

Package ‘metagenomeSeq’

October 8, 2014

Title Statistical analysis for sparse high-throughput sequencing

Version 1.6.0

Date 2013-04-10

Author Joseph Nathaniel Paulson, Mihai Pop, Hector Corrada Bravo

Maintainer Joseph N. Paulson <jpaulson@umiacs.umd.edu>

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, matrixStats, methods, RColorBrewer,gplots

Suggests annotate, biom, parallel, vegan, knitr

Collate 'aggregateByTaxonomy.R' 'allClasses.R' 'biom2MRExperiment.R'
'calculateEffectiveSamples.R' 'cumNorm.R' 'cumNormStat.R'
'cumNormStatFast.R' 'cumNormMat.R' 'correlationTest.R'
'doCountMStep.R' 'doZeroMStep.R' 'doEStep.R' 'exportMat.R'
'exportStats.R' 'fitDO.R' 'fitMeta.R' 'fitPA.R' 'fitZig.R'
'filterData.R' 'getEpsilon.R' 'getCountDensity.R' 'getNegativeLogLikelihoods.R' 'getPi.R' 'getZ.R'
'isItStillActive.R' 'load_biom.R' 'load_meta.R' 'load_metaQ.R'
'load_phenoData.R' 'misc.R' 'MRtable.R' 'MRcoefs.R'
'MRfulltable.R' 'MRExperiment2biom.R' 'plotMRheatmap.R'
'plotCorr.R' 'plotOTU.R' 'plotOrd.R' 'plotRare.R' 'plotGenus.R' 'plotFeature.R' 'zigControl.R'

VignetteBuilder knitr

URL <http://cbcb.umd.edu/software/metagenomeSeq>

biocViews DifferentialExpression, Metagenomics, Visualization

R topics documented:

metagenomeSeq-package	3
aggregateByTaxonomy	3
biom2MRExperiment	4
calculateEffectiveSamples	5
correctIndices	6
correlationTest	6
cumNorm	7
cumNormMat	8
cumNormStat	9
cumNormStatFast	10
doCountMStep	10
doEStep	11
doZeroMStep	12
exportMat	13
exportStats	14
expSummary	14
filterData	15
fitDO	16
fitMeta	17
fitPA	17
fitZig	18
getCountDensity	19
getEpsilon	20
getNegativeLogLikelihoods	20
getPi	21
getZ	22
isItStillActive	22
libSize	23
load_biom	24
load_meta	24
load_metaQ	25
load_phenoData	26
lungData	26
makeLabels	27
mouseData	27
MRcoefs	28
MRcounts	29
MRexperiment	30
MRexperiment2biom	31
MRfulltable	32
MRtable	33
newMRexperiment	34
normFactors	35
plotCorr	36
plotFeature	37
plotGenus	38

plotMRheatmap	39
plotOrd	40
plotOTU	41
plotRare	42
posterior.probs	43
uniqueFeatures	43
zigControl	44

Index	45
--------------	-----------

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

Description

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

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

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

Author(s)

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

References

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

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

Description

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

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, out = "MRExperiment")

aggTax(obj, lvl, alternate = FALSE, norm = FALSE, log = FALSE,
  aggfun = colSums, sl = 1000, 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.
out	Either 'MRExperiment' or 'matrix'

Value

An aggregated count matrix.

Examples

```
# not run
# aggregateByTaxonomy(mouseData, lvl="genus", norm=TRUE, aggfun=colMedians)
# aggTax(mouseData, lvl=phylum, norm=FALSE, aggfun=colSums)
```

biom2MRExperiment *Biome to MRExperiment objects*

Description

Wrapper to convert biome files to MRExperiment objects.

Usage

```
biom2MRExperiment(obj)
```

Arguments

obj	The biome object file.
-----	------------------------

Value

A MRexperiment object.

See Also

[load_meta](#) [load_phenoData](#) [newMRexperiment](#) [load_biom](#)

Examples

```
#library(biom)
#rich_dense_file = system.file("extdata", "rich_dense_otu_table.biom", package = "biom")
#x = read_biom(rich_dense_file)
#biom2MRexperiment(x)
```

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 $\sum_1 1^n$ ($n =$ 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](#)

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

Description

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

Usage

```
correctIndices(n)
```

Arguments

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

Value

A vector of the indices for an upper triangular matrix.

Examples

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

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

correlationTest	<i>Pairwise correlation for each row of a matrix or MRexperiment object</i>
-----------------	---

Description

Calculates the pairwise correlation statistics and associated p-values.

Usage

```
correlationTest(obj, method = "pearson", alternative = "two.sided",
  norm = TRUE, log = TRUE, parallel = FALSE, cores = 2,
  override = FALSE, ...)
```

Arguments

obj	A MRExperiment object or count matrix.
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.
parallel	Parallelize the correlation testing?
cores	Number of cores to make use of if parallel == TRUE.
override	If the number of rows to test is over a thousand the test will not commence (unless override==TRUE).
...	Extra settings for mclapply.

Value

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

Examples

```
data(mouseData)
cors = correlationTest(mouseData[1:10,], norm=FALSE, log=FALSE)
head(cors)
```

cumNorm

Cumulative sum scaling factors.

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

Vector of the sum up to and including a sample's pth quantile

See Also[fitZig](#) [cumNormStat](#)**Examples**

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

`cumNormMat`*Cumulative sum scaling factors.*

Description

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

Usage

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

Arguments

<code>obj</code>	A MRExperiment object.
<code>p</code>	The pth quantile.
<code>s1</code>	The value to scale by (default=1000).

Value

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

See Also[fitZig](#) [cumNorm](#)**Examples**

```
data(mouseData)
head(cumNormMat(mouseData))
```

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

Description

Calculates the percentile for which to sum counts up to and scale by. cumNormStat might be deprecated one day.

Usage

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

Arguments

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

Value

Percentile for which to scale data

See Also

[fitZig](#) [cumNorm](#) [cumNormStatFast](#)

Examples

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

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

Description

Calculates the percentile for which to sum counts up to and scale by. Faster version than available in cumNormStat.

Usage

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

Arguments

obj	A MExperiment object.
pFlag	Plot the median difference quantiles.
rel	Cutoff for the relative difference from one median difference from the reference to the next.
...	Applicable if pFlag == TRUE. Additional plotting parameters.

Value

Percentile for which to scale data

See Also

[fitZig](#) [cumNorm](#) [cumNormStat](#)

Examples

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

doCountMStep	<i>Compute the Maximization step calculation for features still active.</i>
--------------	---

Description

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

Usage

```
doCountMStep(z, y, mmCount, stillActive, fit2 = NULL)
```

Arguments

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

Details

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

Value

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

See Also

[fitZig](#)

doEStep	<i>Compute the Expectation step.</i>
---------	--------------------------------------

Description

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

Usage

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

Arguments

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

Details

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

Value

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

See Also

[fitZig](#)

doZeroMStep

Compute the zero Maximization step.

Description

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

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 $\text{sum}(\text{zeroIndices})$.

See Also[fitZig](#)

`exportMat`*Export the normalized MExperiment dataset as a matrix.*

Description

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

Usage

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

Arguments

<code>obj</code>	A MExperiment object or count matrix.
<code>log</code>	Whether or not to log transform the counts - if MExperiment object.
<code>norm</code>	Whether or not to normalize the counts - if MExperiment object.
<code>sep</code>	Separator for writing out the count matrix.
<code>file</code>	Output file name.

Value

NA

See Also[cumNorm](#)**Examples**

```
# see vignette
```

exportStats	<i>Various statistics of the count data.</i>
-------------	--

Description

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

Usage

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

Arguments

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

Value

None.

See Also

[cumNorm quantile](#)

Examples

```
# see vignette
```

expSummary	<i>Access MRExperiment object experiment data</i>
------------	---

Description

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

Usage

```
expSummary(obj)
```

Arguments

obj	a MRExperiment object.
-----	------------------------

Author(s)

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

Examples

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

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

Description

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

Usage

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

Arguments

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

Value

A MRexperiment object.

Examples

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

`fitDO`*Wrapper to calculate Discovery Odds Ratios on feature values.*

Description

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

Usage

```
fitDO(obj, cl, norm = TRUE, log = TRUE)
```

Arguments

<code>obj</code>	A MRexperiment object with a count matrix, or a simple count matrix.
<code>cl</code>	Group comparison
<code>norm</code>	Whether or not to normalize the counts - if MRexperiment object.
<code>log</code>	Whether or not to log2 transform the counts - if MRexperiment object.

Value

NA

See Also

[cumNorm fitZig](#)

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)
```

fitMeta	<i>Computes a slightly modified form of Metastats.</i>
---------	--

Description

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

Usage

```
fitMeta(obj, mod, useCSSoffset = TRUE, B = 1000, coef = 2, s1 = 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.
s1	The value to scale by (default=1000).

Value

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

fitPA	<i>Wrapper to run fisher's test on presence/absence of a feature.</i>
-------	---

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)
```

Arguments

obj	A MRExperiment object with a count matrix, or a simple count matrix.
cl	Group comparison
thres	Threshold for defining presence/absence.

Value

NA

See Also[cumNorm](#) [fitZig](#)**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)
```

fitZig

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

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

Usage

```
fitZig(obj, mod, zeroMod = NULL, useCSSoffset = TRUE,
       control = zigControl())
```

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.

Value

The fits, posterior probabilities, posterior probabilities used at time of convergence for each feature, ebytes (limma object) fit, among other data.

See Also[cumNorm zigControl](#)**Examples**

```

data(lungData)
k = grep("Extraction.Control",pData(lungData)$SampleType)
lungTrim = lungData[,-k]
k = which(rowSums(MRcounts(lungTrim)>0)<30)
cumNorm(lungTrim)
lungTrim = lungTrim[-k,]
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
settings = zigControl(maxit=1,verbose=FALSE)
fit = fitZig(obj = lungTrim,mod=mod,control=settings)

```

getCountDensity	<i>Compute the value of the count density function from the count model residuals.</i>
-----------------	--

Description

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

Usage

```
getCountDensity(residuals, log = FALSE)
```

Arguments

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

Value

Density values from the count model residuals.

See Also[fitZig](#)

getEpsilon	<i>Calculate the relative difference between iterations of the negative log-likelihoods.</i>
------------	--

Description

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

Usage

```
getEpsilon(nll, nllOld)
```

Arguments

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

Value

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

See Also

[fitZig](#)

getNegativeLogLikelihoods	<i>Calculate the negative log-likelihoods for the various features given the residuals.</i>
---------------------------	---

Description

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

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](#)

<code>getPi</code>	<i>Calculate the mixture proportions from the zero model / spike mass model residuals.</i>
--------------------	--

Description

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

Usage

```
getPi(residuals)
```

Arguments

- `residuals` Residuals from the zero model.

Value

Mixture proportions for each sample.

See Also

[fitZig](#)

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

Description

Calculate the current Z estimate responsibilities (posterior probabilities)

Usage

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

Arguments

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

Value

A list of updated zUsed and nllUSED.

See Also

[fitZig](#)

isItStillActive	<i>Function to determine if a feature is still active.</i>
-----------------	--

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

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(obj)
```

Arguments

obj a MRexperiment object.

Author(s)

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

Examples

```
data(lungData)
head(libSize(lungData))
```

load_biom	<i>Load objects organized in the Biome format.</i>
-----------	--

Description

Wrapper to load Biome formatted object.

Usage

```
load_biom(file)
```

Arguments

file	The biome object filepath.
------	----------------------------

Value

A MRexperiment object.

See Also

[load_meta](#) [load_phenoData](#) [newMRexperiment](#) [biom2MRexperiment](#)

Examples

```
#library(biom)
#rich_dense_file = system.file("extdata", "rich_dense_otu_table.biom", package = "biom")
#x = load_biome(rich_dense_file)
#x
```

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

Description

Load a matrix of OTUs in a tab delimited format

Usage

```
load_meta(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

[load_phenoData](#)

Examples

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

load_metaQ	<i>Load a count dataset associated with a study set up in a Qiime format.</i>
------------	---

Description

Load a matrix of OTUs in Qiime's format

Usage

```
load_metaQ(file)
```

Arguments

file Path and filename of the actual data file.

Value

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

See Also

[load_meta](#) [load_phenoData](#)

Examples

```
# see vignette
```

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

Description

Load a matrix of metadata associated with a study.

Usage

```
load_phenoData(file, tran = FALSE, sep = "\t")
```

Arguments

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

Value

The metadata as a dataframe.

See Also

[load_meta](#)

Examples

```
# see vignette
```

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

Description

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

Usage

```
lungData
```

Format

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

References

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

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

Description

Beginning to transition to better axes for plots

Usage

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

Arguments

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

Value

vector of x,y labels

mouseData	<i>OTU abundance matrix of mice samples from a diet longitudinal study</i>
-----------	--

Description

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

Usage

```
mouseData
```

Format

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

References

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2894525/>

MRcoefs

*Table of top-ranked microbial marker gene from linear model fit***Description**

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

Usage

```
MRcoefs(obj, by = 2, coef = NULL, number = 10, taxa = obj$taxa,
        uniqueNames = FALSE, adjust.method = "fdr", group = 0, eff = 0,
        numberEff = FALSE, file = NULL)
```

Arguments

obj	A list containing the linear model fit produced by lmFit through fitZig.
by	Column number or column name specifying which coefficient or contrast of the linear model is of interest.
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.
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.
group	One of three 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	Restrict samples to have at least a "eff" quantile or number of effective samples.
numberEff	Boolean, whether eff should represent quantile (default/FALSE) or number.
file	Name of output file, including location, to save the table.

Value

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

See Also

[fitZig MRtable](#)

Examples

```

data(lungData)
k = grep("Extraction.Control",pData(lungData)$SampleType)
lungTrim = lungData[,-k]
k = which(rowSums(MRcounts(lungTrim)>0)<10)
lungTrim = lungTrim[-k,]
cumNorm(lungTrim)
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
settings = zigControl(maxit=1,verbose=FALSE)
fit = fitZig(obj = lungTrim,mod=mod,control=settings)
head(MRcoefs(fit))

```

MRcounts

Accessor for the counts slot of a MRexperiment object

Description

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

Usage

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

Arguments

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

Author(s)

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

Examples

```

data(lungData)
head(MRcounts(lungData))

```

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

Description

This is the main class for metagenomeSeq.

Objects from the Class

Objects should be created with calls to `newMRExperiment`.

Extends

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

Methods

Class-specific methods.

[<sample>, <variable>]: 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` datasets 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 `posterior.probs`.

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

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

To populate the various slots in an `MRExperiment` several functions are run. 1) `cumNormStat` calculates the proper percentile to calculate normalization factors. The `cumNormStat` slot is populated. 2) `cumNorm` calculates the actual normalization factors using `p = cumNormStat`.

Other functions will place subsequent matrices (normalized counts (`cumNormMat`), posterior probabilities (`posterior.probs`))

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

Examples

```
# See vignette
```

MRExperiment2biom	<i>MRExperiment to biom objects</i>
-------------------	-------------------------------------

Description

Wrapper to convert MRExperiment objects to biom objects.

Usage

```
MRExperiment2biom(obj, id = NULL, norm = FALSE, log = FALSE, s1 = 1000)
```

Arguments

obj	The MRExperiment object.
id	Optional id for the biom matrix.
norm	Normalized data?
log	Logged data?
s1	scaling factor for normalized counts.

Value

A biom object.

See Also

[load_meta](#) [load_phenoData](#) [newMRExperiment](#) [load_biom](#) [biom2MRExperiment](#)

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

Description

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

Usage

```
MRfulltable(obj, by = 2, coef = NULL, number = 10, taxa = obj$taxa,
  uniqueNames = FALSE, adjust.method = "fdr", group = 0, eff = 0,
  numberEff = FALSE, file = NULL)
```

Arguments

obj	A list containing the linear model fit produced by lmFit through fitZig.
by	Column number or column name specifying which coefficient or contrast of the linear model is of interest.
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.
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.
group	One of four 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	Restrict samples to have at least a "eff" quantile or number of effective samples.
numberEff	Boolean, whether eff should represent quantile (default/FALSE) or number.
file	Name of output file, including location, to save the table.

Value

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

See Also

[fitZig MRcoefs MRtable fitPA](#)

Examples

```
data(lungData)
k = grep("Extraction.Control",pData(lungData)$SampleType)
lungTrim = lungData[,-k]
k = which(rowSums(MRcounts(lungTrim)>0)<10)
lungTrim = lungTrim[-k,]
cumNorm(lungTrim)
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
settings = zigControl(maxit=1,verbose=FALSE)
fit = fitZig(obj = lungTrim,mod=mod,control=settings)
head(MRfulltable(fit))
```

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

Description

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

Usage

```
MRtable(obj, by = 2, coef = NULL, number = 10, taxa = obj$taxa,
        uniqueNames = FALSE, adjust.method = "fdr", group = 0, eff = 0,
        numberEff = FALSE, file = NULL)
```

Arguments

obj	A list containing the linear model fit produced by lmFit through fitZig.
by	Column number or column name specifying which coefficient or contrast of the linear model is of interest.
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.
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.

group	One of three 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	Restrict samples to have at least a "eff" quantile or number of effective samples.
numberEff	Boolean, whether eff should represent quantile (default/FALSE) or number.
file	Name of file, including location, to save the table.

Value

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

See Also

[fitZig MRcoefs](#)

Examples

```
data(lungData)
k = grep("Extraction.Control", pData(lungData)$SampleType)
lungTrim = lungData[,-k]
k = which(rowSums(MRcounts(lungTrim)>0)<10)
lungTrim = lungTrim[-k,]
cumNorm(lungTrim)
smokingStatus = pData(lungTrim)$SmokingStatus
mod = model.matrix(~smokingStatus)
settings = zigControl(maxit=1, verbose=FALSE)
fit = fitZig(obj = lungTrim, mod=mod, control=settings)
head(MRtable(fit))
```

newMRExperiment

Create a MRExperiment object

Description

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

Usage

```
newMRExperiment(counts, phenoData = NULL, featureData = NULL,
  libSize = NULL, normFactors = NULL)
```

Arguments

counts	A matrix or data frame of count data. The count data is representative of the number of reads annotated for a feature (be it gene, OTU, species, etc). Rows should correspond to features and columns to samples.
phenoData	An AnnotatedDataFrame with pertinent sample information.
featureData	An AnnotatedDataFrame with pertinent feature information.
libSize	libSize, library size, is the total number of reads for a particular sample.
normFactors	normFactors, the normalization factors used in either the model or as scaling factors of sample counts for each particular sample.

Details

See [MRExperiment-class](#) and eSet (from the Biobase package) for the meaning of the various slots.

Value

an object of class MRExperiment

Author(s)

Joseph N Paulson, jpaulson@umiacs.umd.edu

Examples

```
cnts = matrix(abs(rnorm(1000)),nc=10)
obj <- newMRExperiment(cnts)
```

normFactors

Access the normalization factors in a MRExperiment object

Description

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

Usage

```
normFactors(obj)
```

Arguments

obj a MRExperiment object.

Author(s)

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

Examples

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

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

Description

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

Usage

```
plotCorr(obj, n, log = TRUE, norm = 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.
log	Whether or not to log2 transform the counts - if MRExperiment object.
norm	Whether or not to normalize the counts - if MRExperiment object.
fun	Function to calculate pair-wise relationships. Default is pearson correlation
...	Additional plot arguments.

Value

NA

See Also

[cumNormMat](#)

Examples

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

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

Description

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

Usage

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

Arguments

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

Value

NA

See Also

[cumNorm](#)

Examples

```
data(mouseData)
classIndex=list(Western=which(pData(mouseData)$diet=="Western"))
classIndex$BK=which(pData(mouseData)$diet=="BK")
otuIndex = 8770

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

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

Description

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

Usage

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

Arguments

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

Value

NA

See Also[cumNorm](#)

Examples

```

data(mouseData)
classIndex=list(controls=which(pData(mouseData)$diet=="BK"))
classIndex$cases=which(pData(mouseData)$diet=="Western")
otuIndex = grep("Strep", fData(mouseData)$fdata)
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.

Usage

```
plotMRheatmap(obj, n, log = TRUE, norm = TRUE, ...)
```

Arguments

obj	A MRexperiment object with count data.
n	The number of features to plot. This chooses the "n" features with greatest variance.
log	Whether or not to log ₂ transform the counts - if MRexperiment object.
norm	Whether or not to normalize the counts - if MRexperiment object.
...	Additional plot arguments.

Value

NA

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)
plotMRheatmap(obj=mouseData, n=200, cexRow = 0.4, cexCol = 0.4, trace="none",
              col = heatmapCols, ColSideColors = heatmapColColors)

```

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

Description

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

Usage

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

Arguments

obj	A MRExperiment object or count matrix.
tran	Transpose the matrix.
comp	Which components to display
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.
log	Whether or not to log2 the counts - if MRExperiment object.
norm	Whether or not to normalize the counts - if MRExperiment object.
ret	Whether or not to output the coordinates.
n	Number of features to make use of in calculating your distances.
...	Additional plot arguments.

Value

NA

See Also

[cumNormMat](#)

Examples

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

plotOTU

Basic plot function of the raw or normalized data.

Description

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

Usage

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

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.
ret	Boolean to return the observed data that would have been plotted.
...	Additional plot arguments.

Value

NA

See Also

[cumNorm](#)

Examples

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

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

Description

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

Usage

```
plotRare(obj, cl = NULL, ret = FALSE, ...)
```

Arguments

obj	A MRexperiment object with count data or matrix.
cl	Vector of classes for various samples.
ret	True/False, return the number of features and the depth of coverage as a vector.
...	Additional plot arguments.

Value

NA

See Also

[plotOrd](#), [plotMRheatmap](#), [plotCorr](#), [plotOTU](#), [plotGenus](#)

Examples

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

posterior.probs	<i>Access the posterior probabilities that results from analysis</i>
-----------------	--

Description

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

Usage

```
posterior.probs(obj)
```

Arguments

obj a MRexperiment object.

Author(s)

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

Examples

```
# see vignette
```

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

Description

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

Usage

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

Arguments

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

Value

Table of features unique to a group

Examples

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

zigControl	<i>Settings for the fitZig function</i>
------------	---

Description

Settings for the fitZig function

Usage

```
zigControl(tol = 1e-04, maxit = 10, verbose = TRUE)
```

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.

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

- *Topic **package**
 - metagenomeSeq-package, 3
- [,MRexperiment-method (MRexperiment), 30
- aggregateByTaxonomy, 3
- aggTax (aggregateByTaxonomy), 3

- biom2MRexperiment, 4, 24, 31

- calculateEffectiveSamples, 5
- colMeans,MRexperiment-method (MRexperiment), 30
- colSums,MRexperiment-method (MRexperiment), 30
- correctIndices, 6
- correlationTest, 6
- corTest (correlationTest), 6
- cumNorm, 7, 8–10, 13, 14, 16, 18, 19, 30, 37, 38, 41, 44
- cumNormMat, 8, 30, 36, 39, 40
- cumNormStat, 8, 9, 10, 30
- cumNormStatFast, 9, 10

- doCountMStep, 10
- doEStep, 11
- doZeroMStep, 12

- exportMat, 13
- exportMatrix (exportMat), 13
- exportStats, 14
- expSummary, 14
- expSummary,MRexperiment-method (expSummary), 14

- filterData, 15
- fitD0, 16
- fitMeta, 17
- fitPA, 17, 33
- fitZig, 5, 8–13, 16, 18, 18–23, 28, 33, 34, 43, 44

- genusPlot (plotGenus), 38
- getCountDensity, 19
- getEpsilon, 20
- getNegativeLogLikelihoods, 20
- getPi, 21
- getZ, 22

- isItStillActive, 22

- libSize, 23, 30
- libSize,MRexperiment-method (libSize), 23
- load_biom, 5, 24, 31
- load_meta, 5, 24, 24–26, 31
- load_metaQ, 25
- load_phenoData, 5, 24, 25, 26, 31
- lungData, 26

- makeLabels, 27
- metagenomeSeq (metagenomeSeq-package), 3
- metagenomeSeq-package, 3
- metagenomicLoader (load_meta), 24
- mouseData, 27
- MRcoefs, 5, 28, 33, 34
- MRcounts, 29, 30
- MRcounts,MRexperiment-method (MRcounts), 29
- MRexperiment, 30
- MRexperiment-class (MRexperiment), 30
- MRexperiment2biom, 31
- MRfulltable, 5, 32
- MRtable, 28, 33, 33

- newMRexperiment, 5, 24, 30, 31, 34
- normFactors, 30, 35
- normFactors,MRexperiment-method (normFactors), 35

- p.adjust, 28, 32, 33
- phenoData (load_phenoData), 26
- plotCorr, 36, 42

plotFeature, [37](#)
plotGenus, [38](#), [42](#)
plotMRheatmap, [39](#), [42](#)
plotOrd, [40](#), [42](#)
plotOTU, [41](#), [42](#), [44](#)
plotRare, [42](#)
posterior.probs, [30](#), [43](#)
posterior.probs, MRexperiment-method
 (posterior.probs), [43](#)

qiimeLoader (load_metaQ), [25](#)
quantile, [14](#)

rowMeans, MRexperiment-method
 (MRexperiment), [30](#)
rowSums, MRexperiment-method
 (MRexperiment), [30](#)

settings2 (zigControl), [44](#)

uniqueFeatures, [43](#)

zigControl, [19](#), [44](#)