Package 'EBSeqHMM'

October 18, 2022

Type Package

Title Bayesian analysis for identifying gene or isoform expression
changes in ordered RNA-seq experiments

Version 1.30.0

Date 2015-03-21

Author Ning Leng, Christina Kendziorski

Depends EBSeq

Maintainer Ning Leng <lengning1@gmail.com>

Description
The EBSeqHMM package implements an auto-regressive hidden Markov model for statistical analysis in ordered RNA-seq experiments (e.g. time course or spatial course data). The EBSeqHMM package provides functions to identify genes and isoforms that have non-constant expression profile over the time points/positions, and cluster them into expression paths.

License Artistic-2.0

Collate 'EBTest_ext.R' 'EBHMMNBfunForMulti.R' 'EBHMMNBfun.R'
'EBHMMNBMultiEM_2chain.R' 'f0.R' 'LikefunNBHMM.R' 'beta.mom.R'
'EBSeqHMMTest.R' 'GetConfidentCalls.R' 'GetDECalls.R'
'GetAllPaths.R' 'PlotExp.R'

BuildVignettes yes

biocViews ImmunoOncology, StatisticalMethod, DifferentialExpression,
MultipleComparison, RNASeq, Sequencing, GeneExpression,
Bayesian, HiddenMarkovModel, TimeCourse

git_url https://git.bioconductor.org/packages/EBSeqHMM

git_branch RELEASE_3_15

git_last_commit d111a93

git_last_commit_date 2022-04-26

Date/Publication 2022-10-18

R **topics documented:**

EBSeqHMM–package   *EBSeqHMM: A Bayesian approach for identifying gene-expression changes in ordered RNA-seq experiments*

Description

The EBSeqHMM package implements an auto-regressive hidden Markov model for statistical analysis in ordered RNA-seq experiments (e.g. time course or spatial course data). The EBSeqHMM package provides functions to identify genes and isoforms that have non-constant expression profile over the time points/positions, and cluster them into expression paths.

Details

Package:	EBSeqHMM
Type:	Package
Version:	0.99.1
Date:	2014-09-16
License:	Artistic-2.0

Author(s)

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

References

Leng, N., Li, Y., Mcintosh, B. E., Nguyen, B. K., Duffin, B., Tian, S., Thomson, J. A., Colin, D., Stewart, R. M., and Kendziorski, C. (2014). Ebseq-hmm: A bayesian approach for identifying gene-expression changes in ordered rna-seq experiments.

See Also

EBSeq

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
EBSeqHMMGeneOut <- EBSeqHMMTest(Data=GeneExampleData, sizeFactors=Sizes, Conditions=Conditions,
         UpdateRd=2)
```

---

beta.mom	*Method of moments estimation (beta distribution)*

Description

Method of moments estimation (beta distribution)

Usage

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

Arguments

qs.in   A vector contains the numbers that will be fitted with a beta distribution.

Details

beta.mom() function can be used to estimate parameters in a Beta function using method of moments

Value

alpha.hat,beta.hat: Returns the estimation of alpha and beta.

Author(s)

Ning Leng

Examples

```
beta.mom(rbeta(10,1,1))
```

EBHMMNBfun   *Baum-Welch algorithm for a single hidden markov chain*

Description

   Baum-Welch algorithm for a single hidden markov chain

Usage

```
EBHMMNBfun(Data,NgVector=NULL,Conditions, sizeFactors,
PriorFC=1.5,homo=TRUE, maxround=5,
Pi0=NULL, Tran=NULL,NoTrend=FALSE, NumTranStage=3,
FCParam=NULL, AlphaIn=NULL,BetaIn=NULL,
StateNames=c("Up","NC","Down"),
EM=TRUE, UpdateParam=TRUE, Print=TRUE,
OnlyQ=FALSE,WithinCondR=TRUE,
PenalizeLowMed=TRUE, PenalizeLowMedQt=.2,PenalizeLowMedVal=10)
```

Arguments

Data	input data, rows are genes/isoforms and columns are samples
NgVector	Ng vector; NULL for gene level data
Conditions	A factor indicates the condition (time/spatial point) which each sample belongs to.
sizeFactors	a vector indicates library size factors
Tran	initial values for transition matrices
Pi0	initial values for starting probabilities
NumTranStage	number of states
PriorFC	target FC for gridient change
StateNames	name of the hidden states
homo	whether the chain is assumed to be homogenious
maxround	max number of iteration
AlphaIn,BetaIn	If the parameters are known and the user doesn't want to estimate them from the data, user may specify them here.
NoTrend	if NoTrend=TRUE, initial transition probabilities will be set to be equal
FCParam	not in use
EM	Whether estimate the prior parameters of the beta distribution by EM
UpdateParam	Whether update starting probabilities and transition probabilities
OnlyQ	If OnlyQ=TRUE, the function will only return estimated q's.
WithinCondR	By defining WithinCondR=TRUE, estimation of r's are obtained within each condition. (WithinCondR=FALSE is not suggested here)
Print	Whether print the elapsed-time while running the test.
PenalizeLowMed,PenalizeLowMedQt,PenalizeLowMedVal	
	Transcripts with median quantile < = PenalizeLowMedQt will be penalized

Details

EBHMMNBfun() function implements the Balm-Welch algorithm that estimates the starting probabilities and transition probabilities of a single hidden Markov model. Here the emission distribution of each gene is assumed to be a Beta-Negative Binomial distribution with parameters (r_g, alpha, beta) , in which alpha and beta are shared by all the genes and r_g is gene specific. If not specified, r_g, alpha and beta will be estimated using method of moments. For isoform data, we assume isoforms from the same Ig group share the same beta^Ig. alpha is shared by all the isoforms and r_gi is isoform specific. The user also needs to specify an expected FC.

Value

MAPTerm: the most likely path of each gene/isoform. MAPTermNum: numeric version of MAPTerm.

AllTerm: all possible expression paths considered in the model. PP: posterior probability of being each expression path.

WhichMax: index of the most likely path. Allf: prior probability of being each path.

Pi0Track: estimated starting probabilities of each iteration.

TranTrack: estimated transition probabilities of each iteration.

AlphaTrack, BetaTrack: estimated alpha and beta(s).

LLAll=PostSumForLL.Sum: log likelihood of the model.

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
tmp <- EBHMMNBfun(Data=GeneExampleData, sizeFactors=Sizes, Conditions=Conditions,
         maxround=2, OnlyQ=TRUE)
```

EBHMMNBfunForMulti   *Baum-Welch algorithm for multiple hidden markov chains*

Description

Baum-Welch algorithm for multiple hidden markov chains

Usage

```
EBHMMNBfunForMulti(Data,PPIn,
NgVector=NULL,Conditions, sizeFactors,
PriorFC=1.5,homo=TRUE, maxround=5,
Pi0=NULL, Tran=NULL, NumTranStage=3,
FCParam=NULL, AlphaIn=NULL,BetaIn=NULL,
StateNames=c("Up","NC","Down"),
EM=TRUE, UpdateParam=TRUE, Print=TRUE,WithinCondR=TRUE,
PenalizeLowMed=TRUE, PenalizeLowMedQt=.2,PenalizeLowMedVal=10)
```

Arguments

`Data`	input data, rows are genes/isoforms and columns are samples
`PPIn`	PPDE for all adjacent comparisons
`NgVector`	Ng vector; NULL for gene level data
`Conditions`	A factor indicates the condition (time/spatial point) which each sample belongs to.
`sizeFactors`	a vector indicates library size factors
`Tran`	initial values for transition matrices
`Pi0`	initial values for starting probabilities
`NumTranStage`	number of states in two chains
`PriorFC`	target FC for gridient change
`StateNames`	name of the hidden states
`homo`	whether the chain is assumed to be homogenious
`maxround`	max number of iteration
`AlphaIn,BetaIn`	If the parameters are known and the user doesn't want to estimate them from the data, user may specify them here.
`FCParam`	not in use
`EM`	Whether estimate the prior parameters of the beta distribution by EM
`UpdateParam`	Whether update starting probabilities and transition probabilities
`WithinCondR`	By defining WithinCondR=TRUE, estimation of r's are obtained within each condition. (WithinCondR=FALSE is not suggested here)
`Print`	Whether print the elapsed-time while running the test.
`PenalizeLowMed,PenalizeLowMedQt,PenalizeLowMedVal`	
	Transcripts with median quantile < = PenalizeLowMedQt will be penalized

Details

EBHMMNBfunForMulti() function implements the Balm-Welch algorithm that estimates the starting probabilities and transition probabilities of a hidden Markov model with multiple chains. Here the emission distribution of each gene is assumed to be a Beta-Negative Binomial distribution with parameters (r_g, alpha, beta) , in which alpha and beta are shared by all the genes and r_g is gene specific. If not specified, r_g, alpha and beta will be estimated using method of moments. For isoform data, we assume isoforms from the same Ig group share the same $beta^{Ig}$. alpha is shared by all the isoforms and r_{gi} is isoform specific. The user also needs to specify an expected FC.

Value

MAPTerm: the most likely path of each gene/isoform.

MAPTermNum: numeric version of MAPTerm.

AllTerm: all possible expression paths considered in the model.

PP: posterior probability of being each expression path.

WhichMax: index of the most likely path.

Allf: prior probability of being each path.

Pi0Track: estimated starting probabilities of each iteration.

TranTrack: estimated transition probabilities of each iteration.

AlphaTrack, BetaTrack: estimated alpha and beta(s).

LLAll=PostSumForLL.Sum: log likelihood of the model.

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
tmp <- EBHMMNBfunForMulti(Data=GeneExampleData, PPIn=matrix(1,ncol=15, nrow=100),sizeFactors=Sizes, Conditions=C
          maxround=2)
```

EBHMMNBMultiEM_2chain   *Run EBSeqHMM model with a fixed expected FC*

Description

Run EBSeqHMM model with a fixed expected FC

Usage

```
EBHMMNBMultiEM_2chain(Data,
NgVector=NULL, Conditions, AllTran=NULL,
AllPi0=NULL, Terms=NULL,
sizeFactors, NumTranStage=c(3,2),PriorFC=2,
StateNames=c("Up","Down"),homo=FALSE,
UpdateRd=5, PIBound=.9, UpdatePI=FALSE,Print=FALSE,
WithinCondR=TRUE,
PenalizeLowMed=TRUE, PenalizeLowMedQt=.1,PenalizeLowMedVal=10)
```

Arguments

`Data`	input data, rows are genes and columns are samples
`NgVector`	Ng vector; NULL for gene level data
`Conditions`	A factor indicates the condition (time/spatial point) which each sample belongs to.
`AllTran`	initial values for transition matrices
`AllPi0`	initial values for starting probabilities
`Terms`	Terms
`sizeFactors`	a vector indicates library size factors
`StateNames`	names of the hidden states
`NumTranStage`	number of states in two chains
`PriorFC`	target FC for gridient change
`homo`	whether the chain is assumed to be homogenious
`UpdateRd`	max number of iteration
`UpdatePI`	whether update the mixture proportion of two chains
`PIBound`	upper bound of the mixture proportion of the two chains
`Print`	Whether print the elapsed-time while running the test.
`WithinCondR`	By defining WithinCondR=TRUE, estimation of r's are obtained within each condition. (WithinCondR=FALSE is not suggested here)
`PenalizeLowMed,PenalizeLowMedQt,PenalizeLowMedVal`	
	Transcripts with median quantile $< =$ PenalizeLowMedQt will be penalized

Details

EBHMMNBMultiEM_2chain() function implements the EBSeqHMM model to perform statistical analysis in an RNA-seq experiment with ordered conditions. EBHMMNBMultiEM_2chain() calls EBHMMNBfunForMulti() function to perform Balm-Welch algorithm that estimates the starting probabilities and transition probabilities. Here the emission distribution of each gene is assumed to be a Beta-Negative Binomial distribution with parameters (r_g, alpha, beta) , in which alpha and beta are shared by all the genes and r_g is gene specific. If not specified, r_g, alpha and beta will be estimated using method of moments. For isoform data, we assume isoforms from the same Ig group share the same beta^Ig. alpha is shared by all the isoforms and r_gi is isoform specific. The user also needs to specify an expected FC. Function EBSeqHMMTest() runs several models with varying FCs and returns the model with maximum likelihood.

Value

Pi0Out: estimated starting probabilities of each iteration.

TranOut: estimated transition probabilities of each iteration.

Pi: estimated probability of being each chain.

Alpha, Beta: estimated alpha and beta(s).

LLSum: log likelihood of the model.

QList: estimated q's.

MgAllPP: marginal PP for all paths.

MgAllMAPChar: Most likely path based on MgAllPP.

MgAllMaxVal: Highest PP based on MgAllPP.

PPMatW: Posterior probabilities of being each of the chains.

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
tmp <- EBHMMNBMultiEM_2chain(Data=GeneExampleData,sizeFactors=Sizes, Conditions=Conditions,
         UpdateRd=2)
```

EBSeqHMMTest	*Identify DE genes and classify them into their most likely path in an RNA-seq experiment with ordered conditions*

Description

Identify DE genes and classify them into their most likely path in an RNA-seq experiment with ordered conditions

Usage

```
EBSeqHMMTest(Data,
NgVector=NULL, Conditions, AllTran=NULL,
AllPi0=NULL, Terms=NULL,
sizeFactors, NumTranStage=c(3,2),FCV=2,
homo=FALSE, UpdateRd=10, PIBound=.9, UpdatePI=FALSE,
Print=FALSE,WithinCondR=TRUE,Qtrm=.75,QtrmCut=10,
PenalizeLowMed=TRUE, PenalizeLowMedQt=.1,PenalizeLowMedVal=10)
```

Arguments

Data	input data, rows are genes and columns are samples
NgVector	Ng vector; NULL for gene level data
Conditions	A factor indicates the condition (time/spatial point) which each sample belongs to.
AllTran	initial values for transition matrices
AllPi0	initial values for starting probabilities

Terms	Terms
FCV	candidate values for expected FC. Default is 2. If user wants to search through a list of candidate FCs, he/she may define FCV as a vector. e.g. By defining FCV=seq(1.4,2,.2), the function will search over (1.4 1.6 1.8 2.0). Note that searching over a number of candidate FCs will increase the running time.
sizeFactors	a vector indicates library size factors
NumTranStage	number of states in two chains
homo	whether the chain is assumed to be homogenious
UpdateRd	max number of iteration
UpdatePI	whether update the mixture proportion of two chains
PIBound	upper bound of the mixture proportion of the two chains
Qtrm,QtrmCut	Transcripts with Qtrm th quantile $<=$ QtrmCut will be removed before testing. The default value is Qtrm = 0.75 and QtrmCut=10. By default setting, transcripts that have >75% of the samples with expression less than 10 won't be tested.
WithinCondR	By defining WithinCondR=TRUE, estimation of r's are obtained within each condition. (WithinCondR=FALSE is not suggested here)
Print	Whether print the elapsed-time while running the test.
PenalizeLowMed,PenalizeLowMedQt,PenalizeLowMedVal	
	Transcripts with median quantile $<=$ PenalizeLowMedQt will be penalized

Details

EBSeqHMMTest() function applies EBSeqHMM model with differentexpected FC's and select the optimal FC that maximizes the log likelohood. EBSeqHMMTest() calls EBHMMNBMultiEM_2chain() function which implements the EBSeqHMM model to perform statistical analysis in an RNA-seq experiment with ordered conditions based on a fixed expected FC. EBSeqHMMTest() runs EBHMMNBMultiEM_2chain() with varying FCs (default is seq(1.4,2,.2)). And it will return the results of the model with optimal FC. Here the emission distribution of each gene is assumed to be a Beta-Negative Binomial distribution with parameters (r_g, alpha, beta) , in which alpha and beta are shared by all the genes and r_g is gene specific. If not specified, r_g, alpha and beta will be estimated using method of moments. For isoform data, we assume isoforms from the same Ig group share the same beta^Ig. alpha is shared by all the isoforms and r_gi is isoform specific. The user also needs to specify an expected FC.

Value

Pi0Out: estimated starting probabilities of each iteration.

TranOut: estimated transition probabilities of each iteration.

Pi: estimated probability of being each chain.

Alpha, Beta: estimated alpha and beta(s).

LLSum: log likelihood of the model.

QList: estimated q's.

MgAllPP: marginal PP for all paths.

MgAllMAPChar: Most likely path based on MgAllPP.

MgAllMaxVal: Highest PP based on MgAllPP.

PPMatW: Posterior probabilities of being each of the chains.

FCLikelihood: log likelihood of each FC.

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
EBSeqHMMGeneOut <- EBSeqHMMTest(Data=GeneExampleData, sizeFactors=Sizes, Conditions=Conditions,
          UpdateRd=2)
```

EBTest_ext   *Extented EBTest function*

Description

Extented EBTest function

Usage

```
EBTest_ext(Data,NgVector=NULL,Conditions,
sizeFactors, maxround, Pool=FALSE, NumBin=1000,
ApproxVal=10^-10, Alpha=NULL, Beta=NULL,
PInput=NULL,RInput=NULL,PoolLower=.25,
PoolUpper=.75,OnlyCalcR=FALSE,Print=TRUE)
```

Arguments

Data	Input data, rows are genes/isoforms and columns are samples. Data should come from a two condition experiment
NgVector	Ng vector; NULL for gene level data
Conditions	A factor indicates the condition (time/spatial point) which each sample belongs to. Only two levels are allowed.
sizeFactors	a vector indicates library size factors
maxround	number of iteration
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 FCs as the candidate genes (default is 25 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 may specify them here.

Print   Whether print the elapsed-time while running the test.

OnlyCalcR   if OnlyCalcR=TRUE, the function will only return estimation of r's.

Details

EBSeq_ext() function is an extension of EBTest() function, which is used to calculate the conditional probability $P(X\_g,t \mid X\_g,t-1)$. In EBSeqHMM, we assume the conditional distribution is Beta-Negative Binomial.

Value

See [EBTest](#)

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
Data=GeneExampleData[,1:6]
CondVector <- rep(paste("t",1:2,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2"))
Sizes <- MedianNorm(Data[1:10,])
Out <- EBTest_ext(Data=Data[1:10,], sizeFactors=Sizes, Conditions=Conditions,
        maxround=1)
```

---

f0   *Calculate the prior predictive distribution of the Beta-Negative Binomial model*

Description

Calculate the prior predictive distribution of the Beta-Negative Binomial model

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 set as TRUE, the output will in log scale.

Details

Function f0() will calculate the Beta-Negative Binomial prior predictive probability for a given set of parameters

Value

output a numeric vector, each element shows the prior predictive probability of one gene/isoform

Author(s)

Ning Leng

Examples

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

GeneExampleData	*Simulated gene level data set with 5 ordered conditions*

Description

'GeneExampleData' gives the gene level simulated data with 5 ordered conditions, triplicates for each condition. The data set was simulated following the Negative Binomial distribution. The parameters of each gene (mean and overdispersion) were sampled from the empirical estimates from an empirical RNA-Seq data set from Thomson lab at Morgridge Institute for Research.

Format

GeneExampleData is a matrix with 100 genes (rows) and 15 samples (columns).

See Also

IsoExampleList

Examples

```
data(GeneExampleData)
str(GeneExampleData)
```

GetAllPaths	*Obtain all possible gene paths for an RNA-seq experiments with ordered conditions*

Description

Obtain all possible gene paths for an RNA-seq experiments with ordered conditions

Usage

```
GetAllPaths(EBSeqHMMOut, OnlyDynamic=TRUE)
```

Arguments

EBSeqHMMOut   output from EBSeqHMMTest function

OnlyDynamic   if specifies as TRUE, only dynamic paths will be shown

Details

GetAllPaths() function may be used to generate all possible expression paths of a particular design.

Value

output: a vector of paths. For example, Up-Up-Up-Up, Up-Up-EE-EE, Up-Down-Up-EE, etc.

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
EBSeqHMMGeneOut <- EBSeqHMMTest(Data=GeneExampleData, sizeFactors=Sizes, Conditions=Conditions,
        UpdateRd=2)
AllPaths <- GetAllPaths(EBSeqHMMGeneOut)
```

GetConfidentCalls *Obtain confident gene calls for classifying genes into expression paths*

Description

Obtain confident gene calls for classifying genes into expression paths

Usage

```
GetConfidentCalls(EBSeqHMMOut, FDR=.05, cutoff=0.5, OnlyDynamic=TRUE,Paths=NULL)
```

Arguments

EBSeqHMMOut	output from EBSeqHMMTest function
FDR	Target FDR, default is 0.05.
cutoff	cutoff to use for defining a confident call. Genes with PP_path greater or equal to cutoff will be called as a confident call. Default is 0.5.
OnlyDynamic	if specifies as T, only dynamic paths will be shown
Paths	paths that are of interest. Default is NULL. If it is not specified, all possible paths will be considered.

Details

Function GetConfidentCalls() can be used to obtain a list of DE genes/isoforms with user specific cutoffs. To obtain a list of DE genes/isoforms with a target FDR alpha, the user may specify FDR=alpha. To further choose genes/isoforms with high posterior probability of being its most likely path, the user may specify the option cutoff (default is 0.5). Then genes or isoforms with PP(most likely path) $> = 0.5$ will be selected

Value

Overall: a list of genes/isoforms that are identified as DE under the target FDR, shown are their names and PPs; EachPath: a list object, each sublist contains confident calls (genes/isoforms) that have PP(path)>=cutoff for a particular expression path, shown are their names and PPs; NumEach: length of each sublist in EachPath. EachPathName: gene/isoform names in each of the sublists in EachPath

Note

Output: output a list of genes that are classified to a expression path as a confident assignment.

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
EBSeqHMMGeneOut <- EBSeqHMMTest(Data=GeneExampleData, sizeFactors=Sizes, Conditions=Conditions,
          UpdateRd=2)
GeneDECalls <- GetDECalls(EBSeqHMMGeneOut, FDR=.05)
GeneConfCalls <- GetConfidentCalls(EBSeqHMMGeneOut, FDR=.05,cutoff=.5, OnlyDynamic=TRUE)
```

GetDECalls   *Obtain DE gene/isoform list at a certain FDR*

Description

Obtain DE gene/isoform list at a certain FDR

Usage

```
GetDECalls(EBSeqHMMOut,FDR=.05)
```

Arguments

EBSeqHMMOut   output from EBSeqHMMTest function

FDR   Target FDR; default is 0.05

Details

Function GetDECalls() can be used to obtain a list of DE genes/isoforms with user specific cutoffs.
To obtain a list of DE genes/isoforms with a target FDR alpha, the user may specify FDR=alpha.

Value

a list of genes/isoforms that are identified as DE under the target FDR, shown are their names and
PPs;

Note

Output: output a list of genes that are DE in at least one condition in an RNA-seq experiment with
multiple ordered conditions

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
EBSeqHMMGeneOut <- EBSeqHMMTest(Data=GeneExampleData, sizeFactors=Sizes, Conditions=Conditions,
          UpdateRd=2)
GeneDECalls <- GetDECalls(EBSeqHMMGeneOut, FDR=.05)
```

IsoExampleList *Simulated isoform level data set with 5 ordered conditions*

Description

'IsoExampleList' gives the isoform level simulated data with 5 ordered conditions, triplicates for
each condition. The data set was simulated following the Negative Binomial distribution. The
parameters of each isoform (mean and overdispersion) were sampled from the isoform level em-
pirical estimates from an empirical RNA-Seq data set from Thomson lab at Morgridge Institute for
Research.

Format

IsoExampleList is a list with three components. IsoExampleList$IsoExampleData contains a matrix
with 200 isoform (rows) and 15 samples (columns). IsoExampleList$IsoNames contains a vector
of isoform names. IsoformExampleList$IsosGeneNames contains a vector indicating the gene each
isoform belongs to.

See Also

GeneExampleData

Examples

```
data(IsoExampleList)
str(IsoExampleList)
```

LikefunNBHMM *Likelihood function of the Beta-Negative Binomial HMM Model*

Description

Likelihood function of the Beta-Negative Binomial HMM Model

Usage

```
LikefunNBHMM(ParamPool, InputPool)
```

Arguments

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

Details

The likelihood function of the Beta-Negative Binomial HMM model used in EBSeqHMM. EBSeqHMM uses optim() function to obtain the optimal estimates that minimizes the likelihood.

Value

optimal estimates of the parameters of interest

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
tmp <- GeneExampleData[1:10,]
In <- list(tmp,1,5,10,3,tmp,rep(1,15),as.factor(rep(1:5,each=3)), 10,cbind(rep(.5,10),rep(1,10),rep(2,10)))
Start <- c(1,1)
LikefunNBHMM(Start,In)
```

PlotExp *Plot expression of a single gene*

Description

Plot expression of a single gene

Usage

```
PlotExp(NormalizedData, Conditions, Name)
```

Arguments

NormalizedData	Expression data after adjusting for library size factors
Conditions	sample conditions
Name	name of the gene/isoform of interest

Details

PlotExp() function will generate line plots for genes or isoforms of interest.

Value

PlotExp() funtion will generate line plots for genes or isoforms of interest.

Author(s)

Ning Leng

Examples

```
data(GeneExampleData)
CondVector <- rep(paste("t",1:5,sep=""),each=3)
Conditions <- factor(CondVector, levels=c("t1","t2","t3","t4","t5"))
Sizes <- MedianNorm(GeneExampleData)
NormData <- GetNormalizedMat(GeneExampleData, Sizes)
PlotExp(NormData, Conditions, "Gene_1")
```

Index