Package ‘metagenomeSeq’

March 26, 2020

Title  Statistical analysis for sparse high-throughput sequencing
Version  1.29.4
Date  2019-07-12
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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
Depends  R(>= 3.0), Biobase, limma, glmnet, methods, RColorBrewer
Suggests  annotate, BiocGenerics, biomformat, knitr, gss, testthat (>= 0.8), vegan, interactiveDisplay, IHW
Imports  parallel, matrixStats, foreach, Matrix, gplots, graphics, grDevices, stats, utils, Wrench
VignetteBuilder  knitr
URL  https://github.com/nosson/metagenomeSeq/
BugReports  https://github.com/nosson/metagenomeSeq/issues
biocViews  ImmunoOncology, Classification, Clustering, GeneticVariability, DifferentialExpression, Microbiome, Metagenomics, Normalization, Visualization, MultipleComparison, Sequencing, Software
RoxygenNote  7.1.0
git_url  https://git.bioconductor.org/packages/metagenomeSeq
git_branch  master
git_last_commit  690e3b3
git_last_commit_date  2020-03-24
Date/Publication  2020-03-25
R topics documented:

- `metagenomeSeq-package` ......................................................... 3
- `aggregateBySample` ............................................................... 4
- `aggregateByTaxonomy` ............................................................. 5
- `biom2MRexperiment` ................................................................. 6
- `calcNormFactors` ................................................................. 7
- `calcPosComponent` ............................................................... 7
- `calcShrinkParameters` ............................................................. 8
- `calcStandardError` ............................................................... 8
- `calculateEffectiveSamples` .................................................... 9
- `calcZeroAdjustment` ............................................................. 9
- `calcZeroComponent` ............................................................ 10
- `correctIndices` ................................................................. 10
- `correlationTest` ................................................................. 11
- `cumNorm` ........................................................................ 12
- `cumNormMat` ............................................................... 13
- `cumNormStat` ................................................................. 14
- `cumNormStatFast` ............................................................ 14
- `doCountMStep` ................................................................. 15
- `doEStep` ................................................................. 16
- `doZeroMStep` ................................................................. 17
- `exportMat` ................................................................. 17
- `exportStats` ................................................................. 18
- `expSummary` ................................................................. 19
- `extractMR` ................................................................. 19
- `filterData` ................................................................. 20
- `fitDO` ................................................................. 21
- `fitFeatureModel` ............................................................... 22
- `fitFeatureModelResults-class` .................................................. 23
- `fitLogNormal` ............................................................... 23
- `fitMultipleTimeSeries` ............................................................. 24
- `fitPA` ................................................................. 25
- `fitSSTimeSeries` ............................................................... 26
- `fitTimeSeries` ............................................................... 27
- `fitZeroLogNormal` ............................................................. 29
- `fitZig` ................................................................. 30
- `fitZigResults-class` ............................................................... 31
- `getCountDensity` ............................................................... 32
- `getEpsilon` ................................................................. 32
- `getNegativeLogLikelihoods` .................................................... 33
- `getPi` ................................................................. 34
- `getZ` ................................................................. 34
- `isItStillActive` ............................................................... 35
- `libSize` ................................................................. 36
- `libSize<-` ................................................................. 36
- `loadBiom` ................................................................. 37
- `loadMeta` ................................................................. 38
- `loadMetaQ` ................................................................. 38
- `loadPhenoData` ............................................................... 39
- `lungData` ................................................................. 40
- `makeLabels` ............................................................... 40
metagenomeSeq-package

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

```r
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

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

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

Description

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

Usage

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

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

Arguments

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

Value
An aggregated count matrix.

Examples

data(mouseData)
aggregateByTaxonomy(mouseData[1:100,],lvl="class",norm=TRUE,aggfun=colSums)
# not run
# aggregateByTaxonomy(mouseData,lvl="class",norm=TRUE,aggfun=colMedians)
# aggTax(mouseData,lvl='phylum',norm=FALSE,aggfun=colSums)

biom2MRexperiment  Biom to MRexperiment objects

Description
Wrapper to convert biom files to MRexperiment objects.

Usage

biom2MRexperiment(obj)

Arguments

obj  The biom object file.

Value
A MRexperiment object.

See Also
loadMeta loadPhenoData newMRexperiment loadBiom

Examples

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

Cumulative sum scaling (css) normalization factors

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

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

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

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

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 (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
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

```r
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.
cumNorm

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**

```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).

**Value**

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

**See Also**

`fitZig` `cumNorm`

**Examples**

```r
data(mouseData)
head(cumNormMat(mouseData))
```
**cumNormStat**

Cumulative sum scaling percentile selection

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

**Usage**

```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 row means for reference.

**Usage**

```r
cumNormStatFast(obj, pFlag = FALSE, rel = 0.1, ...)
```

**See Also**
`fitZig`, `cumNorm`, `cumNormStat`
Arguments

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

Value

Percentile for which to scale data

See Also

- `fitZig`
- `cumNorm`
- `cumNormStat`

Examples

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

doCountMStep  

docCountMStep  

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

```r
doCountMStep(z, y, mmCount, stillActive, fit2 = NULL, dfMethod = "modified")
```

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 $Sy_{ij}$ 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 $log f_count(y;mu_i,sigma_i^2) + delta_{ij} log pi_j(S_j) + (1-delta_{ij}) log (1-pi_j (S_j))$. The responsibilities are defined as $z_{ij} = pr(delta_{ij}=1 | data)$.
doEStep

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

See Also
fitZig

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

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 $f_{\text{zig}}(y_{ij} = \pi_j(S_j) \cdot f_0(y_{ij}) + (1-\pi_j(S_j)) \cdot f_{\text{count}}(y_{ij}) \cdot \mu_i \cdot \sigma_i^2)$. The log-likelihood in this extended model is $\log f_{\text{count}}(y_{ij};\mu_i,\sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$. The responsibilities are defined as $z_{ij} = \frac{\pi_j \cdot I_0(y_{ij})}{\pi_j \cdot I_0(y_{ij}) + (1-\pi_j) \cdot f_{\text{count}}(y_{ij})}$. The log-likelihood is $(1-\delta_{ij}) \log f_{\text{count}}(y_{ij};\mu_i,\sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$.

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. $\pi_j = \sum_i^M \frac{1}{M} z_{ij}$ $\pi_j$ 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 $f_z(y_{ij} = \pi_j(S_j)c \cdot f_0(y_{ij})+(1-\pi_j(S_j))c \cdot f_{count}(y_{ij};\mu_i,\sigma_i^2)$. The log-likelihood in this extended model is $(1-\delta_{ij}) \log f_{count}(y_{ij};\mu_i,\sigma_i^2) + \delta_{ij} \log \pi_j(S_j) + (1-\delta_{ij}) \log (1-\pi_j(S_j))$. The responsibilities are defined as $z_{ij} = \Pr(\delta_{ij}=1 | data)$.

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"
)
exportStats

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

t ests

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 quantitative
### 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, jpmoulson@umiacs.umd.edu

### Examples

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

---

### extractMR

*Extract the essentials of an MRexperiment.*

### Description

Extract the essentials of an MRexperiment.

### Usage

```r
extractMR(obj)
```
filterData

Arguments

obj

MReperiment-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))

Description

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

Usage

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

Arguments

obj
A MReperiment object or count matrix.

present
Features with at least 'present' positive samples.

depth
Samples with at least this much depth of coverage

Value

A MReperiment object.

Examples

data(mouseData)
filterData(mouseData)
fitDO

Wrapper to calculate Discovery Odds Ratios on feature values.

Description

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

Usage

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

Arguments

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

Value

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

See Also

cumNorm fitZig fitPA fitMeta

Examples

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

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

Description

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

Usage

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 - permutted 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)
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

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 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.
fitPA

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

Value

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

See Also

cumNorm fitZig fitDO fitMeta

Examples

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

*Discover differentially abundant time intervals using SS-Anova*

**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**

```r
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
fitTimeSeries

norm When aggregating counts to normalize or not.
log Log2 transform.
s1 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"),
)
```r
lvl = NULL,
include = c("class", "time:class"),
C = 0,
B = 1000,
norm = TRUE,
log = TRUE,
s1 = 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.
- `s1` 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.
fitZeroLogNormal

See Also
cumNorm fitSSTimeSeries plotTimeSeries

Examples

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

fitZeroLogNormal

Compute the log fold-change estimates for the zero-inflated log-normal model

Description

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

Usage

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

Arguments

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

Value

A list of objects including:

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

See Also
cumNorm fitFeatureModel
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_{\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: $(1-\delta_{ij}) \log f_{\text{count}}(y_{ij} ; \mu_i, \sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$. The responsibilities are defined as $z_{ij} = \Pr(\delta_{ij}=1 | \text{data})$.

Usage

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

Arguments

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

Value

A list of objects including:

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

See Also
cumNorm zigControl

Examples

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

**Description**

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

**Usage**

```r
generateDensity(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

**Description**

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

**Usage**

```r
generateEpsilon(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

---

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 $(1-\delta_{ij}) \log f_{count}(y;\mu_i,\sigma_i^2) + \delta_{ij} \log \pi_j(s_j) + (1-\delta_{ij}) \log (1-\pi_j(s_j))$. 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**

\[ F(x) = \frac{1}{1 + \exp(-(x-m)/s)} \] (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**

```r
getPi(residuals)
```

**Arguments**

- `residuals` : Residuals from the zero model.

**Value**

Mixture proportions for each sample.

**See Also**

`fitZig`

getZ

*Calculate the current Z estimate responsibilities (posterior probabilities)*

**Description**

Calculate the current Z estimate responsibilities (posterior probabilities)

**Usage**

```r
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.
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

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**

```r
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**

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

**Arguments**

- `object` : a `MRexperiment` object
- `value` : vector of library sizes

**Value**

vector library sizes
loadBiom

Author(s)

Joseph N. Paulson

Examples

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

loadBiom

Load objects organized in the Biom format.

Description

Wrapper to load Biom formatted object.

Usage

loadBiom(file)

Arguments

file The biom object filepath.

Value

A MRexperiment object.

See Also

loadMeta loadPhenoData newMRexperiment biom2MRexperiment

Examples

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

Load a count dataset associated with a study.

Description

Load a matrix of OTUs in a tab delimited format.

Usage

loadMeta(file, sep = "\t")

Arguments

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

Value

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

See Also

loadPhenoData

Examples

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

loadMetaQ

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

Description

Load a matrix of OTUs in Qiime's format.

Usage

loadMetaQ(file)

Arguments

file
Path and filename of the actual data file.

Value

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

See Also

loadMeta loadPhenoData

Examples

# see vignette

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

**Format**

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

**Value**

MRexperiment-class object of 16S lung samples.

**References**


**makeLabels** | *Function to make labels simpler*

**Description**

Beginning to transition to better axes for plots

**Usage**

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

**Arguments**

- **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
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

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 "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.
MRcounts

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.
one The value to scale by (default=1000).

Value

Normalized or raw counts
MRexperiment

Author(s)
Joseph N. Paulson, jpaulson@umiacs.umd.edu

Examples

```r
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 $p = \text{cumNormStat}$.

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

```r
# See vignette
```

---

### Description

Wrapper to convert MRexperiment objects to biom objects.

### Usage

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

### Arguments

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

### Value

A biom object.

### See Also

`loadMeta`, `loadPhenoData`, `newMRexperiment`, `loadBiom`, `biom2MRexperiment`
MRfulltable  

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

Description

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

Usage

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

Arguments

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

See Also

fitZig fitFeatureModel MRcoefs MRtable fitPA

Examples

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

fit = fitFeatureModel(obj = lungTrim,mod=mod)
head(MRfulltable(fit))

MRihw runs IHW within a MRcoefs() call

Description

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

Usage

MRihw(obj, ...)

Arguments

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**

```r
## 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**

```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-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.
counts Filter features to have at least 'counts' of counts.
file Name of file, including location, to save the table.
nullMRexperiment

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

See Also
fitZig fitFeatureModel MRcoefs MRfulltable

Examples

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

newMRexperiment

Create a MRexperiment object

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

Usage

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

Arguments

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

phenoData An AnnotatedDataFrame with pertinent sample information.

featureData An AnnotatedDataFrame with pertinent feature information.

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

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

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)

data(lungData)
head(normFactors(lungData))

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

```r
## 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

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

---

**plotBubble**

Basic plot of binned vectors.

### Description

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

plotBubble(
  yvector,
  xvector,
  sigvector = NULL,
  nbbreaks = 10,
  ybreak = quantile(yvector, p = seq(0, 1, length.out = nbbreaks)),
  xbreak = quantile(xvector, p = seq(0, 1, length.out = nbbreaks)),
  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).
  nbbreaks      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

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

Arguments

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

Value

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

See Also

`fitTimeSeries`

Examples

```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
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.
plotFeature

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**

```r
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`
plotMRheatmap

Examples

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

otuIndex = grep("Strep", pData(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
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

```r
data(mouseData)
cl = pData(mouseData)[,3]
plotOrd(mouseData,tran=TRUE,useDist=TRUE,pch=21,bg=factor(cl),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.
plotRare

### 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")

---

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

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

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

**Value**
Library size and number of detected features

**See Also**
plotOrd, plotMRheatmap, plotCorr, plotOTU, plotGenus

**Examples**

data(mouseData)
c1 = factor(pData(mouseData)[,3])
res = plotRare(mouseData,cl=c1,pch=21,bg=c1)
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)
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

Access the posterior probabilities that results from analysis

Description

Accessing the posterior probabilities following a run through fitZig

Usage

posteriorProbs(obj)

Arguments

obj

a MReperiment 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 MReperiment or matrix and return matrix

Description

Function to check if object is a MReperiment class or matrix

Usage

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

**Arguments**

<table>
<thead>
<tr>
<th>obj</th>
<th>a MReperiment or matrix object</th>
</tr>
</thead>
<tbody>
<tr>
<td>norm</td>
<td>return a normalized MReperiment matrix</td>
</tr>
<tr>
<td>log</td>
<td>return a log transformed MReperiment matrix</td>
</tr>
<tr>
<td>sl</td>
<td>scaling value</td>
</tr>
</tbody>
</table>

**Value**

Matrix

**Examples**

```r
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**

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

**Arguments**

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

```r
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

# 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"),
  ...
)


**trapz**

**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**

```r
# Not run
```

---

**Trapezoidal Integration**

**Description**

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

**Usage**

`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

# 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) #=> 1.999999969

---

**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
)
```

**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
uniqueFeatures

Value

MRexperiment that contains fitTimeSeries data, featureData, and phenoData

See Also

fitTimeSeries fitMultipleTimeSeries

Examples

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

uniqueFeatures Table of features unique to a group

Description

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

Usage

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**

*Computes normalization factors using wrench instead of cumNorm*

**Description**

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

**Usage**

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

**Arguments**

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

**Value**

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

**See Also**

`cumNorm` `fitZig`

**Examples**

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

**zigControl**

*Settings for the fitZig function*

**Description**

Settings for the fitZig function

**Usage**

```r
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)
Index

<table>
<thead>
<tr>
<th>package</th>
<th>index</th>
</tr>
</thead>
<tbody>
<tr>
<td>metagenomeSeq-package</td>
<td>3</td>
</tr>
<tr>
<td>[.,MRexperiment,ANY,ANY,ANY-method (MRexperiment)</td>
<td>45</td>
</tr>
<tr>
<td>[.,MRexperiment-method (MRexperiment)</td>
<td>45</td>
</tr>
<tr>
<td>aggregateBySample</td>
<td>4</td>
</tr>
<tr>
<td>aggregateByTaxonomy</td>
<td>5</td>
</tr>
<tr>
<td>aggSamp (aggregateBySample)</td>
<td>4</td>
</tr>
<tr>
<td>aggTax (aggregateByTaxonomy)</td>
<td>5</td>
</tr>
<tr>
<td>biom2MRexperiment</td>
<td>6, 37, 46</td>
</tr>
<tr>
<td>calcNormFactors</td>
<td>7</td>
</tr>
<tr>
<td>calcPosComponent</td>
<td>7</td>
</tr>
<tr>
<td>calcShrinkParameters</td>
<td>8</td>
</tr>
<tr>
<td>calculateEffectiveSamples</td>
<td>9</td>
</tr>
<tr>
<td>calcZeroAdjustment</td>
<td>9</td>
</tr>
<tr>
<td>calcZeroComponent</td>
<td>10</td>
</tr>
<tr>
<td>colMeans,MRexperiment-method (MRexperiment)</td>
<td>45</td>
</tr>
<tr>
<td>colSums,MRexperiment-method (MRexperiment)</td>
<td>45</td>
</tr>
<tr>
<td>correctIndices</td>
<td>10, 12</td>
</tr>
<tr>
<td>correlationTest</td>
<td>10, 11</td>
</tr>
<tr>
<td>corTest (correlationTest)</td>
<td>11</td>
</tr>
<tr>
<td>cumNorm, 7, 12, 13–15, 18, 21, 22, 25, 27, 29, 31, 46, 57, 58, 62, 66–68, 71, 72</td>
<td></td>
</tr>
<tr>
<td>cumNormMat, 13, 46, 56, 59, 60</td>
<td></td>
</tr>
<tr>
<td>cumNormStat, 12, 14, 15, 46</td>
<td></td>
</tr>
<tr>
<td>cumNormStatFast, 7, 14, 14</td>
<td></td>
</tr>
<tr>
<td>deprecated_metagenomeSeq_function (metagenomeSeq-deprecated)</td>
<td>42</td>
</tr>
<tr>
<td>doCountMStep</td>
<td>15</td>
</tr>
<tr>
<td>doEStep</td>
<td>16</td>
</tr>
<tr>
<td>doZeroMStep</td>
<td>17</td>
</tr>
<tr>
<td>exportMat</td>
<td>17</td>
</tr>
<tr>
<td>exportMatrix (exportMat)</td>
<td>17</td>
</tr>
<tr>
<td>exportStats</td>
<td>18</td>
</tr>
<tr>
<td>expSummary</td>
<td>19</td>
</tr>
<tr>
<td>expSummary,MRexperiment-method (expSummary)</td>
<td>19</td>
</tr>
<tr>
<td>extractMR</td>
<td>19</td>
</tr>
<tr>
<td>filterData</td>
<td>20</td>
</tr>
<tr>
<td>fitDO, 21, 25</td>
<td></td>
</tr>
<tr>
<td>fitFeatureModel, 7–10, 22, 29, 44, 48, 51</td>
<td></td>
</tr>
<tr>
<td>fitFeatureModelResults-class, 23</td>
<td></td>
</tr>
<tr>
<td>fitLogNormal</td>
<td>23</td>
</tr>
<tr>
<td>fitMeta, 21, 25</td>
<td></td>
</tr>
<tr>
<td>fitMeta (metagenomeSeq-deprecated), 42</td>
<td></td>
</tr>
<tr>
<td>fitMultipleTimeSeries, 24, 70</td>
<td></td>
</tr>
<tr>
<td>fitPA, 21, 25, 48</td>
<td></td>
</tr>
<tr>
<td>fitSSTimeSeries, 25, 26, 29</td>
<td></td>
</tr>
<tr>
<td>fitTimeSeries, 24, 25, 27, 53, 63, 66–68, 70</td>
<td></td>
</tr>
<tr>
<td>fitZeroLogNormal, 7–10, 29</td>
<td></td>
</tr>
<tr>
<td>fitZig, 7, 9, 12–17, 21, 25, 30, 32–36, 44, 48, 51, 64, 71, 72</td>
<td></td>
</tr>
<tr>
<td>fitZigResults-class, 31</td>
<td></td>
</tr>
<tr>
<td>genusPlot (plotGenus)</td>
<td>58</td>
</tr>
<tr>
<td>getCountDensity</td>
<td>32</td>
</tr>
<tr>
<td>getEpsilon</td>
<td>32</td>
</tr>
<tr>
<td>getNegativeLogLikelihoods</td>
<td>33</td>
</tr>
<tr>
<td>getPi</td>
<td>34</td>
</tr>
<tr>
<td>getZ, 31, 34</td>
<td></td>
</tr>
<tr>
<td>isItStillActive</td>
<td>35</td>
</tr>
<tr>
<td>libSize, 36, 45</td>
<td></td>
</tr>
<tr>
<td>libSize,MRexperiment-method (MRexperiment), 45</td>
<td></td>
</tr>
<tr>
<td>libSize&lt;-, 36</td>
<td></td>
</tr>
<tr>
<td>libSize&lt;-,MRexperiment,numeric-method (libSize&lt;-,)</td>
<td>36</td>
</tr>
<tr>
<td>load_biom (metagenomeSeq-deprecated), 42</td>
<td></td>
</tr>
<tr>
<td>load_meta (metagenomeSeq-deprecated), 42</td>
<td></td>
</tr>
<tr>
<td>load_metaQ (metagenomeSeq-deprecated), 42</td>
<td></td>
</tr>
<tr>
<td>load_phenoData</td>
<td>6, 37–39, 39, 46</td>
</tr>
<tr>
<td>loadBiom, 6, 37, 46</td>
<td></td>
</tr>
<tr>
<td>loadMeta, 6, 37, 38, 39, 46</td>
<td></td>
</tr>
<tr>
<td>loadMetaQ, 38</td>
<td></td>
</tr>
<tr>
<td>loadPhenoData, 6, 37–39, 39, 46</td>
<td></td>
</tr>
<tr>
<td>lungData, 40</td>
<td></td>
</tr>
<tr>
<td>Function</td>
<td>Page(s)</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>makeLabels</td>
<td>40</td>
</tr>
<tr>
<td>mergeMRexperiments</td>
<td>41</td>
</tr>
<tr>
<td>mergeTable</td>
<td>41</td>
</tr>
<tr>
<td>metagenomeSeq (metagenomeSeq-package)</td>
<td>3</td>
</tr>
<tr>
<td>metagenomeSeq-deprecated</td>
<td>42</td>
</tr>
<tr>
<td>metagenomicLoader (loadMeta)</td>
<td>38</td>
</tr>
<tr>
<td>MRcoefs</td>
<td>9, 43, 48, 51</td>
</tr>
<tr>
<td>MRcounts</td>
<td>44, 45</td>
</tr>
<tr>
<td>MRcounts, MRexperiment-method</td>
<td>(MRcounts), 44</td>
</tr>
<tr>
<td>MRexperiment</td>
<td>45</td>
</tr>
<tr>
<td>MRexperiment-class (MRexperiment)</td>
<td>45</td>
</tr>
<tr>
<td>MRfulltable</td>
<td>9, 44, 47, 51</td>
</tr>
<tr>
<td>MRihw</td>
<td>43, 48</td>
</tr>
<tr>
<td>MRihw, fitFeatureModelResults-method</td>
<td>49</td>
</tr>
<tr>
<td>MRihw, fitZigResults-method</td>
<td>49</td>
</tr>
<tr>
<td>MRtable</td>
<td>44, 48, 50</td>
</tr>
<tr>
<td>newMRexperiment</td>
<td>6, 37, 45, 46, 51</td>
</tr>
<tr>
<td>normFactors</td>
<td>45, 52, 71</td>
</tr>
<tr>
<td>normFactors, MRexperiment-method</td>
<td>(MRexperiment), 45</td>
</tr>
<tr>
<td>normFactors&lt;-</td>
<td>.53</td>
</tr>
<tr>
<td>normFactors&lt;-, MRexperiment, numeric-method</td>
<td>(normFactors&lt;-), 53</td>
</tr>
<tr>
<td>p.adjust</td>
<td>21, 25, 43, 47, 50</td>
</tr>
<tr>
<td>phenoData (loadPhenoData)</td>
<td>39</td>
</tr>
<tr>
<td>plotBubble</td>
<td>53</td>
</tr>
<tr>
<td>plotClassTimeSeries</td>
<td>55</td>
</tr>
<tr>
<td>plotCorr</td>
<td>56, 62</td>
</tr>
<tr>
<td>plotFeature</td>
<td>56</td>
</tr>
<tr>
<td>plotGenus</td>
<td>58, 62</td>
</tr>
<tr>
<td>plotMRheatmap</td>
<td>54, 59, 62</td>
</tr>
<tr>
<td>plotOrd</td>
<td>60, 62</td>
</tr>
<tr>
<td>plotOTU</td>
<td>61, 62, 72</td>
</tr>
<tr>
<td>plotRare</td>
<td>62</td>
</tr>
<tr>
<td>plotTimeSeries</td>
<td>27, 29, 63</td>
</tr>
<tr>
<td>posteriorProbs</td>
<td>45, 46, 64</td>
</tr>
<tr>
<td>posteriorProbs, MRexperiment-method</td>
<td>(posteriorProbs), 64</td>
</tr>
<tr>
<td>qiimeLoader (loadMetaQ)</td>
<td>38</td>
</tr>
<tr>
<td>quantile</td>
<td>18</td>
</tr>
<tr>
<td>returnAppropriateObj</td>
<td>64</td>
</tr>
<tr>
<td>rowMeans, MRexperiment-method</td>
<td>(MRexperiment), 45</td>
</tr>
<tr>
<td>rowSums, MRexperiment-method</td>
<td>(MRexperiment), 45</td>
</tr>
</tbody>
</table>