Package ‘metagenomeSeq’

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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.
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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.
aggregateBySample

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

References

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=matrixStats::rowMedians)
# not run
# aggregateBySample(mouseData, fct="diet", aggfun=matrixStats::rowMedians)
# aggSamp(mouseData, fct='diet',aggfun=rowMaxs)
aggregateByTaxonomy

**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**

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

```r
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**

```r
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
Cumulative sum scaling (css) normalization factors

Description
Return a vector of 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
Calculates 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 (logisitic) 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**

`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.
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**
```r
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**
```r
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**
```r
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

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
```r
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
```r
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 MReperiment 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**
```r
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

- **z**: Matrix (m x n) of estimate responsibilities (probabilities that a count comes from a spike distribution at 0).
- **y**: Matrix (m x n) of count observations.
- **mmCount**: Model matrix for the count distribution.
- **stillActive**: Boolean vector of size M, indicating whether a feature converged or not.
- **fit2**: Previous fit of the count model.
- **dfMethod**: 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 $f_{\text{zig}}(y_{ij} = \pi_j(S_j) f_0(y_{ij}) + (1-\pi_j (S_j)) f_{\text{count}}(y_{ij};\mu_i,\sigma_i^2)$. 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 $z_{ij} = \text{pr}(\delta_{ij}=1 | \text{data})$.

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 $z_{ij} = \frac{\pi_j}{\pi_j + (1-\pi_j)} f_{\text{count}}(y_{ij};\mu_i,\sigma_i^2)$.

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

Usage

doZeroMStep(z, zeroIndices, mmZero)

Arguments

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

zeroIndices
Index (matrix m x n) of counts that are zero/non-zero.

mmZero
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

```r
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"))
```

---

### 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

```r
expSummary(obj)
```

### Arguments

- **obj**: a MRexperiment object.

### Value

Experiment summary table

### Author(s)

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

### Examples

```r
data(mouseData)  
expSummary(mouseData)
```
extractMR

Extract the essentials of an MRexperiment.

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

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

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
**fitFeatureModel**

**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)
```

**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.
- **sl**: The value to scale by (default=1000).

Value

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

Examples

```r
# 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

```r
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

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

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 ssFit ssIntervalCandidate ssPerm ssPermAnalysis plotTimeSeries

Examples

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

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

*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**

```r
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**

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

**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 $f_{z\text{ig}}(y_{ij} = \pi_j(S_j)*f_0(y_{ij}) + (1-\pi_j(S_j)) * f_{\text{count}}(y_{ij}; \mu_i, \sigma_i^2)$. 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**

`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 $z_{ij} = \Pr( \delta_{ij}=1 | \text{data})$
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: $f(x) = 1/(\sqrt{2 \pi \sigma}) \exp(-((x - \mu)^2/(2 \sigma^2)))$. 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 $f_z(y_{ij} = \pi_j(S_j) \cdot f_0(y_{ij}) + (1-\pi_j(S_j)) \cdot f_{\text{count}}(y_{ij};\mu_i,\sigma_i^2)$.

**Usage**

```r
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**

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

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

**Usage**

```r
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.
getNegativeLogLikelihoods

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

See Also
fitZig

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

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

geti(residuals)

Arguments

residuals Residuals from the zero model.

Value

Mixture proportions for each sample.

See Also

fitZig

geti

Calculate the current Z estimate responsibilities (posterior probabilities)

Description

Calculate the current Z estimate responsibilities (posterior probabilities)

Usage

geti(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.
**isItStillActive**

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**

```r
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

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

---

**loadBiom**  
Load objects organized in the Biom format.

Description

Wrapper to load Biom formatted object.

Usage

```r
loadBiom(file)
```

Arguments

- `file`  
The biom object filepath.

Value

A MRexperiment object.

See Also

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

Examples

```r
#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'.

See Also

loadMeta loadPhenoData

Examples

# see vignette

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

```r
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

```r
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

```r
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
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.

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**

```r
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/FALSE) 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, jpaulson@umiacs.umd.edu

Examples

data(lungData)
head(MRcounts(lungData))
MRexperiment

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**

**MRexperiment to biom objects**

**Examples**

# See vignette

```
MRexperiment2biom
```

**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

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**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**

```
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))
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, options are "ihw-abundance" (median feature count per row) and "ihw-ubiquity" (number of non-zero features per row). For obj of class fitZigResults, 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

```r
## 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`, options are "ihw-abundance" (median feature count per row) and "ihw-ubiquity" (number of non-zero features per row). For obj of class `fitZigResults`, 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

```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**

```r
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
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
plotBubble

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

```r
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**

```r
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**

```r
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  

**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)
notuIndex=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**

```r
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**

```r
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**

```r
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 = 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**

```r
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  Access the posterior probabilities that results from analysis

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 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

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

#### Usage
```r
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
```r
# Not run
```

### ssPerm

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

#### Usage
```r
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  

**Trapezoidal Integration**

**Description**

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

**Usage**

```r
trapz(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**

```r
# Calculate the area under the sine curve from 0 to pi:
n <- 101
x <- seq(0, pi, len = n)
y <- sin(x)
trapz(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
trapz(x, y) - h^2/12 * (cb - ca)  #=> 1.99999969
```

ts2MRexperiment  

**With a list of fitTimeSeries results, generate an MRexperiment that can be plotted with metaviz**

**Description**

With a list of fitTimeSeries results, generate an MRexperiment that can be plotted with metaviz.

**Usage**

```r
ts2MRexperiment(obj, sampleNames = NULL, sampleDescription = "timepoints", taxonomyLevels = NULL, taxonomyHierarchyRoot = "bacteria", taxonomyDescription = "taxonomy", featuresOfInterest = NULL, featureDataOfInterest = NULL)
```
uniqueFeatures

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>obj</td>
<td>Output of fitMultipleTimeSeries</td>
</tr>
<tr>
<td>sampleNames</td>
<td>Sample names for plot</td>
</tr>
<tr>
<td>sampleDescription</td>
<td>Description of samples for plot axis label</td>
</tr>
<tr>
<td>taxonomyLevels</td>
<td>Feature names for plot</td>
</tr>
<tr>
<td>taxonomyHierarchyRoot</td>
<td>Root of feature hierarchy for MRexperiment</td>
</tr>
<tr>
<td>taxonomyDescription</td>
<td>Description of features for plot axis label</td>
</tr>
<tr>
<td>featuresOfInterest</td>
<td>The features to select from the fitMultipleTimeSeries output</td>
</tr>
<tr>
<td>featureDataOfInterest</td>
<td>featureData for the resulting MRexperiment</td>
</tr>
</tbody>
</table>

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
```

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.
Value

Table of features unique to a group

Examples

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

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

Settings for the fitZig function

**Usage**

```r
gizControl(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 `gizControl`.

**See Also**

`fitZig` `cumNorm` `plotOTU`

**Examples**

```r
control = gizControl(tol=1e-10,maxit=10,verbose=FALSE)
```
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