

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 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.

License Artistic-2.0

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metagenomeSeq-package *Statistical analysis for sparse high-throughput sequencing*

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.

Author(s)

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

References

Paulson, Joseph N., O. Colin Stine, Hector Corrada Bravo, and Mihai Pop. "Differential abundance analysis for microbial marker-gene surveys." *Nature methods* (2013).

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

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

```
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

```
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 | <i>Aggregates a MRexperiment object or counts matrix to a particular level.</i> |
|---------------------|---|

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 log ₂ 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)

```

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 | <i>Cumulative sum scaling (css) normalization factors</i> |
|-----------------|---|

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.

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

Calculat 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 | <i>Calculate the correct indices for the output of correlationTest</i> |
|----------------|--|

Description

Consider the upper triangular portion of a matrix of size $n \times n$. 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

```
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

```
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 | <i>Correlation of each row of a matrix or MRExperiment object</i> |
|-----------------|---|

Description

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

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,]
cormat = as.matrix(dist(mat)) # Creating a matrix
cormat[cormat>0] = 0 # Creating an empty matrix
ind = correctIndices(nrow(mat))
cormat[upper.tri(cormat)][ind] = cors[,1]
table(cormat[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 | <i>Cumulative sum scaling factors.</i> |
|------------|--|

Description

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

Usage

```
cumNormMat(obj, p = cumNormStatFast(obj), s1 = 1000)
```

Arguments

| | |
|-----|---------------------------------------|
| obj | A matrix or MRExperiment object. |
| p | The pth quantile. |
| s1 | The value to scale by (default=1000). |

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 | <i>Cumulative sum scaling percentile selection</i> |
|-------------|--|

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 MExperiment 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 MExperiment 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")
```

Arguments

| | |
|--------------------------|--|
| <code>z</code> | Matrix (m x n) of estimate responsibilities (probabilities that a count comes from a spike distribution at 0). |
| <code>y</code> | Matrix (m x n) of count observations. |
| <code>mmCount</code> | Model matrix for the count distribution. |
| <code>stillActive</code> | Boolean vector of size M, indicating whether a feature converged or not. |
| <code>fit2</code> | Previous fit of the count model. |
| <code>dfMethod</code> | Either 'default' or 'modified' (by responsibilities) |

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 $f_{zig}(y_{ij} = \pi_j(S_j) * f_0(y_{ij}) + (1 - \pi_j(S_j)) * f_{count}(y_{ij}; \mu_i, \sigma_i^2))$. 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 $z_{ij} = \text{pr}(\delta_{ij} = 1 | \text{data})$.

Value

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

See Also

[fitZig](#)

doEStep

Compute the Expectation step.

Description

Estimates the responsibilities $z_{ij} = \frac{\pi_j \cdot I_0(y_{ij})}{\pi_j \cdot I_0(y_{ij}) + (1 - \pi_j) \cdot f_{\text{count}}(y_{ij})}$

Usage

```
doEStep(countResiduals, zeroResiduals, zeroIndices)
```

Arguments

countResiduals Residuals from the count model.
 zeroResiduals Residuals from the zero model.
 zeroIndices Index (matrix m x n) of counts that are zero/non-zero.

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 $(1 - \delta_{ij}) \log f_{\text{count}}(y_{ij}; \mu_i, \sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1 - \delta_{ij}) \log (1 - \pi_j(s_j))$. The responsibilities are defined as $z_{ij} = \text{pr}(\delta_{ij} = 1 \mid \text{data})$.

Value

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

See Also

[fitZig](#)

doZeroMStep

*Compute the zero Maximization step.***Description**

Performs Maximization step calculation for the mixture components. Uses least squares to fit the parameters of the mean of the logistic distribution. $\pi_j = \sum_i \frac{1}{M} M_{z_{ij}}$ 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 $f_{\text{zig}}(y_{ij} = \pi_j(S_j) \cdot f_0(y_{ij}) + (1 - \pi_j(S_j)) \cdot f_{\text{count}}(y_{ij}; \mu_i, \sigma_i^2)$. 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 $z_{ij} = \text{pr}(\delta_{ij} = 1 | \text{data})$.

Usage

```
doZeroMStep(z, zeroIndices, mmZero)
```

Arguments

| | |
|--------------------------|--|
| <code>z</code> | Matrix (m x n) of estimate responsibilities (probabilities that a count comes from a spike distribution at 0). |
| <code>zeroIndices</code> | Index (matrix m x n) of counts that are zero/non-zero. |
| <code>mmZero</code> | 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. |

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

```
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

```
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

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

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, jpaulson@umiacs.umd.edu

Examples

```
data(mouseData)
expSummary(mouseData)
```

| | |
|-----------|---|
| extractMR | <i>Extract the essentials of an MRexperiment.</i> |
|-----------|---|

Description

Extract the essentials of an MRexperiment.

Usage

```
extractMR(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

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

| | |
|------------|---|
| filterData | <i>Filter datasets according to no. features present in features with at least a certain depth.</i> |
|------------|---|

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

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

Arguments

| | |
|---------|--|
| obj | A MRExperiment object or count matrix. |
| present | Features with at least 'present' positive samples. |
| depth | Sampls with at least this much depth of coverage |

Value

A MRExperiment object.

Examples

```
data(mouseData)
filterData(mouseData)
```

| | |
|-------|--|
| fitDO | <i>Wrapper to calculate Discovery Odds Ratios on feature values.</i> |
|-------|--|

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

```
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 | <i>Computes differential abundance analysis using a zero-inflated log-normal model</i> |
|-----------------|--|

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

```
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
- permutedfits - permuted z-score estimates under the null

See Also

[cumNorm](#)

Examples

```
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
 permutedFits permuted z-score estimates under the null

| | |
|--------------|---|
| fitLogNormal | <i>Computes a log-normal linear model and permutation based p-values.</i> |
|--------------|---|

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

```
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. |
| sl | 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)<30)
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,lv1='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 |

Value

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

See Also

[cumNorm](#) [fitZig](#) [fitD0](#) [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)
```

 fitSSTimeSeries

Discover differentially abundant time intervals using SS-Anova

Description

Calculate time intervals of interest using SS-Anova fitted models. Fitting is performed using 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. |
| 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 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](#) [ssFit](#) [ssIntervalCandidate](#) [ssPerm](#) [ssPermAnalysis](#) [plotTimeSeries](#)

Examples

```
data(mouseData)
res = fitSSTimeSeries(obj=mouseData,feature="Actinobacteria",
  class="status",id="mouseID",time="relativeTime",lvl='class',B=2)
```

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

```

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 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](#) [plotTimeSeries](#)

Examples

```
data(mouseData)
res = fitTimeSeries(obj=mouseData,feature="Actinobacteria",
  class="status",id="mouseID",time="relativeTime",lvl='class',B=2)
```

| | |
|------------------|---|
| fitZeroLogNormal | <i>Compute the log fold-change estimates for the zero-inflated log-normal model</i> |
|------------------|---|

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 τ^2 for positive component
- tauZero - estimated τ^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

Computes the weighted fold-change estimates and t-statistics.

Description

Wrapper to actually run the Expectation-maximization algorithm and estimate f_{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 $z_{ij} = \text{pr}(\delta_{ij} = 1 \mid \text{data})$.

Usage

```
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 $z_{ij} = \text{pr}(\text{delta}_{ij}=1 \mid \text{data})$
 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 *Compute the value of the count density function from the count model residuals.*

Description

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 $\text{delta}_{ij} = 1$ 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 $(1 - \text{delta}_{ij}) \log f_{\text{count}}(y; \mu_i, \sigma_i^2) + \text{delta}_{ij} \log \pi_j(s_j) + (1 - \text{delta}_{ij}) \log (1 - \pi_j(s_j))$. The responsibilities are defined as $z_{ij} = \text{pr}(\text{delta}_{ij}=1 \mid \text{data})$.

Usage

```
getCountDensity(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 | <i>Calculate the relative difference between iterations of the negative log-likelihoods.</i> |
|------------|--|

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 $z_{ij} = \text{pr}(\delta_{ij}=1 \mid \text{data})$.

Usage

```
getEpsilon(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 $z_{ij} = \text{pr}(\delta_{ij}=1 \mid \text{data and current values})$.

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

```
getPi(residuals)
```

Arguments

residuals Residuals from the zero model.

Value

Mixture proportions for each sample.

See Also

[fitZig](#)

| | |
|------|--|
| getZ | <i>Calculate the current Z estimate responsibilities (posterior probabilities)</i> |
|------|--|

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](#)

`isItStillActive` *Function to determine if a feature is still active.*

Description

In the Expectation Maximization routine features posterior probabilities routinely converge based on a tolerance threshold. This function checks whether or not the feature's negative log-likelihood (measure of the fit) has changed or not.

Usage

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

Arguments

| | |
|-----------------------------|--|
| <code>eps</code> | Vector of size M (features) representing the relative difference between the new nll and old nll. |
| <code>tol</code> | The threshold tolerance for the difference |
| <code>stillActive</code> | A vector of size M booleans saying if a feature is still active or not. |
| <code>stillActiveNLL</code> | A vector of size M recording the negative log-likelihoods of the various features, updated for those still active. |
| <code>nll</code> | 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 | <i>Load objects organized in the Biom format.</i> |
|----------|---|

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 | <i>Load a count dataset associated with a study.</i> |
|----------|--|

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'.

See Also

[loadMeta](#) [loadPhenoData](#)

Examples

```
# see vignette
```

| | |
|---------------|--|
| loadPhenoData | <i>Load a clinical/phenotypic dataset associated with a study.</i> |
|---------------|--|

Description

Load a matrix of metadata associated with a study.

Usage

```
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

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

| | |
|----------|---|
| lungData | <i>OTU abundance matrix of samples from a smoker/non-smoker study</i> |
|----------|---|

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.

Format

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

Value

MRExperiment-class object of 16S lung samples.

References

<http://www.ncbi.nlm.nih.gov/pubmed/21680950>

| | |
|------------|--|
| makeLabels | <i>Function to make labels simpler</i> |
|------------|--|

Description

Beginning to transition to better axes for plots

Usage

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

Arguments

| | |
|------|-------------------------|
| x | string for the x-axis |
| y | string for the y-axis |
| norm | is the data normalized? |
| log | is the data logged? |

Value

vector of x,y labels

Examples

```
metagenomeSeq::makeLabels(norm=TRUE, log=TRUE)
```

mergeMRExperiments *Merge two MRExperiment objects together*

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

```
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 *Merge two tables*

Description

Merge two tables

Usage

```
mergeTable(x, y)
```

Arguments

| | |
|---|----------|
| x | Table 1. |
| y | Table 2. |

Value

Merged table

metagenomeSeq-deprecated

Deprecated 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

OTU abundance matrix of mice samples from a diet longitudinal study

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.

Format

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

Value

MExperiment-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 "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none". See p.adjust for more details. Additionally, options using independent hypothesis weighting (IHW) are available. See MRihw 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/FALSE) or number. |
| counts | Filter features to have 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](#) [MRtable](#) [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(MRcoefs(fit))
####
fit = fitFeatureModel(obj = lungTrim,mod=mod)
head(MRcoefs(fit))
```

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. |
| s1 | The value to scale by (default=1000). |

Value

Normalized or raw counts

Author(s)

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

Examples

```
data(lungData)
head(MRcounts(lungData))
```

| | |
|--------------|---|
| MRexperiment | <i>Class "MRexperiment" – a modified eSet object for the data from high-throughput sequencing experiments</i> |
|--------------|---|

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.

Examples

```
# See vignette
```

```
MRexperiment2biom      MRexperiment to biom objects
```

Description

Wrapper to convert MRexperiment objects to biom objects.

Usage

```
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 | <i>Table of top microbial marker gene from linear model fit including sequence information</i> |
|-------------|--|

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

```
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 | 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 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 *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

| | |
|-----|---|
| obj | Either a fitFeatureModelResults or fitZigResults object |
| ... | other parameters |

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

| | |
|--------------|--|
| obj | Either a fitFeatureModelResults or fitZigResults object |
| p | a vector of pvalues extracted from obj |
| adjustMethod | Value specifying which adjustment method and which covariate to use for IHW pvalue adjustment. For obj of class fitFeatureModelResults-class , options are "ihw-abundance" (median feature count per row) and "ihw-ubiquity" (number of non-zero features per row). For obj of class fitZigResults-class , options are "ihw-abundance" (weighted mean per feature) and "ihw-ubiquity" (number of non-zero features per row). |
| alpha | pvalue significance level specified for IHW call. Default is 0.1 |

 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

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

Arguments

| | |
|--------------|--|
| obj | Either a fitFeatureModelResults or fitZigResults object |
| p | a vector of pvalues extracted from obj |
| adjustMethod | Value specifying which adjustment method and which covariate to use for IHW pvalue adjustment. For obj of class fitFeatureModelResults-class , options are "ihw-abundance" (median feature count per row) and "ihw-ubiquity" (number of non-zero features per row). For obj of class fitZigResults-class , options are "ihw-abundance" (weighted mean per feature) and "ihw-ubiquity" (number of non-zero features per row). |
| alpha | pvalue significance level specified for IHW call. Default is 0.1 |

 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

```
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 | <i>Create a MRExperiment object</i> |
|-----------------|-------------------------------------|

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

```
normFactors(object)
```

Arguments

object a MRExperiment object

Value

Normalization scaling factors

Author(s)

Joseph N. Paulson

Examples

```
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

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

Arguments

| | |
|--------|---|
| object | a MRExperiment object |
| value | vector of normalization scaling factors |

Value

Normalization scaling factors

Author(s)

Joseph N. Paulson

Examples

```
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

```
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

```
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

```
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 | <i>Basic correlation plot function for normalized or unnormalized counts.</i> |
|----------|---|

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

| | |
|-------------------------|---|
| <code>obj</code> | A MRExperiment object with count data. |
| <code>otuIndex</code> | The row to plot |
| <code>classIndex</code> | A list of the samples in their respective groups. |
| <code>col</code> | A vector to color samples by. |
| <code>sort</code> | Boolean, sort or not. |
| <code>sortby</code> | Default is sort by library size, alternative vector for sorting |
| <code>norm</code> | Whether or not to normalize the counts - if MRExperiment object. |
| <code>log</code> | Whether or not to log2 transform the counts - if MRExperiment object. |
| <code>sl</code> | Scaling factor - if MRExperiment and norm=TRUE. |
| <code>...</code> | 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 log ₂ 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

Basic heatmap plot function for normalized counts.

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

```
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

```

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 | <i>Plot of rarefaction effect</i> |
|----------|-----------------------------------|

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)
cl = factor(pData(mouseData)[,3])
res = plotRare(mouseData,cl=cl,pch=21,bg=cl)
tmp=lapply(levels(cl), function(lv) lm(res[,"ident"]~res[,"libSize"]-1, subset=c1==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

| | |
|------|---|
| res | Output of fitTimeSeries function |
| C | Value for which difference function has to be larger or smaller than (default 0). |
| xlab | X-label. |
| ylab | Y-label. |
| main | Main label. |
| ... | Extra plotting arguments. |

Value

Plot of difference in abundance for significant features.

See Also

[fitTimeSeries](#)

Examples

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

| | |
|----------------|--|
| posteriorProbs | <i>Access the posterior probabilities that results from analysis</i> |
|----------------|--|

Description

Accessing the posterior probabilities following a run through [fitZig](#)

Usage

```
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 MExperiment or matrix and return matrix

Description

Function to check if object is a MExperiment class or matrix

Usage

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

Arguments

| | |
|------|---|
| obj | a MExperiment or matrix object |
| norm | return a normalized MExperiment matrix |
| log | return a log transformed MExperiment matrix |
| sl | scaling value |

Value

Matrix

Examples

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

```
ssFit
```

smoothing-splines anova fit

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. |

Value

A list of permuted 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 (permuted) 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 permuted 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 permuted area estimates for time intervals of interest.

See Also

[cumNorm](#) [fitTimeSeries](#) [ssFit](#) [ssPerm](#) [ssIntervalCandidate](#)

Examples

```
# Not run
```

| | |
|--------|--------------------------------|
| trapez | <i>Trapezoidal Integration</i> |
|--------|--------------------------------|

Description

Compute the area of a function with values 'y' at the points 'x'. Function comes from the pracma package.

Usage

```
trapez(x, y)
```

Arguments

| | |
|---|---------------------------------------|
| x | x-coordinates of points on the x-axis |
| y | y-coordinates of function values |

Value

Approximated integral of the function from 'min(x)' to 'max(x)'. Or a matrix of the same size as 'y'.

Examples

```
# Calculate the area under the sine curve from 0 to pi:
n <- 101
x <- seq(0, pi, len = n)
y <- sin(x)
trapez(x, y)          #=> 1.999835504

# Use a correction term at the boundary: -h^2/12*(f'(b)-f'(a))
h <- x[2] - x[1]
ca <- (y[2]-y[1]) / h
cb <- (y[n]-y[n-1]) / h
trapez(x, y) - h^2/12 * (cb - ca) #=> 1.999999969
```

| | |
|-----------------|---|
| ts2MRExperiment | <i>With a list of fitTimeSeries results, generate an MRExperiment that can be plotted with metavizr</i> |
|-----------------|---|

Description

With a list of fitTimeSeries results, generate an MRExperiment that can be plotted with metavizr

Usage

```
ts2MRExperiment(
  obj,
  sampleNames = NULL,
  sampleDescription = "timepoints",
  taxonomyLevels = NULL,
  taxonomyHierarchyRoot = "bacteria",
  taxonomyDescription = "taxonomy",
  featuresOfInterest = NULL,
  featureDataOfInterest = NULL
)
```

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

```
data(mouseData)
res = fitMultipleTimeSeries(obj=mouseData,lv1='phylum',class="status",
  id="mouseID",time="relativeTime",B=1)
obj = ts2MRExperiment(res)
obj
```

| | |
|----------------|--|
| uniqueFeatures | <i>Table of features unique to a group</i> |
|----------------|--|

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

```
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. |

Value

Table of features unique to a group

Examples

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

| | |
|------------|---|
| wrenchNorm | <i>Computes normalization factors using wrench instead of cumNorm</i> |
|------------|---|

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

```
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

```
data(mouseData)
mouseData <- wrenchNorm(mouseData, condition = mouseData$diet)
head(normFactors(mouseData))
```

zigControl

Settings for the fitZig function

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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