edgeR

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`DGEList-class` *Digital Gene Expression data - class*

Description

A simple list-based class for storing read counts from digital gene expression technologies.

Slots/List Components

Objects of this class contain the following list components:

`data`:	numeric matrix containing the read counts.
`lib.size`:	numeric vector containing the total number of reads for each library (column of `code`).
`group`:	vector giving the experimental group/condiction.

Methods

This class inherits directly from class `list` so any operation appropriate for lists will work on objects of this class. `DGEList` objects also have a `show` method.

Author(s)

Mark Robinson

`EBList-class` *differential expression of Digital Gene Expression data - class*

Description

A simple list-based class for storing results of the approximate empirical Bayes rule parameters

Slots/List Components

Objects of this class contain the following list components:

`sigma2.0.est`:	numeric scale `sigma_0^2` estimate.
`alpha`:	numeric scalar alpha estimate.

`score`:	numeric scalar (likelihood) score.
`inos`:	numeric vector containing the (likelihood) information.
`exact`:	list containing info with respect to quantile adjustment.

Methods

This class inherits directly from class `list` so any operation appropriate for lists will work on objects of this class. `EBList` objects also have a `show` method.

Author(s)

Mark Robinson

`alpha.approxeb` *Estimate the prior weight, alpha*

Description

Estimate the prior weight, using an approximate empirical Bayes rule

Usage

```
alpha.approxeb(object, verbose=TRUE)
```

Arguments

`object`	`DGEList` object containing the raw data with elements `data` (table of counts), `group` (vector indicating group) and `lib.size` (vector of library sizes)
`verbose`	whether to write comments, default `true`

Value

`EBList` object with elements `p` (overall proportion), `p1` (estimates for first group), `p2` (estimates for second group)

Author(s)

Mark Robinson

Examples

```
y<-matrix(rnbinom(20,size=1,mu=10),nrow=5)
d<-DGEList(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000:1001),2))
alpha<-alpha.approxeb(d)
```

`approx.expected.info` *Approximate of expected information (Fisher information)*

Description

Using a linear fit (for simplicity), the expected information from the conditional log likelihood of the dispersion parameter of the negative binomial is calculated over all genes.

Usage

```
approx.expected.info(object, d, qA, robust = FALSE)
```

Arguments

`object`	`DGEList` object containing the raw data with elements `data` (table of counts), `group` (vector indicating group) and `lib.size` (vector of library sizes)
`d`	delta parameter for negative binomial - `phi/(phi+1)`
`qA`	list from output of `quantileAdjust`
`robust`	logical on whether to use a robust fit, default `FALSE`

Value

vector of Fisher information approximates (with length same as the number of rows of the original data)

Author(s)

Mark Robinson

Examples

```
set.seed(0)
y<-matrix(rnbinom(40,size=1,mu=10),ncol=4)
d<-list(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000:1001),2))
qA<-quantileAdjust(d,alpha=100)
exp.inf<-approx.expected.info(d,1/(1 + qA$r[1]),qA)
```

`condLogLikDerDelta` *Conditional log-likelihood in terms of delta*

Description

Conditional log-likelihood paramterized in terms of delta (`phi / (phi+1)`)

Usage

```
condLogLikDerDelta(y, delta, grid = TRUE, der = 1, doSum = TRUE)
```

Arguments

`y`	matrix with count data (or pseudo-data)
`delta`	delta (`phi / (phi+1)`)parameter of negative binomial
`grid`	logical, whether to calculate a grid over the values of delta
`der`	derative, either 0 (the function), 1 (first derivative) or 2 (second derivative)
`doSum`	logical, whether to sum over samples or not (default `TRUE`

Value

vector of matrix of function/derivative evaluations

Author(s)

Mark Robinson

Examples

```
y1<-matrix(rnbinom(10,size=1,mu=10),nrow=5)
v1<-seq(.1,.9,length=9)
ll1<-condLogLikDerDelta(y1,v1,grid=TRUE,der=0,doSum=FALSE)
ll2<-condLogLikDerDelta(y1,delta=.5,grid=FALSE,der=0)
```

`condLogLikDerSize` *Conditional log-likelihood in terms of size*

Description

Conditional log-likelihood paramterized in terms of size (`1 / phi`)

Usage

```
condLogLikDerSize(y,r,der=1)
```

Arguments

`y`	list containing the raw data with elements `data` (table of counts), `group` (vector indicating group) and `lib.size` (vector of library sizes)
`r`	size parameter of negative binomial distribution
`der`	derative, either 0 (the function), 1 (first derivative) or 2 (second derivative)

Value

vector of matrix of function/derivative evaluations

Author(s)

Mark Robinson

Examples

```
y1<-matrix(rnbinom(10,size=1,mu=10),nrow=5)
ll2<-condLogLikDerSize(y1,r=10,der=0)
```

`deDGE` *Compute moderated differential expression scores for digital gene expression (DGE) data*

Description

Runs weighted likelihood calculation for moderated estimates of dispersion, and tests for differences in 'tag' abundance between groups

Usage

```
deDGE(object,alpha=500,doPoisson=FALSE,verbose=TRUE)
```

Arguments

`object`	`DGEList` containing elements `data` (matrix: rows-tags, columns-libraries), `lib.size` `group` indicating class
`alpha`	weight to put on the individual tag's likelihood
`doPoisson`	logical, whether to fit Poisson model instead of Negative Binomial, default `FALSE`
`verbose`	logical, whether to write comments, default `TRUE`

Value

`deDGEList` with elements `lr` (likelihood ratio test), `r` (estimates of 1/overdispersion), `ps` (list containing proportion estimates)

Author(s)

Mark Robinson

References

Robinson MD, Smyth GK. 'Small-sample estimation of negative binomial dispersion, with applications to SAGE data.' Biostatistics. 2008 Apr;9(2):321-32.

Robinson MD, Smyth GK. 'Moderated statistical tests for assessing differences in tag abundance.' Bioinformatics. 2007 Nov 1;23(21):2881-7.

Examples

```
# generate raw data from NB, create list object
y<-matrix(rnbinom(20,size=1,mu=10),nrow=5)
d<-DGEList(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000:1001),2))

# find alpha and call main procedure to find differences
alpha<-alpha.approxeb(d)
ms<-deDGE(d,alpha=alpha$alpha)
```

`deDGEList-class` *differential expression of Digital Gene Expression data - class*

Description

A simple list-based class for storing results of differential expression analysis for DGE data

Slots/List Components

Objects of this class contain the following list components:

`ps`:	list containing estimates of `p` parameter.
`r`:	numeric vector of size parameter (`1/phi`) where `phi` is negative binomial dispersion.
`pseudo`:	numeric matrix with the pseudo-counts.
`M`:	numeric scalar with the library size that pseudo counts are mapped to.
`exact`:	numeric vector of exact p-values (unadjusted).

Methods

This class inherits directly from class `list` so any operation appropriate for lists will work on objects of this class. `deDGEList` objects also have a `show` method.

Author(s)

Mark Robinson

`estimatePs` *Estimate expression proportions*

Description

Estimate expression proportions (maximum likelihood with size fixed) based on negative binomial for each tag and sample group (only 2 groups implemented at this point)

Usage

```
estimatePs(y1, y2, lib.size1, lib.size2, r, tol = 1e-10, maxit = 30)
```

Arguments

`y1`	matrix of counts for first group
`y2`	matrix of counts for second group
`lib.size1`	vector of library sizes for first group
`lib.size2`	vector of library sizes for second group
`r`	size parameter of negative binomial
`tol`	tolerance between iterations
`maxit`	maximum number of iterations

Value

list with elements `p` (overall proportion), `p1` (estimates for first group), `p2` (estimates for second group)

Author(s)

Mark Robinson

Examples

```
y1<-matrix(rnbinom(10,size=1,mu=10),nrow=5)
y2<-matrix(rnbinom(10,size=1,mu=5),nrow=5)
ps<-estimatePs(y1,y2,c(1000,1001),c(1000,1001),r=1)
```

`exactTestNB` *An exact test for differences between two negative binomial groups*

Description

An exact test for differences between two negative binomial groups

Usage

```
exactTestNB(y, g, mus, r, verbose=TRUE)
```

Arguments

`y`	data (e.g. quantile adjusted pseudodata) to compute Fisher exact statistics on
`g`	group indicator, must be same length as nrow(y)
`mus`	vector of means under the null hypothesis (of no difference between groups)
`r`	preset or estimated negative binomial `size` parameter. If you want to run a Poisson test, set r very large (e.g. 1000)
`verbose`	whether to write comments, default `true`

Value

list with elements `lr` (likelihood ratio test), `r` (estimates of 1/overdispersion), `ps` (list containing proportion estimates)

Author(s)

Mark Robinson

Examples

```
y<-matrix(rnbinom(20,mu=10,size=1.5),nrow=5)
group<-c(1,1,2,2)
mus<-rep(10,5)
f<-exactTestNB(y,group,mus,r=1.5)
```

`findMaxD2` *Maximizes the negative binomial likelihood*

Description

Maximizes the negative binomial likelihood (a weighted version using the common likelihood given weight alpha) for each tag

Usage

```
findMaxD2(x, alpha = 0.5, grid = TRUE, tol = 1e-05, n.iter = 5, grid.length = 20
```

Arguments

`x`	list with elements `data`, `lib.size` and `group`
`alpha`	weight given to common likelihood, set to 0 for individual estimates or large (e.g. 100) for common likelihood
`grid`	logical, whether to use a grid search (default = `TRUE`); if `FALSE` use Newton-Rhapson steps
`tol`	if `grid=FALSE`, tolerance for Newton-Rhapson iterations
`n.iter`	if `grid=FALSE`, number of Newton-Rhapson iterations
`grid.length`	length of the grid to maximize over; default `200`

Value

list with elements `lr` (likelihood ratio test), `r` (estimates of 1/overdispersion), `ps` (list containing proportion estimates)

Author(s)

Mark Robinson

Examples

```
y<-matrix(rnbinom(1000,mu=10,size=2),ncol=4)
d<-list(data=y,group=c(1,1,2,2),lib.size=c(1000:1003))
cml1<-findMaxD2(d,alpha=10)
cml2<-findMaxD2(d,alpha=0)
```

`getData` *Extract data table from DGEList object*

Description

Returns the `data` slot of a DGEList object

Usage

```
getData(object)
```

Arguments

`object`	matrix of counts for first group

Value

matrix of data (presumably integers)

Author(s)

Mark Robinson

Examples

```
# generate raw data from NB, create list object
y<-matrix(rnbinom(20,size=1,mu=10),nrow=5)
d<-DGEList(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000:1001),2))
# should be 5x4
print(dim(getData(d)))
```

`interpolateHelper` *Quantile Adjustment interpolator*

Description

Helper function to interpolate the quantile function

Usage

```
interpolateHelper(mu, p, r, d,verbose=TRUE)
```

Arguments

`mu`	matrix of means
`p`	matrix of percentiles
`r`	scalar, vector or matrix of `size` parameters
`d`	original data matrix
`verbose`	whether to write comments, default `true`

Value

matrix with quantile-adjusted pseudo data

Author(s)

Mark Robinson

Examples

```
y<-matrix(rnbinom(10000,size=2,mu=10),ncol=4)
d<-list(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000,1010),2))
ps<-estimatePs(d$data[,1:2],d$data[,3:4],d$lib.size[1:2],d$lib.size[3:4],r=2)
N<-prod(d$lib.size)^(1/ncol(d$data))
perc<-pnbinom(d$data-1,size=2,mu=outer(ps$p,d$lib.size))+dnbinom(d$data,size=2,mu=outer(p
pseudo<-interpolateHelper(outer(ps$p,rep(N,4)),perc,r=2,d$data)
```

`logLikDerP` *Log-likelihood for proportion*

Description

Log-likelihood and derivatives for the proportion parameter of negative binomial (mean = library size * proportion)

Usage

```
logLikDerP(p, y, lib.size, r, der = 0)
```

Arguments

`p`	vector of proportion parameters to be evaluated
`y`	matrix of data
`lib.size`	vector of library sizes
`r`	size parameter of negative binomial distribution
`der`	derative, either 0 (the function), 1 (first derivative) or 2 (second derivative)

Value

vector of evaluations

Author(s)

Mark Robinson

Examples

```
y<-matrix(rnbinom(20,size=1.5,mu=10),nrow=5)
d<-list(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000:1001),2))

this.p<-rowMeans( y/ outer(rep(1,nrow(y)),d$lib.size) )
d1p<-logLikDerP(this.p,y,d$lib.size,r=1.5,der=1)
```

`plotMA` *MA-like plot for deDGEList objects*

Description

Plots

Usage

```
plotMA(object,xlab="A",ylab="M",ylim=NULL,pch=19,...)
```

Arguments

`object`	`deDGEList` object, as output from `deDGE`
`xlab`	x-axis label
`ylab`	y-axis label
`ylim`	limits on y-axis, if left at `NULL`, scaled to be symmetric about 0
`pch`	plot character
`...`	further arguments to the `plot` command

Value

A plot to the current device

Author(s)

Mark Robinson

See Also

`deDGE`

Examples

```
# generate raw data from NB, create list object
y<-matrix(rnbinom(20,size=1,mu=10),nrow=5)
d<-DGEList(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000:1001),2))

# find alpha and call main procedure to find differences
alpha<-alpha.approxeb(d)
ms<-deDGE(d,alpha=alpha$alpha)

# plot it
plotMA(ms)
```

`quantileAdjust` *Normalizes a dataset by using a quantile adjustment*

Description

The function adjusts (you might say normalizes) a dataset, creating pseudodata that represents quantile-adjusted data as if all samples had the same library size, while estimating the dispersion parameter.

Usage

```
quantileAdjust(object, N = prod(object$lib.size)^(1/ncol(object$data)), alpha =
```

Arguments

`object`	list containing the raw data with elements `data` (table of counts), `group` (vector indicating group) and `lib.size` (vector of library sizes)
`N`	library size to normalize to; default is the geometric mean of the original library sizes
`alpha`	weight to put on the individual tag's likelihood
`null.hypothesis`	logical, whether to calculate the means and percentile under the null hypothesis; default is `TRUE`
`n.iter`	number of iterations in estimating the size parameter
`r.init`	initialized value of the size parameter; if `NULL`, then the common value on unadjusted data is used
`tol`	tolerance in estimating the size parameter
`verbose`	whether to write comments, default `true`

Value

list containing several elements used in downstream function calls. `r` is the dispersion estimate, `pseudo` is the quantile-adjusted pseudodata, `ps` is a list containing the abundance estimates, `N` is the common library size and `p` and `mu` are the percentiles and means, respectively that the quantile is based on

Author(s)

Mark Robinson

Examples

```
set.seed(0)
y<-matrix(rnbinom(40,size=1,mu=10),ncol=4)
d<-list(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000:1001),2))
qA<-quantileAdjust(d,alpha=100)
```

`readDGE` *Read a list of files containing DGE data*

Description

Reads a list of text files, one for each sample. Files should be tab-delimited with an identifier (could be tag sequence) as the first column and counts as the second column. The function creates one big table with 0s where necessary.

Usage

```
readDGE(files,...)
```

Arguments

`files`	character vector of filenames
`...`	option arguments to send to `read.table`

Value

list with elements `data` (table of counts), `lib.size` (library sizes)

Author(s)

Mark Robinson

Examples

```
#  Read all .txt files from current working directory

## Not run:
files <- dir(pattern="*\\.txt$")
RG <- readDGE(files,sep="\t",header=TRUE,comment.char="",stringsAsFactors=FALSE)
## End(Not run)
```

`tau2.0.objective` *Objective function for tau2*

Description

Objective function for tau2 which is used in the rule of how much to squeeze the dispersion parameters towards the common value

Usage

```
tau2.0.objective(tau2.0, info.g, score.g)
```

Arguments

`tau2.0`	scalar, value for tau2
`info.g`	observed information for each gene
`score.g`	observed score (first derivative of log-likelihood) for each gene

Value

scalar, value of objective function at tau2.0

Author(s)

Mark Robinson

Examples

```
y<-matrix(rnbinom(20,size=1,mu=10),nrow=5)
x<-list(data=y,group=rep(1:2,each=2),lib.size=rep(1000:1001,each=2))
scores <- condLogLikDerDelta(y, delta=0.5, der = 1, doSum = TRUE)
qA <- quantileAdjust(x, alpha = 10, null.hypothesis = TRUE)
exp.inf <- approx.expected.info(x, d=0.5, qA)
sigma2.0.est <- optimize(tau2.0.objective, c(0, 500), info.g = exp.inf, score.g = scores)
```

`topTags` *Displays the top differentially expressed tags in a table*

Description

Displays/Returns the top DE tags in a data frame

Usage

```
topTags(object,n=10,adj.method= "BH")
```

Arguments

`object`	`deDGEList`, output from `deDGE`
`n`	number of tags to display/return
`adj.method`	method used to adjust P-values, using `p.adjust`

Value

Data frame containing the relative level of expression, log fold changes, unadjusted and adjusted P-values

Author(s)

Mark Robinson

References

Robinson MD, Smyth GK. 'Small-sample estimation of negative binomial dispersion, with applications to SAGE data.' Biostatistics. 2008 Apr;9(2):321-32.

Robinson MD, Smyth GK. 'Moderated statistical tests for assessing differences in tag abundance.' Bioinformatics. 2007 Nov 1;23(21):2881-7.

Examples

```
# generate raw data from NB, create list object
y<-matrix(rnbinom(80,size=1,mu=10),nrow=20)
d<-DGEList(data=y,group=rep(1:2,each=2),lib.size=rep(c(1000:1001),2))
rownames(d$data)<-paste("tagno",1:nrow(d$data),sep=".")

# find alpha and call main procedure to find differences
alpha<-alpha.approxeb(d)
ms<-deDGE(d,alpha=alpha$alpha)

# look at top 10
topTags(ms)
```

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