otuIndex**: The row to plot
- **classIndex**: A list of the samples in their respective groups.
- **col**: A vector to color samples by.
- **sort**: Boolean, sort or not.
- **sortby**: Default is sort by library size, alternative vector for sorting
- **norm**: Whether or not to normalize the counts - if MRexperiment object.
- **log**: Whether or not to log2 transform the counts - if MRexperiment object.
- **sl**: Scaling factor - if MRexperiment and norm=TRUE.
- **...**: Additional plot arguments.

Value

counts and classindex

See Also

cumNorm

Examples

data(mouseData)
classIndex=list(Western=which(pData(mouseData)$diet=="Western"))
classIndex$BK=which(pData(mouseData)$diet=="BK")

otuIndex = 8770

par(mfrow=c(2,1))
dates = pData(mouseData)$date
plotFeature(mouseData,norm=FALSE,log=FALSE,otuIndex,classIndex, col=dates,sortby=dates,ylab="Raw reads")
plotGenus

Basic plot function of the raw or normalized data.

Description
This function plots the abundance of a particular OTU by class. The function uses the estimated posterior probabilities to make technical zeros transparent.

Usage
plotGenus(obj, otuIndex, classIndex, norm = TRUE, log = TRUE, no = 1:length(otuIndex), labs = TRUE, xlab = NULL, ylab = NULL, jitter = TRUE, jitter.factor = 1, pch = 21, ...)

Arguments
obj An MRexperiment object with count data.
.otuIndex A list of the otus with the same annotation.
.classIndex A list of the samples in their respective groups.
.norm Whether or not to normalize the counts - if MRexperiment object.
.log Whether or not to log2 transform the counts - if MRexperiment object.
.no Which of the otuIndex to plot.
.labs Whether to include group labels or not. (TRUE/FALSE)
.xlab xlabel for the plot.
.ylab ylabel for the plot.
.jitter Boolean to jitter the count data or not.
.jitter.factor Factor value for jitter
.pch Standard pch value for the plot command.
... Additional plot arguments.

Value
plotted data

See Also
cumNorm

Examples

data(mouseData)
classIndex=list(controls=which(pData(mouseData)$diet=='BK'))
classIndex$cases=which(pData(mouseData)$diet=='Western')
otuIndex = grep("Strep",fData(mouseData)$family)
otuIndex=otuIndex[order(rowSums(MRcounts(mouseData)[otuIndex,]),decreasing=TRUE)]
plotGenus(mouseData,otuIndex,classIndex,no=1:2,xaxt="n",norm=FALSE,ylab="Strep normalized log(cpt)")
plotMRheatmap

Description

This function plots a heatmap of the 'n' features with greatest variance across rows (or other statistic).

Usage

plotMRheatmap(obj, n, norm = TRUE, log = TRUE, fun = sd, ...)

Arguments

obj A MRexperiment object with count data.

n The number of features to plot. This chooses the 'n' features of greatest positive statistic.

norm Whether or not to normalize the counts - if MRexperiment object.

log Whether or not to log2 transform the counts - if MRexperiment object.

fun Function to select top 'n' features.

... Additional plot arguments.

Value

plotted matrix

See Also

cumNormMat

Examples

data(mouseData)
trials = pData(mouseData)$diet
heatmapColColors=brewer.pal(12,"Set3")[as.integer(factor(trials))];
heatmapCols = colorRampPalette(brewer.pal(9, "RdBu"))(50)
#### version using sd
plotMRheatmap(obj=mouseData,n=200,cexRow = 0.4,cexCol = 0.4,trace="none",
          col = heatmapCols,ColSideColors = heatmapColColors)
#### version using MAD
plotMRheatmap(obj=mouseData,n=50,fun=mad,cexRow = 0.4,cexCol = 0.4,trace="none",
          col = heatmapCols,ColSideColors = heatmapColColors)
plotOrd

Plot of either PCA or MDS coordinates for the distances of normalized or unnormalized counts.

Description

This function plots the PCA / MDS coordinates for the "n" features of interest. Potentially uncovering batch effects or feature relationships.

Usage

plotOrd(obj, tran = TRUE, comp = 1:2, norm = TRUE, log = TRUE, usePCA = TRUE, useDist = FALSE, distfun = stats::dist, dist.method = "euclidian", n = NULL, ...)

Arguments

- **obj**: A MRexperiment object or count matrix.
- **tran**: Transpose the matrix.
- **comp**: Which components to display
- **norm**: Whether or not to normalize the counts - if MRexperiment object.
- **log**: Whether or not to log2 the counts - if MRexperiment object.
- **usePCA**: TRUE/FALSE whether to use PCA or MDS coordinates (TRUE is PCA).
- **useDist**: TRUE/FALSE whether to calculate distances.
- **distfun**: Distance function, default is stats::dist
- **dist.method**: If useDist==TRUE, what method to calculate distances.
- **n**: Number of features to make use of in calculating your distances.
- **...**: Additional plot arguments.

Value

coordinates

See Also

cumNormMat

Examples

data(mouseData)
c1 = pData(mouseData)[,3]
plotOrd(mouseData, tran=TRUE, useDist=TRUE, pch=21, bg=factor(c1), usePCA=FALSE)
**plotOTU**

*Basic plot function of the raw or normalized data.*

**Description**

This function plots the abundance of a particular OTU by class. The function uses the estimated posterior probabilities to make technical zeros transparent.

**Usage**

```r
plotOTU(obj, otu, classIndex, log = TRUE, norm = TRUE,
        jitter.factor = 1, pch = 21, labs = TRUE, xlab = NULL,
        ylab = NULL, jitter = TRUE, ...)
```

**Arguments**

- `obj` A MRexperiment object with count data.
- `otu` The row number/OTU to plot.
- `classIndex` A list of the samples in their respective groups.
- `log` Whether or not to log2 transform the counts - if MRexperiment object.
- `norm` Whether or not to normalize the counts - if MRexperiment object.
- `jitter.factor` Factor value for jitter.
- `pch` Standard pch value for the plot command.
- `labs` Whether to include group labels or not. (TRUE/FALSE)
- `xlab` xlabel for the plot.
- `ylab` ylabel for the plot.
- `jitter` Boolean to jitter the count data or not.
- `...` Additional plot arguments.

**Value**

Plotted values

**See Also**

- `cumNorm`

**Examples**

```r
data(mouseData)
classIndex=list(controls=which(pData(mouseData)$diet=="BK"),
classIndex$cases=which(pData(mouseData)$diet=="Western")
# you can specify whether or not to normalize, and to what level
plotOTU(mouseData,otu=9083,classIndex,norm=FALSE,main="9083 feature abundances")
```
plotRare  
Plot of rarefaction effect

Description
This function plots the number of observed features vs. the depth of coverage.

Usage
plotRare(obj, cl = NULL, ...)

Arguments
obj  A MRexperiment object with count data or matrix.
cl   Vector of classes for various samples.
...  Additional plot arguments.

Value
Library size and number of detected features

See Also
plotOrd, plotMRheatmap, plotCorr, plotOTU, plotGenus

Examples

data(mouseData)
c1 = factor(pData(mouseData)[,3])
res = plotRare(mouseData,c1=pch=21,bg=cl)
tmp=lapply(levels(cl), function(lv) lm(res[,"ident"]~res[,"libSize"]-1, subset=cl==lv))
for(i in 1:length(levels(cl))){
  abline(tmp[[i]], col=i)
}
legend("topleft", c("Diet 1","Diet 2"), text.col=c(1,2),box.col=NA)

plotTimeSeries  
Plot difference function for particular bacteria

Description
Plot the difference in abundance for significant features.

Usage
plotTimeSeries(res, C = 0, xlab = "Time", ylab = "Difference in abundance", main = "SS difference function prediction", ...)
Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>res</td>
<td>Output of <code>fitTimeSeries</code> function</td>
</tr>
<tr>
<td>C</td>
<td>Value for which difference function has to be larger or smaller than (default 0).</td>
</tr>
<tr>
<td>xlab</td>
<td>X-label.</td>
</tr>
<tr>
<td>ylab</td>
<td>Y-label.</td>
</tr>
<tr>
<td>main</td>
<td>Main label.</td>
</tr>
<tr>
<td>...</td>
<td>Extra plotting arguments.</td>
</tr>
</tbody>
</table>

Value

Plot of difference in abundance for significant features.

See Also

`fitTimeSeries`

Examples

```r
data(mouseData)
res = fitTimeSeries(obj=mouseData,feature="Actinobacteria", class="status",id="mouseID",time="relativeTime",lvl='class',B=10)
plotTimeSeries(res)
```

Description

Accessing the posterior probabilities following a run through `fitZig`

Usage

```r
posteriorProbs(obj)
```

Arguments

- `obj` a `MRexperiment` object.

Value

Matrix of posterior probabilities

Author(s)

Joseph N. Paulson
Examples

# This is a simple demonstration
data(lungData)
k = grep("Extraction.Control", pData(lungData)$SampleType)
lungTrim = lungData[,-k]
k = which(rowSums(MRcounts(lungTrim)>0)<30)
lungTrim = cumNorm(lungTrim)
lungTrim = lungTrim[-k,]
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
# The maxit is not meant to be 1 -- this is for demonstration/speed
settings = zigControl(maxit=1,verbose=FALSE)
fit = fitZig(obj = lungTrim, mod=mod, control=settings)
head(posteriorProbs(lungTrim))

returnAppropriateObj Check if MRexperiment or matrix and return matrix

Description

Function to check if object is a MRexperiment class or matrix

Usage

returnAppropriateObj(obj, norm, log, sl = 1000)

Arguments

obj a MRexperiment or matrix object
norm return a normalized MRexperiment matrix
log return a log transformed MRexperiment matrix
sl scaling value

Value

Matrix

Examples

data(lungData)
head(returnAppropriateObj(lungData,norm=FALSE,log=FALSE))
Description

Sets up a data-frame with the feature abundance, class information, time points, sample ids and returns the fitted values for the fitted model.

Usage

ssFit(formula, abundance, class, time, id, include = c("class", "time:class"), pd, ...)

Arguments

formula Formula for ssanova. Of the form: abundance ~ ... where ... includes any pData slot value.
abundance Numeric vector of abundances.
class Class membership (factor of group membership).
time Time point vector of relative times (same length as abundance).
id Sample / patient id.
include Parameters to include in prediction.
pd Extra variable.
... Extra parameters for ssanova function (see ?ssanova).

Value

A list containing:

data : Inputed data

* fit : The interpolated / fitted values for timePoints
* se : The standard error for CI intervals
* timePoints : The time points interpolated over

See Also

cumNorm fitTimeSeries ssPermAnalysis ssPerm ssIntervalCandidate

Examples

# Not run
ssIntervalCandidate  
*calculate interesting time intervals*

**Description**

Calculates time intervals of interest using SS-Anova fitted confidence intervals.

**Usage**

ssIntervalCandidate(fit, standardError, timePoints, positive = TRUE, C = 0)

**Arguments**

- `fit`  
  SS-Anova fits.
- `standardError`  
  SS-Anova se estimates.
- `timePoints`  
  Time points interpolated over.
- `positive`  
  Positive region or negative region (difference in abundance is positive/negative).
- `C`  
  Value for which difference function has to be larger or smaller than (default 0).

**Value**

Matrix of time point intervals of interest

**See Also**

cumNorm fitTimeSeries ssFit ssPerm ssPermAnalysis

**Examples**

# Not run

---

ssPerm  
*class permutations for smoothing-spline time series analysis*

**Description**

Creates a list of permuted class memberships for the time series permutation tests.

**Usage**

ssPerm(df, B)

**Arguments**

- `df`  
  Data frame containing class membership and sample/patient id label.
- `B`  
  Number of permutations.
ssPermAnalysis

Value
A list of permutted class memberships

See Also
cumNorm fitTimeSeries ssFit ssPermAnalysis ssIntervalCandidate

Examples
# Not run

ssPermAnalysis

smoothing-splines anova fits for each permutation

Description
Calculates the fit for each permutation and estimates the area under the null (permutted) model for interesting time intervals of differential abundance.

Usage
ssPermAnalysis(data, formula, permList, intTimes, timePoints, include = c("class", "time:class"), ...)

Arguments
data Data used in estimation.
formula Formula for ssanova. Of the form: abundance ~ ... where ... includes any pData slot value.
permList A list of permutted class memberships
intTimes Interesting time intervals.
timePoints Time points to interpolate over.
include Parameters to include in prediction.
... Options for ssanova

Value
A matrix of permutted area estimates for time intervals of interest.

See Also
cumNorm fitTimeSeries ssFit ssPerm ssIntervalCandidate

Examples
# Not run
trapz \hspace{1cm} \textit{Trapezoidal Integration}

\textbf{Description} \\
Compute the area of a function with values \('y'\) at the points \('x'\). Function comes from the pracma package.

\textbf{Usage} \\
\texttt{trapz(x, y)}

\textbf{Arguments} \\
x \hspace{1cm} x-coordinates of points on the x-axis \\
y \hspace{1cm} y-coordinates of function values

\textbf{Value} \\
Approximated integral of the function from \(\text{min}(x)\) to \(\text{max}(x)\). Or a matrix of the same size as \(\text{y}\).

\textbf{Examples} \\
# Calculate the area under the sine curve from 0 to pi:
\begin{verbatim}
n <- 101
x <- seq(0, pi, len = n)
y <- sin(x)
trapz(x, y) #=> 1.999835504
\end{verbatim}

# Use a correction term at the boundary: \(-h^2/12*(f'(b)-f'(a))\)
\begin{verbatim}
h <- x[2] - x[1]
ca <- (y[2]-y[1]) / h
cb <- (y[n]-y[n-1]) / h
trapz(x, y) - h^2/12 * (cb - ca) #=> 1.999999969
\end{verbatim}

\textbf{ts2MRexperiment} \hspace{1cm} \textit{With a list of fitTimeSeries results, generate an MRexperiment that can be plotted with metaviz}

\textbf{Description} \\
With a list of fitTimeSeries results, generate an MRexperiment that can be plotted with metaviz

\textbf{Usage} \\
\texttt{ts2MRexperiment(obj, sampleNames = NULL, sampleDescription = "timepoints", taxonomyLevels = NULL, taxonomyHierarchyRoot = "bacteria", taxonomyDescription = "taxonomy", featuresOfInterest = NULL, featureDataOfInterest = NULL)
**uniqueFeatures**

**Arguments**

- **obj**: Output of `fitMultipleTimeSeries`.
- **sampleNames**: Sample names for plot.
- **sampleDescription**: Description of samples for plot axis label.
- **taxonomyLevels**: Feature names for plot.
- **taxonomyHierarchyRoot**: Root of feature hierarchy for MRexperiment.
- **taxonomyDescription**: Description of features for plot axis label.
- **featuresOfInterest**: The features to select from the `fitMultipleTimeSeries` output.
- **featureDataOfInterest**: featureData for the resulting MRexperiment.

**Value**

MRexperiment that contains fitTimeSeries data, featureData, and phenoData.

**See Also**

- `fitTimeSeries`
- `fitMultipleTimeSeries`

**Examples**

```r
data(mouseData)
res = fitMultipleTimeSeries(obj=mouseData,lvl='phylum',class="status",
                           id="mouseID",time="relativeTime",B=1)
obj = ts2MRexperiment(res)
obj
```

---

### uniqueFeatures

**Table of features unique to a group**

**Description**

Creates a table of features, their index, number of positive samples in a group, and the number of reads in a group. Can threshold features by a minimum no. of reads or no. of samples.

**Usage**

```r
uniqueFeatures(obj, cl, nsamples = 0, nreads = 0)
```

**Arguments**

- **obj**: Either a MRexperiment object or matrix.
- **cl**: A vector representing assigning samples to a group.
- **nsamples**: The minimum number of positive samples.
- **nreads**: The minimum number of raw reads.
wrenchNorm

**Value**

Table of features unique to a group

**Examples**

```r
data(mouseData)
head(uniqueFeatures(mouseData[1:100,], cl=pData(mouseData)[,3]))
```

---

**Description**

Calculates normalization factors using method published by M. Sentil Kumar et al. (2018) to compute normalization factors which considers compositional bias introduced by sequencers.

**Usage**

```r
wrenchNorm(obj, condition)
```

**Arguments**

- `obj` an MRexperiment object
- `condition` case control label that wrench uses to calculate normalization factors

**Value**

an MRexperiment object with updated normalization factors. Accessible by `normFactors`.

**See Also**

`cumNorm` `fitZig`

**Examples**

```r
data(mouseData)
mouseData <- wrenchNorm(mouseData, condition = mouseData$diet)
head(normFactors(mouseData))
```
Description
Settings for the fitZig function

Usage
zigControl(tol = 1e-04, maxit = 10, verbose = TRUE, dfMethod = "modified", pvalMethod = "default")

Arguments
tol The tolerance for the difference in negative log likelihood estimates for a feature to remain active.
maxit The maximum number of iterations for the expectation-maximization algorithm.
verbose Whether to display iterative step summary statistics or not.
dfMethod Either 'default' or 'modified' (by responsibilities).
pvalMethod Either 'default' or 'bootstrap'.

Value
The value for the tolerance, maximum no. of iterations, and the verbose warning.

Note
fitZig makes use of zigControl.

See Also
fitZig cumNorm plotOTU

Examples
control = zigControl(tol=1e-10,maxit=10,verbose=FALSE)
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