

Package ‘EBSeq’

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Type Package

Title An R package for gene and isoform differential expression analysis of RNA-seq data

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Author Ning Leng, Christina Kendziorski

Maintainer Ning Leng <lengning1@gmail.com>

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Description Differential Expression analysis at both gene and isoform level using RNA-seq data

License Artistic-2.0

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'Likefun.R' 'LogN.R' 'LogNMulti.R' 'LikefunMulti.R' 'EBTest.R'
'GetPatterns.R' 'EBMultiTest.R' 'GetPP.R' 'PostFC.R'
'GetPPMat.R' 'GetMultiPP.R' 'GetMultiFC.R' 'PlotPostVsRawFC.R'
'crit_fun.R' 'DenNHist.R' 'GetNormalizedMat.R' 'PlotPattern.R'
'PolyFitPlot.R' 'QQP.R' 'QuantileNorm.R' 'RankNorm.R'
'GetDEResults.R'

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EBSeq_NingLeng-package

EBSeq: RNA-Seq Differential Expression Analysis on both gene and isoform level

Description

In 'EBSeq_NingLeng-package,' a Negative Binomial-beta model was built to analyze the RNASeq data. We used the empirical bayes method and EM algrithom.

Details

Package:	EBSeq_NingLeng
Type:	Package
Version:	1.0
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License:	What license is it under?
LazyLoad:	yes

Author(s)

Ning Leng, Christina Kendziorski
Maintainer: Ning Leng <nleng@wisc.edu>

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

EBTest, EBMultiTest

Examples

```
data(GeneMat)
GeneMat.small = GeneMat[c(1:10,511:550),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data=GeneMat.small,
Conditions=as.factor(rep(c("C1","C2"), each=5)),
sizeFactors=Sizes, maxround=5)
```

beta.mom

Fit the beta distribution by method of moments

Description

'beta.mom' fits the beta distribution by method of moments.

Usage

```
beta.mom(qs.in)
```

Arguments

qs.in A vector contains the numbers that are assumed to follow a beta distribution.

Value

alpha.hat Returns the estimation of alpha.
beta.hat Returns the estimation of beta.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

DenNHist, DenNHistTable

Examples

```
#tmp = rbeta(5, 5, 100)
#param = beta.mom(tmp)
```

crit_fun

Calculate the soft threshold for a target FDR

Description

'crit_fun' calculates the soft threshold for a target FDR.

Usage

```
crit_fun(PPEE, thre)
```

Arguments

PPEE	The posterior probabilities of being EE.
thre	The target FDR.

Details

Regarding a target FDR α , both hard threshold and soft threshold could be used. If the hard threshold is preferred, user could simply take the transcripts with PP(DE) greater than $(1-\alpha)$. Using the hard threshold, any DE transcript in the list is with $FDR \leq \alpha$.

If the soft threshold is preferred, user could take the transcripts with PP(DE) greater than $\text{crit_fun}(\text{PPEE}, \alpha)$. Using the soft threshold, the list of DE transcripts is with average FDR α .

Value

The adjusted FDR threshold of target FDR.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```

data(GeneMat)
GeneMat.small = GeneMat[c(1:10, 500:600),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1", "C2"), each=5)),
sizeFactors = Sizes, maxround = 5)
PP = GetPPMat(EBOut)
DEfound = rownames(PP)[which(PP[, "PPDE"] >= 0.95)]
str(DEfound)

SoftThre = crit_fun(PP[, "PPEE"], 0.05)
DEfound_soft = rownames(PP)[which(PP[, "PPDE"] >= SoftThre)]

```

DenNHist

Density plot to compare the empirical q 's and the simulated q 's from the fitted beta distribution.

Description

'DenNHist' gives the density plot that compares the empirical q 's and the simulated q 's from the fitted beta distribution.

Usage

```
DenNHist(EBOut, GeneLevel = F)
```

Arguments

EBOut The output of EBTest or EBMultiTest.
GeneLevel Indicate whether the results are from data at gene level.

Value

For data with $n1$ conditions and $n2$ uncertainty groups, $n1*n2$ plots will be generated. Each plot represents a subset of the data. The empirical estimation of q 's will be represented as blue histograms and the density of the fitted beta distribution will be represented as the green line.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

beta.mom, QQP, EBTest, EBMultiTest

Examples

```

data(GeneMat)
GeneMat.small = GeneMat[c(500:1000),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1", "C2"), each=5)),
sizeFactors = Sizes, maxround = 5)
par(mfrow = c(2,2))
DenNHist(EBOut)

```

EBMultiTest

Using EM algorithm to calculate the posterior probabilities of interested patterns in a multiple condition study

Description

'EBMultiTest' is built based on the assumption of NB-Beta Empirical Bayes model. It utilizes the EM algorithm to give the posterior probability of the interested patterns.

Usage

```

EBMultiTest(Data, NgVector = NULL, Conditions, AllParti = NULL,
sizeFactors, maxround, Pool = F, NumBin = 1000,
ApproxVal=10^-10, PoolLower=.25, PoolUpper = .75, Print=T,Qtrm=1,QtrmCut=0)

```

Arguments

Data	A data matrix contains expression values for each transcript (gene or isoform level). In which rows should be transcripts and columns should be samples.
NgVector	A vector indicates the uncertainty group assignment of each isoform. e.g. if we use number of isoforms in the host gene to define the uncertainty groups, suppose the isoform is in a gene with 2 isoforms, Ng of this isoform should be 2. The length of this vector should be the same as the number of rows in Data. If it's gene level data, Ngvector could be left as NULL.
Conditions	A vector indicates the condition in which each sample belongs to.
AllParti	A matrix indicates the interested patterns. Columns should be conditions and rows should be patterns. The matrix could be obtained by the GetPatterns function. If AllParti=NULL, all possible patterns will be used.
sizeFactors	The normalization factors. It should be a vector with lane specific numbers (the length of the vector should be the same as the number of samples, with the same order as the columns of Data).
maxround	Number of iterations. The default value is 5. Users should always check the convergency by looking at the Alpha and Beta in output. If the hyper-parameter estimations are not converged in 5 iterations, larger number is suggested.
Pool	While working without replicates, user could define the Pool = TRUE in the EBTest function to enable pooling.
NumBin	By defining NumBin = 1000, EBSeq will group the genes with similar means together into 1,000 bins.

PoolLower, PoolUpper	<p>With the assumption that only subset of the genes are DE in the data set, we take genes whose FC are in the PoolLower - PoolUpper quantile of the FC's as the candidate genes (default is 25%-75%).</p> <p>For each bin, the bin-wise variance estimation is defined as the median of the cross condition variance estimations of the candidate genes within that bin.</p> <p>We use the cross condition variance estimations for the candidate genes and the bin-wise variance estimations of the host bin for the non-candidate genes.</p>
ApproxVal	The variances of the transcripts with mean < var will be approximated as mean/(1-ApproxVal).
Print	Whether print the elapsed-time while running the test.
Qtrm, QtrmCut	Transcripts with Qtrm th quantile \leq QtrmCut will be removed before testing. The default value is Qtrm = 1 and QtrmCut=0. By default setting, transcripts with all 0's won't be tested.

Value

Alpha	Fitted parameter alpha of the prior beta distribution. Rows are the values for each iteration.
Beta	Fitted parameter beta of the prior beta distribution. Rows are the values for each iteration.
P, PFromZ	The bayes estimator of following each pattern of interest. Rows are the values for each iteration.
Z, PoissonZ	The Posterior Probability of following each pattern of interest for each transcript. (Maybe not in the same order of input).
RList	The fitted values of r for each transcript.
MeanList	The mean of each transcript. (across conditions).
VarList	The variance of each transcript. (across conditions).
QList	The fitted q values of each transcript within each condition.
SPMean	The mean of each transcript within each condition (adjusted by the normalization factors).
SPEstVar	The estimated variance of each transcript within each condition (adjusted by the normalization factors).
PoolVar	The variance of each transcript (The pooled value of within condition EstVar).
DataList	A List of data that grouped with Ng and bias.
PPpattern	The Posterior Probability of following each pattern (columns) for each transcript (rows). Transcripts with expression 0 for all samples are not shown in this matrix.
f	The likelihood of likelihood of prior predictive distribution of being each pattern for each transcript.
AllParti	The matrix describe the patterns.
PPpatternWith0	The Posterior Probability of following each pattern (columns) for each transcript (rows). Transcripts with expression 0 for all samples are shown in this matrix with PP(any_patrn)=NA.
ConditionOrder	The condition assignment for C1Mean, C2Mean, etc.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

EBTest, GetMultiPP, GetMultiFC

Examples

```
data(MultiGeneMat)
MultiGeneMat.small = MultiGeneMat[201:210,]
Conditions = c("C1", "C1", "C2", "C2", "C3", "C3")
PosParti = GetPatterns(Conditions)
Parti = PosParti[-3,]
MultiSize = MedianNorm(MultiGeneMat.small)
MultiOut = EBMultiTest(MultiGeneMat.small, NgVector = NULL,
Conditions = Conditions, AllParti = Parti,
sizeFactors = MultiSize, maxround = 5)
MultiPP = GetMultiPP(MultiOut)
```

EBTest

Using EM algorithm to calculate the posterior probabilities of being DE

Description

Base on the assumption of NB-Beta Empirical Bayes model, the EM algorithm is used to get the posterior probability of being DE.

Usage

```
EBTest(Data, NgVector = NULL, Conditions, sizeFactors, maxround,
Pool = F, NumBin = 1000, ApproxVal = 10^-10, Alpha = NULL,
Beta = NULL, PInput = NULL, RInput = NULL,
PoolLower = .25, PoolUpper = .75, Print = T, Qtrm = 1, QtrmCut=0)
```

Arguments

Data	A data matrix contains expression values for each transcript (gene or isoform level). In which rows should be transcripts and columns should be samples.
NgVector	A vector indicates the uncertainty group assignment of each isoform. e.g. if we use number of isoforms in the host gene to define the uncertainty groups, suppose the isoform is in a gene with 2 isoforms, Ng of this isoform should be 2. The length of this vector should be the same as the number of rows in Data. If it's gene level data, Ngvector could be left as NULL.
Conditions	A factor indicates the condition which each sample belongs to.

sizeFactors	The normalization factors. It should be a vector with lane specific numbers (the length of the vector should be the same as the number of samples, with the same order as the columns of Data).
maxround	Number of iterations. The default value is 5. Users should always check the convergency by looking at the Alpha and Beta in output. If the hyper-parameter estimations are not converged in 5 iterations, larger number is suggested.
Pool	While working without replicates, user could define the Pool = TRUE in the EBTest function to enable pooling.
NumBin	By defining NumBin = 1000, EBSeq will group the genes with similar means together into 1,000 bins.
PoolLower, PoolUpper	With the assumption that only subset of the genes are DE in the data set, we take genes whose FC are in the PoolLower - PoolUpper quantile of the FC's as the candidate genes (default is 25%-75%). For each bin, the bin-wise variance estimation is defined as the median of the cross condition variance estimations of the candidate genes within that bin. We use the cross condition variance estimations for the candidate genes and the bin-wise variance estimations of the host bin for the non-candidate genes.
ApproxVal	The variances of the transcripts with mean < var will be approximated as mean/(1-ApproxVal).
Alpha, Beta, PInput, RInput	If the parameters are known and the user doesn't want to estimate them from the data, user could specify them here.
Print	Whether print the elapsed-time while running the test.
Qtrm, QtrmCut	Transcripts with Qtrm th quantile <= QtrmCut will be removed before testing. The default value is Qtrm = 1 and QtrmCut=0. By default setting, transcripts with all 0's won't be tested.

Details

For each transcript g_i within condition, the model assumes: $X_{g_i} \sim \text{NB}(r_{g_i} * l_s, q_{g_i})$
 $q_{g_i} \sim \text{Beta}(\alpha, \beta^{N_g})$ In which the l_s is the sizeFactors of samples.

The function will test "H0: $q_{g_i}^{C1} = q_{g_i}^{C2}$ " and "H1: $q_{g_i}^{C1} \neq q_{g_i}^{C2}$."

Value

Alpha	Fitted parameter alpha of the prior beta distribution. Rows are the values for each iteration.
Beta	Fitted parameter beta of the prior beta distribution. Rows are the values for each iteration.
P, PFromZ	The bayes estimator of being DE. Rows are the values for each iteration.
Z, PoissonZ	The Posterior Probability of being DE for each transcript(Maybe not in the same order of input).
RList	The fitted values of r for each transcript.
MeanList	The mean of each transcript (across conditions).
VarList	The variance of each transcript (across conditions).
QListi1	The fitted q values of each transcript within condition 1.
QListi2	The fitted q values of each transcript within condition 2.

C1Mean	The mean of each transcript within Condition 1 (adjusted by normalization factors).
C2Mean	The mean of each transcript within Condition 2 (adjusted by normalization factors).
C1EstVar	The estimated variance of each transcript within Condition 1 (adjusted by normalization factors).
C2EstVar	The estimated variance of each transcript within Condition 2 (adjusted by normalization factors).
PoolVar	The variance of each transcript (The pooled value of within condition EstVar).
DataList	A List of data that grouped with Ng.
PPDE	The Posterior Probability of being DE for each transcript (The same order of input).
f0, f1	The likelihood of the prior predictive distribution of being EE or DE (in log scale).
AllZeroIndex	The transcript with expression 0 for all samples (which are not tested).
PPMat	A matrix contains posterior probabilities of being EE (the first column) or DE (the second column). Rows are transcripts. Transcripts with expression 0 for all samples are not shown in this matrix.
PPMatWith0	A matrix contains posterior probabilities of being EE (the first column) or DE (the second column). Rows are transcripts. Transcripts with expression 0 for all samples are shown as PP(EE) = PP(DE) = NA in this matrix. The transcript order is exactly the same as the order of the input data.
ConditionOrder	The condition assignment for C1Mean, C2Mean, etc.
Conditions	The input conditions.
DataNorm	Normalized expression matrix.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

EBMultiTest, PostFC, GetPPMat

Examples

```
data(GeneMat)
str(GeneMat)
GeneMat.small = GeneMat[c(1:10, 511:550), ]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1", "C2"), each = 5)),
sizeFactors = Sizes, maxround = 5)
PP = GetPPMat(EBOut)
```

f0

The Prior Predictive Distribution of being EE

Description

'f0' gives the Prior Predictive Distribution of being EE.

Usage

```
f0(Input, AlphaIn, BetaIn, EmpiricalR, NumOfGroups, log)
```

Arguments

Input	Expression Values.
AlphaIn, BetaIn, EmpiricalR	The parameters estimated from last iteration of EM.
NumOfGroups	How many transcripts within each Ng group.
log	If true, will give the log of the output.

Value

The function will return the prior predictive distribution values of being EE.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

f1

Examples

```
#  
#f0(matrix(rnorm(100,100,1),ncol=10), .5, .6,  
# matrix(rnorm(100,200,1),ncol=10), 100, TRUE)
```

f1

The Prior Predictive Distribution of being DE

Description

'f1' gives the Prior Predictive Distribution of DE.

Usage

```
f1(Input1, Input2, AlphaIn, BetaIn, EmpiricalRSP1,  
EmpiricalRSP2, NumOfGroup, log)
```

Arguments

Input1	Expressions from Condition1.
Input2	Expressions from Condition2.
AlphaIn, BetaIn, EmpiricalRSP1, EmpiricalRSP2	The parameters estimated from last iteration of EM.
NumOfGroup	How many transcripts within each Ng group.
log	If true, will give the log of the output.

Value

The function will return the prior predictive distribution values of being DE.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

f0

Examples

```
#f1(matrix(rnorm(100,100,1),ncol=10),  
# matrix(rnorm(100,100,1),ncol=10), .5, .6,  
# matrix(rnorm(100,200,1),ncol=10),  
# matrix(rnorm(100,200,1),ncol=10), 100, TRUE)
```

GeneMat

*The simulated data for two condition gene DE analysis***Description**

'GeneMat' gives the simulated data for two condition gene DE analysis.

Usage

```
data(GeneMat)
```

Source

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

IsoList

Examples

```
data(GeneMat)
```

GetDEResults

*Obtain Differential Expression Analysis Results in a Two-condition Test***Description**

Obtain DE analysis results in a two-condition test using the output of EBTest()

Usage

```
GetDEResults(EBPrelim, FDR=0.05, Method="robust",
             FDRMethod="hard", Threshold_FC=0.7,
             Threshold_FCRatio=0.3, SmallNum=0.01)
```

Arguments

EBPrelim	Output from the function EBTest().
FDR	Target FDR, default is 0.05.
FDRMethod	"hard" or "soft". Giving a target FDR alpha, either hard threshold and soft threshold may be used. If the hard threshold is preferred, DE transcripts are defined as the the transcripts with PP(DE) greater than (1-alpha). Using the hard threshold, any DE transcript in the list has FDR <= alpha. If the soft threshold is preferred, the DE transcripts are defined as the transcripts with PP(DE) greater than crit_fun(PPEE, alpha). Using the soft threshold, the list of DE transcripts has average FDR alpha.

Based on results from our simulation studies, hard thresholds provide a better-controlled empirical FDR when sample size is relatively small (Less than 10 samples in each condition). User may consider the soft threshold when sample size is large to improve power.

Method	"robust" or "classic". Using the "robust" option, EBSeq is more robust to genes with outliers and genes with extremely small variances. Using the "classic" option, the results will be more comparable to those obtained by using the GetPPMat() function from earlier version ($\leq 1.7.0$) of EBSeq. Default is "robust".
Threshold_FC	Threshold for the fold change (FC) statistics. The default is 0.7. The FC statistics are calculated as follows. First the posterior FC estimates are calculated using PostFC() function. The FC statistics is defined as $\exp(-\log \text{posterior FC})$ and therefore is always less than or equal to 1. The default threshold was selected as the optimal threshold learned from our simulation studies. By setting the threshold as 0.7, the expected FC for a DE transcript is less than 0.7 (or greater than $1/0.7=1.4$). User may specify their own threshold here. A higher (less conservative) threshold may be used here when sample size is large. Our simulation results indicated that when there are more than or equal to 5 samples in each condition, a less conservative threshold will improve the power when the FDR is still well-controlled. The parameter will be ignored if Method is set as "classic".
Threshold_FCRatio	Threshold for the fold change ratio (FCRatio) statistics. The default is 0.3. The FCRatio statistics are calculated as follows. First we get another revised fold change statistic called Median-FC statistic for each transcript. For each transcript, we calculate the median of normalized expression values within each condition. The MedianFC is defined as $\exp(-\log((C1\text{Median}+SmallNum)/(C2\text{Median}+SmallNum)))$. Note a small number is added to avoid Inf and NA. See SmallNum for more details. The FCRatio is calculated as $\exp(-\log(FC\text{statistics}/MedianFC))$. Therefore it is always less than or equal to 1. The default threshold was selected as the optimal threshold learned from our simulation studies. By setting the threshold as 0.3, the FCRatio for a DE transcript is expected to be larger than 0.3.
SmallNum	When calculating the FCRatio (or Median-FC), a small number is added for each transcript in each condition to avoid Inf and NA. Default is 0.01.

Details

GetDEResults() function takes output from EBTest() function and output a list of DE transcripts under a target FDR. It also provides posterior probability estimates for each transcript.

Value

DEfound	A list of DE transcripts.
PPMat	Posterior probability matrix. Transcripts are following the same order as in the input matrix. Transcripts that were filtered by magnitude (in EBTest function), FC, or FCR are assigned with NA for both PPDE and PPEE.
Status	Each transcript will be assigned with one of the following values: "DE", "EE", "Filtered: Low Expression", "Filtered: Fold Change" and "Filtered: Fold Change Ratio". Transcripts are following the same order as in the input matrix.

Author(s)

Ning Leng, Yuan Li

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

EBTest

Examples

```
data(GeneMat)
str(GeneMat)
GeneMat.small = GeneMat[c(1:10,511:550),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1","C2"), each = 5)),
sizeFactors = Sizes, maxround = 5)
Out = GetDEResults(EBOut)
```

GetMultiFC

Calculate the Fold Changes for Multiple Conditions

Description

'GetMultiFC' calculates the Fold Changes for each pair of conditions in a multiple condition study.

Usage

```
GetMultiFC(EBMultiOut, SmallNum = 0.01)
```

Arguments

EBMultiOut	The output of EBMultiTest function.
SmallNum	A small number will be added for each transcript in each condition to avoid Inf and NA. Default is 0.01.

Details

Provide the FC (adjusted by the normalization factors) for each pair of comparisons. A small number will be added for each transcript in each condition to avoid Inf and NA. Default is set to be 0.01.

Value

FCMat	The FC of each pair of comparison (adjusted by the normalization factors).
Log2FCMat	The log ₂ FC of each pair of comparison (adjusted by the normalization factors).
PostFCMat	The posterior FC of each pair of comparison.
Log2PostFCMat	The log ₂ posterior FC of each pair of comparison.
CondMean	The mean of each transcript within each condition (adjusted by the normalization factors).
ConditionOrder	The condition assignment for C1Mean, C2Mean, etc.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

EBMultiTest, PostFC

Examples

```
data(MultiGeneMat)
MultiGeneMat.small = MultiGeneMat[201:210,]

Conditions = c("C1", "C1", "C2", "C2", "C3", "C3")

PosParti = GetPatterns(Conditions)
Parti = PosParti[-3,]

MultiSize = MedianNorm(MultiGeneMat.small)

MultiOut = EBMultiTest(MultiGeneMat.small,
  NgVector=NULL, Conditions=Conditions,
  AllParti=Parti, sizeFactors=MultiSize,
  maxround=5)

MultiFC = GetMultiFC(MultiOut)
```

GetMultiPP

Posterior Probability of Each Transcript

Description

'GetMultiPP' generates the Posterior Probability of being each pattern of each transcript based on the EBMultiTest output.

Usage

```
GetMultiPP(EBout)
```

Arguments

EBout The output of EBMultiTest function.

Value

PP The poster probabilities of being each pattern.
 MAP Gives the most likely pattern.
 Patterns The Patterns.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

GetPPMat

Examples

```
data(MultiGeneMat)
MultiGeneMat.small = MultiGeneMat[201:210,]

Conditions = c("C1", "C1", "C2", "C2", "C3", "C3")
PosParti = GetPatterns(Conditions)
Parti = PosParti[-3,]
MultiSize = MedianNorm(MultiGeneMat.small)

MultiOut = EBMultiTest(MultiGeneMat.small,
  NgVector=NULL, Conditions=Conditions,
  AllParti=Parti, sizeFactors=MultiSize,
  maxround=5)
MultiPP = GetMultiPP(MultiOut)
```

 GetNg

Ng Vector

Description

'GetNg' generates the Ng vector for the isoform level data. (While using the number of isoform in the host gene to define the uncertainty groups.)

Usage

```
GetNg(IsoformName, GeneName, TrunThre = 3)
```

Arguments

IsoformName	A vector contains the isoform names.
GeneName	The gene names of the isoforms in IsoformNames (Should be in the same order).
TrunThre	The number of uncertainty groups the user wish to define. The default is 3.

Value

GeneNg	The number of isoforms that are contained in each gene.
GeneNgTrun	The truncated Ng of each gene. (The genes contain more than 3 isoforms are with Ng 3.)
IsoformNg	The Ng of each isoform.
IsoformNgTrun	The truncated Ng of each isoform (could be used to define the uncertainty group assignment).

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
data(IsoList)

IsoMat = IsoList$IsoMat
IsoNames = IsoList$IsoNames
IsosGeneNames = IsoList$IsosGeneNames
IsoSizes = MedianNorm(IsoMat)
NgList = GetNg(IsoNames, IsosGeneNames)

#IsoNgTrun = NgList$IsoformNgTrun
#IsoEBOut = EBTest(Data = IsoMat, NgVector = IsoNgTrun,
# Conditions = as.factor(rep(c("C1", "C2"), each=5)),
# sizeFactors = IsoSizes, maxround = 5)
```

GetNormalizedMat *Calculate normalized expression matrix*

Description

'GetNormalizedMat' calculates the normalized expression matrix. (Note: this matrix is only used for visualization etc. EBTest and EBMultiTest request **un-adjusted** expressions and normalization factors.)

Usage

```
GetNormalizedMat(Data, Sizes)
```

Arguments

Data	The data matrix with transcripts in rows and lanes in columns.
Sizes	A vector contains the normalization factor for each lane.

Value

The function will return a normalized matrix.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
data(GeneMat)
str(GeneMat)
Sizes = MedianNorm(GeneMat)
NormData = GetNormalizedMat(GeneMat, Sizes)
```

GetPatterns

Generate all possible patterns in a multiple condition study

Description

'GetPatterns' generates all possible patterns in a multiple condition study.

Usage

```
GetPatterns(Conditions)
```

Arguments

Conditions The names of the Conditions in the study.

Value

A matrix describe all possible patterns.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
Conditions = c("C1", "C1", "C2", "C2", "C3", "C3")
PosParti = GetPatterns(Conditions)
```

GetPP

Generate the Posterior Probability of each transcript.

Description

'GetPP' generates the Posterior Probability of being DE of each transcript based on the EBTest output.

Usage

```
GetPP(EBout)
```

Arguments

EBout The output of EBTest function.

Value

The poster probabilities of being DE.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

GetPPMat

Examples

```
data(GeneMat)
GeneMat.small = GeneMat[c(1:10,500:550),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1","C2"), each=5)),
sizeFactors = Sizes, maxround = 5)
PPDE = GetPP(EBOut)
str(PPDE)
head(PPDE)
```

GetPPMat	<i>Posterior Probability of Transcripts</i>
----------	---

Description

'GetPPMat' generates the Posterior Probability of being each pattern of each transcript based on the EBTest output.

Usage

```
GetPPMat(EBout)
```

Arguments

EBout The output of EBTest function.

Value

The poster probabilities of being EE (first column) and DE (second column).

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeg: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
data(GeneMat)
GeneMat.small = GeneMat[c(500:550),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1", "C2"), each=5)),
sizeFactors = Sizes, maxround = 5)
PP = GetPPMat(EBOut)
str(PP)
head(PP)
```

`IsoList`*The simulated data for two condition isoform DE analysis*

Description

'IsoList' gives the simulated data for two condition isoform DE analysis.

Usage

```
data(IsoList)
```

Source

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

GeteMat

Examples

```
data(IsoList)
```

`IsoMultiList`*The simulated data for multiple condition isoform DE analysis*

Description

'IsoMultiList' gives a set of simulated data for multiple condition isoform DE analysis.

Usage

```
data(IsoMultiList)
```

Source

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

IsoList

Examples

```
data(IsoMultiList)
```

Likefun

Likelihood Function of the NB-Beta Model

Description

'Likefun' specifies the Likelihood Function of the NB-Beta Model.

Usage

```
Likefun(ParamPool, InputPool)
```

Arguments

ParamPool	The parameters that will be estimated in EM.
InputPool	The control parameters that will not be estimated in EM.

Value

The function will return the log-likelihood.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeg: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
#x1 = c(.6, .7, .3)
#Input = matrix(rnorm(100,100,1), ncol=10)
#RIn = matrix(rnorm(100,200,1), ncol=10)
#InputPool = list(Input[,1:5], Input[,6:10], Input,
# rep(.1,100), 1, RIn, RIn[,1:5], RIn[,6:10], 100)
#Likefun(x1, InputPool)
```

LikefunMulti

Likelihood Function of the NB-Beta Model In Multiple Condition Test

Description

'LikefunMulti' specifies the Likelihood Function of the NB-Beta Model In Multiple Condition Test.

Usage

```
LikefunMulti(ParamPool, InputPool)
```

Arguments

ParamPool The parameters that will be estimated in EM.
 InputPool The control parameters that will not be estimated in EM.

Value

The function will return the log-likelihood.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
#x1 = c(.6, .7, .3)
#Input = matrix(rnorm(100,100,1),ncol=10)
#RIn = matrix(rnorm(100,200,1),ncol=10)
#InputPool = list(list(Input[,1:5],Input[,6:10]),
# Input, cbind(rep(.1, 10), rep(.9,10)), 1,
# RIn, list(RIn[,1:5],RIn[,6:10]),
# 10, rbind(c(1,1),c(1,2)))
#LikefunMulti(x1, InputPool)
```

LogN

The function to run EM (one round) algorithm for the NB-beta model.

Description

'LogN' specifies the function to run (one round of) the EM algorithm for the NB-beta model.

Usage

```
LogN(Input, InputSP, EmpiricalR, EmpiricalRSP, NumOfEachGroup,
AlphaIn, BetaIn, PIn, NoneZeroLength)
```

Arguments

Input, InputSP The expressions among all the samples.
 NumOfEachGroup Number of genes in each Ng group.
 AlphaIn, PIn, BetaIn, EmpiricalR, EmpiricalRSP
 The parameters from the last EM step.
 NoneZeroLength Number of Ng groups.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
#Input = matrix(rnorm(100,100,1), ncol=10)
#rownames(Input) = paste("g",1:10)
#RIn = matrix(rnorm(100,200,1), ncol=10)
#res = LogN(Input, list(Input[,1:5], Input[,6:10]),
# RIn, list(RIn[,1:5], RIn[,6:10]),
# 10, .6, .7, .3, 1)
```

LogNMulti

EM algorithm for the NB-beta model in the multiple condition test

Description

'LogNMulti' specifies the function to run (one round of) the EM algorithm for the NB-beta model in the multiple condition test.

Usage

```
LogNMulti(Input, InputSP, EmpiricalR, EmpiricalRSP,
NumOfEachGroup, AlphaIn, BetaIn, PIn,
NoneZeroLength, AllParti, Conditions)
```

Arguments

Input, InputSP The expressions among all the samples.
 NumOfEachGroup Number of genes in each Ng group.
 AlphaIn, PIn, BetaIn, EmpiricalR, EmpiricalRSP
 The parameters from the last EM step.
 NoneZeroLength Number of Ng groups.
 AllParti The patterns of interests.
 Conditions The condition assignment for each sample.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
#
#Input = matrix(rnorm(100,100,1),ncol=10)
#rownames(Input) = paste("g",1:10)
#RIn = matrix(rnorm(100,200,1), ncol=10)
#res = LogNMulti(Input, list(Input[,1:5], Input[,6:10]),
# RIn, list(RIn[,1:5], RIn[,6:10]), 10, .6, .7,
# c(.3,.7), 1, rbind(c(1,1), c(1,2)),
# as.factor(rep(c("C1","C2"), each=5)))
```

MedianNorm

Median Normalization

Description

'MedianNorm' specifies the median-by-ratio normalization function from Anders et. al., 2010.

Usage

```
MedianNorm(Data, alternative = FALSE)
```

Arguments

Data	The data matrix with transcripts in rows and lanes in columns.
alternative	if alternative = TRUE, the alternative version of median normalization will be applied. The alternative method is similar to median-by-ratio normalization, but can deal with the cases when all of the genes/isoforms have at least one zero counts (in which case the median-by-ratio normalization will fail). In more details, in median-by-ratio normalization (denote l_1 as libsize for sample 1 as an example, assume total S samples): $\text{hat}l_1 = \text{median}_g [X_{g1} / (X_{g1} * X_{g2} * \dots * X_{gS})^{1/S}] \quad (1)$ which estimates $l_1 / (l_1 * l_2 * \dots * l_S)^{1/S}$. Since we have the constrain that $(l_1 * l_2 * \dots * l_S) = 1$, equation (1) estimates l_1 . Note (1) could also be written as: $\text{hat}l_1 = \text{median}_g [(X_{g1}/X_{g1} * X_{g1}/X_{g2} * \dots * X_{g1}/X_{gS})^{1/S}]$ In the alternative method, we estimate $l_1/l_1, l_1/l_2, \dots, l_1/l_S$ individually by taking $\text{median}_g(X_{g1}/X_{g1}), \text{median}_g(X_{g1}/X_{g2}) \dots$. Then estimate $l_1 = l_1 / (l_1 * l_2 * \dots * l_S)^{1/S}$ by taking the geomean of these estimates: $\text{hat}l_1 = [\text{median}_g(X_{g1}/X_{g1}) * \text{median}_g(X_{g1}/X_{g2}) * \text{median}_g(X_{g1}/X_{g3}) * \dots * \text{median}_g(X_{g1}/X_{gS})]^{1/S}$

Value

The function will return a vector contains the normalization factor for each lane.

Author(s)

Ning Leng

References

Simon Anders and Wolfgang Huber. Differential expression analysis for sequence count data. *Genome Biology* (2010) 11:R106 (open access)

See Also

QuantileNorm

Examples

```
data(GeneMat)
Sizes = MedianNorm(GeneMat)
#EBOut = EBTest(Data = GeneMat,
# Conditions = as.factor(rep(c("C1", "C2"), each=5)),
# sizeFactors = Sizes, maxround = 5)
```

MultiGeneMat

The simulated data for multiple condition gene DE analysis

Description

'MultiGeneMat' generates a set of the simulated data for multiple condition gene DE analysis.

Usage

```
data(MultiGeneMat)
```

Source

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

GeneMat

Examples

```
data(MultiGeneMat)
```

PlotPattern	<i>Visualize the patterns</i>
-------------	-------------------------------

Description

'PlotPattern' generates the visualized patterns before the multiple condition test.

Usage

```
PlotPattern(Patterns)
```

Arguments

Patterns The output of GetPatterns function.

Value

A heatmap to visualize the patterns of interest.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
Conditions = c("C1", "C1", "C2", "C2", "C3", "C3")
Patterns = GetPatterns(Conditions)
PlotPattern(Patterns)
```

PlotPostVsRawFC	<i>Plot Posterior FC vs FC</i>
-----------------	--------------------------------

Description

'PlotPostVsRawFC' helps the users visualize the posterior FC vs FC in a two condition study.

Usage

```
PlotPostVsRawFC(EBOut, FCOut)
```

Arguments

EBOut The output of EBMultiTest function.

FCOut The output of PostFC function.

Value

A figure shows fold change vs posterior fold change.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

PostFC

Examples

```
data(GeneMat)
GeneMat.small = GeneMat[c(500:600),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1", "C2"), each=5)),
sizeFactors = Sizes, maxround = 5)
FC = PostFC(EBOut)
PlotPostVsRawFC(EBOut, FC)
```

PolyFitPlot

Fit the mean-var relationship using polynomial regression

Description

'PolyFitPlot' fits the mean-var relationship using polynomial regression.

Usage

```
PolyFitPlot(X, Y, nterms, xname = "Estimated Mean",
yname = "Estimated Var", pdfname = "",
xlim = c(-1,5), ylim = c(-1,7), ChangeXY = F,
col = "red")
```

Arguments

X	The first group of values want to be fitted by the polynomial regression (e.g Mean of the data).
Y	The second group of values want to be fitted by the polynomial regression (e.g. variance of the data). The length of Y should be the same as the length of X.
nterms	How many polynomial terms want to be used.
xname	Name of the x axis.

yname	Name of the y axis.
pdfname	Name of the plot.
xlim	The x limits of the plot.
ylim	The y limits of the plot.
ChangeXY	If ChangeXY is setted to be TRUE, X will be treated as the dependent variable and Y will be treated as the independent one. Default is FALSE.
col	Color of the fitted line.

Value

The PolyFitPlot function provides a smooth scatter plot of two variables and their best fitting line of polynomial regression.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

Examples

```
data(IsoList)
str(IsoList)
IsoMat = IsoList$IsoMat
IsoNames = IsoList$IsoNames
IsosGeneNames = IsoList$IsosGeneNames
IsoSizes = MedianNorm(IsoMat)
NgList = GetNg(IsoNames, IsosGeneNames)

IsoNgTrun = NgList$IsoformNgTrun
#IsoEBOut = EBTest(Data = IsoMat.small,
# NgVector = IsoNgTrun,
# Conditions = as.factor(rep(c("C1", "C2"), each=5)),
# sizeFactors = IsoSizes, maxround = 5)

#par(mfrow=c(2,2))
#PolyFitValue = vector("list",3)

#for(i in 1:3)
# PolyFitValue[[i]] = PolyFitPlot(IsoEBOut$C1Mean[[i]],
# IsoEBOut$C1EstVar[[i]], 5)

#PolyAll = PolyFitPlot(unlist(IsoEBOut$C1Mean),
# unlist(IsoEBOut$C1EstVar), 5)

#lines(log10(IsoEBOut$C1Mean[[1]][PolyFitValue[[1]]$sort]),
# PolyFitValue[[1]]$fit[PolyFitValue[[1]]$sort],
# col="yellow", lwd=2)
#lines(log10(IsoEBOut$C1Mean[[2]][PolyFitValue[[2]]$sort]),
# PolyFitValue[[2]]$fit[PolyFitValue[[2]]$sort],
```

```
# col="pink", lwd=2)
#lines(log10(IsoEBOut$C1Mean[[3]][PolyFitValue[[3]]$sort]),
# PolyFitValue[[3]]$fit[PolyFitValue[[3]]$sort],
# col="green", lwd=2)

#legend("topleft",c("All Isoforms","Ng = 1","Ng = 2","Ng = 3"),
# col = c("red","yellow","pink","green"),
# lty=1, lwd=3, box.lwd=2)
```

PostFC	<i>Calculate the posterior fold change for each transcript across conditions</i>
--------	--

Description

'PostFC' calculates the posterior fold change for each transcript across conditions.

Usage

```
PostFC(EBoutput, SmallNum = 0.01)
```

Arguments

EBoutput	The ourput from function EBTest.
SmallNum	A small number will be added for each transcript in each condition to avoid Inf and NA. Default is 0.01.

Value

Provide both FC and posterior FC across two conditions. FC is calculated as $(\text{MeanC1} + \text{SmallNum}) / (\text{MeanC2} + \text{SmallNum})$. And Posterior FC is calculated as:

```
# Post alpha P_a_C1 = alpha + r_C1 * n_C1
# Post beta P_b_C1 = beta + Mean_C1 * n_C1
# P_q_C1 = P_a_C1 / (P_a_C1 + P_b_C1)
# Post FC = ((1-P_q_C1)/P_q_c1) / ((1-P_q_c2)/P_q_c2)
```

PostFC	The posterior FC across two conditions.
RealFC	The FC across two conditions (adjusted by the normalization factors).
Direction	The diretion of FC calculation.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

EBTest, GetMultiFC

Examples

```
data(GeneMat)
GeneMat.small = GeneMat[c(500:550),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1", "C2"), each=5)),
sizeFactors = Sizes, maxround = 5)
FC=PostFC(EBOut)
```

QQP

The Quantile-Quantile Plot to compare the empirical q's and simulated q's from fitted beta distribution

Description

'QQP' gives the Quantile-Quantile Plot to compare the empirical q's and simulated q's from fitted beta distribution.

Usage

```
QQP(EBOut, GeneLevel = F)
```

Arguments

EBOut The output of EBTest or EBMultiTest.
GeneLevel Indicate whether the results are from data at gene level.

Value

For data with n1 conditions and n2 uncertainty groups, n1*n2 plots will be generated. Each plot represents a subset of the data.

Author(s)

Ning Leng

References

Ning Leng, John A. Dawson, James A. Thomson, Victor Ruotti, Anna I. Rissman, Bart M.G. Smits, Jill D. Haag, Michael N. Gould, Ron M. Stewart, and Christina Kendziorski. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* (2013)

See Also

EBTest, EBMultiTest, DenNHist

Examples

```
data(GeneMat)
GeneMat.small = GeneMat[c(500:1000),]
Sizes = MedianNorm(GeneMat.small)
EBOut = EBTest(Data = GeneMat.small,
Conditions = as.factor(rep(c("C1", "C2"), each=5)),
sizeFactors = Sizes, maxround = 5)
par(mfrow=c(2,2))
QQP(EBOut)
```

QuantileNorm

Quantile Normalization

Description

'QuantileNorm' gives the quantile normalization.

Usage

```
QuantileNorm(Data, Quantile)
```

Arguments

Data	The data matrix with transcripts in rows and lanes in columns.
Quantile	The quantile the user wishes to use. Should be a number between 0 and 1.

Details

Use a quantile point to normalize the data.

Value

The function will return a vector contains the normalization factor for each lane.

Author(s)

Ning Leng

References

Bullard, James H., et al. Evaluation of statistical methods for normalization and differential expression in mRNA-Seq experiments. *BMC bioinformatics* 11.1 (2010): 94.

See Also

MedianNorm

Examples

```
data(GeneMat)
Sizes = QuantileNorm(GeneMat, .75)
#EBOut = EBTest(Data = GeneMat,
# Conditions = as.factor(rep(c("C1", "C2"), each=5)),
# sizeFactors = Sizes, maxround = 5)
```

RankNorm

Rank Normalization

Description

'RankNorm' gives the rank normalization.

Usage

```
RankNorm(Data)
```

Arguments

Data The data matrix with transcripts in rows and lanes in columns.

Value

The function will return a matrix contains the normalization factor for each lane and each transcript.

Author(s)

Ning Leng

See Also

MedianNorm, QuantileNorm

Examples

```
data(GeneMat)
Sizes = RankNorm(GeneMat)
# Run EBSeq
# EBres = EBTest(Data = GeneData, NgVector = rep(1,10^4),
# Vect5End = rep(1,10^4), Vect3End = rep(1,10^4),
# Conditions = as.factor(rep(c(1,2), each=5)),
# sizeFactors = Sizes, maxround=5)
```

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