

# Package ‘tweeDEseq’

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**Type** Package

**Title** RNA-seq data analysis using the Poisson-Tweedie family of distributions

**Description** Differential expression analysis of RNA-seq using the Poisson-Tweedie (PT) family of distributions. PT distributions are described by a mean, a dispersion and a shape parameter and include Poisson and NB distributions, among others, as particular cases. An important feature of this family is that, while the Negative Binomial (NB) distribution only allows a quadratic mean-variance relationship, the PT distributions generalize this relationship to any order.

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compareCountDist	<i>Compare count data distributions</i>
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## Description

Compares the empirical and estimated distributions for different count data models

## Usage

```
compareCountDist(x, plot=TRUE, ...)
```

## Arguments

x	numeric vector containing the read counts.
plot	If TRUE (the default) then the plot with the ECDF function for the counts and the three different Poisson-Tweedie distributions is produced, otherwise no graphical output is given and this only makes sense if one is interested in the returned value (see value section below).
...	Further arguments to be passed to the plot function.

**Details**

This function serves the purpose of comparing a empirical distribution of counts with three Poisson-Tweedie distributions arising from estimating mean, dispersion and setting  $a = 1$  for comparing against a Poisson,  $a = 0$  for comparing against a negative binomial and estimating the shape parameter  $a$  from data too. The legend shows the values of the  $a$  parameter and the P-value of the likelihood ratio test on whether the expression profile follows a negative binomial distribution ( $H_0 : a = 0$ ).

**Value**

List with the following components:

a	shape parameter estimated from the input data x.
p.value	P-value for the test that the data follows a negative binomial distribution, i.e., $H_0 : a = 0$ .

**References**

Esnaola M, Puig P, Gonzalez D, Castelo R and Gonzalez JR (2013). A flexible count data model to fit the wide diversity of expression profiles arising from extensively replicated RNA-seq experiments. BMC Bioinformatics 14: 254

**See Also**

[qqchisq testShapePT](#)

**Examples**

```
# Generate 500 random counts following a Poisson Inverse Gaussian
# distribution with mean = 20 and dispersion = 5
randomCounts <- rPT(n = 500, mu = 20, D = 5, a = 0.5)

xx <- compareCountDist(randomCounts, plot=FALSE)
xx
```

---

dPT

*The Poisson-Tweedie family of distributions*


---

**Description**

Density function and random generation for the Poisson-Tweedie family of distributions.

**Usage**

```
dPT(x, mu, D, a, tol = 1e-15)
rPT(n, mu, D, a, max = 10*sqrt(mu*D), tol = 1e-4)
```

**Arguments**

x	an object of class 'mlePT' or a non-negative vector containing the integers in which the distribution should be evaluated.
mu	numeric positive scalar giving the mean of the distribution.
D	numeric positive scalar giving the dispersion of the distribution.
a	numeric scalar smaller than 1 giving the shape parameter of the distribution.
tol	numeric scalar giving the tolerance.
n	integer scalar giving number of random values to return.
max	numeric scalar containing the maximum number of counts to be used in the sampling process.

**Value**

If 'x' is of class 'mlePT', 'dPT' will return the Poisson-Tweedie distribution with parameters equal to the ones estimated by 'mlePoissonTweedie' evaluated on the data that was used to estimate the parameters. If 'x' is a numeric vector, 'dPT' will return the density of the specified Poisson-Tweedie distribution evaluated on 'x'.

'rPT' generates random deviates.

**References**

Esnaola M, Puig P, Gonzalez D, Castelo R and Gonzalez JR (2013). A flexible count data model to fit the wide diversity of expression profiles arising from extensively replicated RNA-seq experiments. *BMC Bioinformatics* 14: 254

A.H. El-Shaarawi, R. Zhu, H. Joe (2010). Modelling species abundance using the Poisson-Tweedie family. *Environmetrics* 22, pages 152-164.

P. Hougaard, M.L. Ting Lee, and G.A. Whitmore (1997). Analysis of overdispersed count data by mixtures of poisson variables and poisson processes. *Biometrics* 53, pages 1225-1238.

**See Also**

[compareCountDist](#) [testShapePT](#)

**Examples**

```
# To compute the density function in 1:100 of the Polya-Aeppli
# distribution with mean = 20 and dispersion = 5
dPT(x = 1:100, mu = 20, D = 5, a = -1)

# To generate 100 random counts of the same distribution with same
# parameters
rPT(n = 100, mu = 20, D = 5, a = -1)
```

---

filterCounts	<i>Count data filtering</i>
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---

## Description

Filter count data to remove lowly expressed genes.

## Usage

```
filterCounts(counts,  
             cpm.cutoff=0.5,  
             n.samples.cutoff=2,  
             mean.cpm.cutoff=0,  
             lib.sizes=NULL)
```

## Arguments

counts	numeric data.frame or matrix containing the count data.
cpm.cutoff	expression level cutoff defined as the minimum number of counts per million. By default this is set to 0.5 counts per million.
n.samples.cutoff	minimum number of samples where a gene should meet the counts per million cutoff (cpm.cutoff) in order to be kept as part of the count data matrix. When n.samples.cutoff is a number between 0 and 1, then it is interpreted as the fraction of samples that should meet the counts per million cutoff (cpm.cutoff).
mean.cpm.cutoff	minimum mean of counts per million cutoff that a gene should meet in order to be kept. When the value of this argument is larger than 0 then it overrules the other arguments cpm.cutoff and n.samples.cutoff.
lib.sizes	vector of the total number of reads to be considered per sample/library. If lib.sizes=NULL (default) then these quantities are estimated as the column sums in the input matrix of counts.

## Details

This function removes genes with very low expression level defined in terms of a minimum number of counts per million occurring in a minimum number of samples. Such a policy was described by Davis McCarthy in a message at the bioc-sig-sequencing mailing list. By default, this function keeps genes that are expressed at a level of 0.5 counts per million or greater in at least two samples. Alternatively, one can use the mean.cpm.cutoff to set a minimum mean expression level through all the samples.

## Value

A matrix of filtered genes.

**Author(s)**

J.R. Gonzalez and R. Castelo

**References**

Davis McCarthy, <https://stat.ethz.ch/pipermail/bioc-sig-sequencing/2011-June/002072.html>.

**See Also**

[normalizeCounts](#)

**Examples**

```
# Generate a random matrix of counts
counts <- matrix(rPT(n=1000, a=0.5, mu=10, D=5), ncol = 40)

dim(counts)

# Filter genes with requiring the minimum expression level on every sample
filteredCounts <- filterCounts(counts, n.samples.cutoff=dim(counts)[2])

dim(filteredCounts)
```

---

glmPT

*Fit Poisson-Tweedie generalized linear model.*

---

**Description**

'glmPT' is used to fit generalized linear models for the Poisson-Tweedie family of distributions.

**Usage**

```
tweedEglm(formula, counts, data, mc.cores = 1, a = NULL, offset = NULL, ...)
glmPT(formula, data, offset = NULL, a = NULL, ...)
```

**Arguments**

formula	an object of class 'formula': a symbolic description of the model to be fitted.
counts	Matrix or data.frame of counts for the 'tweedEglm'.
data	an optional data frame, list or environment containing the variables in the model. If not found in 'data', the variables are taken from 'environment(formula)', typically the environment from which 'glm' is called.
mc.cores	number of cpu cores to be used. This option is only available when the 'multi-core' package is installed and loaded first. In such a case, if the default value of 'mc.cores=1' is not changed, all available cores will be used.

offset	this can be used to specify an <code>_a priori_</code> known component to be included in the linear predictor during fitting.
a	numeric vector (for <code>'tweedEglm'</code> ) or numeric scalar (for <code>'glmPT'</code> ) smaller than 1. If specified the PT shape parameter will be fixed. For <code>'tweedEglm'</code> , if the provided <code>'a'</code> is a scalar this value will be used for all rows of <code>'counts'</code> (genes).
...	additional arguments to be passed to the <code>'optim'</code> <code>'control'</code> options.

**Value**

An object of class `'glmPT'` containing the following information:

call	the matched call.
contrasts	(where relevant) the contrasts used.
convergence	A character string giving any additional information returned by the optimizer, or <code>'NULL'</code> .
counts	A two-element integer vector giving the number of calls to <code>'fn'</code> and <code>'gr'</code> respectively. This excludes those calls needed to compute the Hessian, if requested, and any calls to <code>'fn'</code> to compute a finite-difference approximation to the gradient.
df	Number of estimated parameters.
fitted.values	The fitted mean values, obtained by transforming the linear predictors by the inverse of the link function.
hessian	A symmetric matrix giving an estimate of the Hessian at the solution found.
message	A character string giving any additional information returned by the optimizer, or <code>'NULL'</code> .
ncov	An integer giving the number of variables in the model.
par	A vector giving the estimated parameters.
residuals	The residuals in the final iteration of the IWLS fit.
se	A vector giving the standard error of the estimated parameters.
value	Value of the log-likelihood of the model in the last iteration.
...	Further arguments to be passed to the <code>'glm.fit'</code> function.

**Author(s)**

Mikel Esnaola <mesnaola@creal.cat>

**Examples**

```
counts <- matrix(rPT(n = 1000, a = 0.5, mu = 10, D = 5), ncol = 40)
g <- factor(rep(c(0,1), 20))
mod1 <- glmPT(counts[1,]~g)
mod1
summary(mod1)
anova(mod1)

mod2 <- tweedEglm(~ g, counts)
mod2
```

---

`gofTest`*Test the goodness of fit of every row in a matrix of counts*

---

**Description**

Function to test the goodness of fit of every row in a matrix of counts

**Usage**

```
gofTest(counts, a = 0, mc.cores = 1)
```

**Arguments**

<code>counts</code>	matrix of counts
<code>a</code>	numeric scalar smaller than 1. The function will test whether the shape parameter is equal to the introduced 'a' (default is 0).
<code>mc.cores</code>	number of cpu cores to be used. This option is only available when the 'multi-core' package is installed and loaded first. In such a case, if the default value of <code>mc.cores=1</code> is not changed, all available cores will be used.

**Details**

By default `a = 0`, and therefore the function tests for every row of the input matrix of counts whether the count data follows a Negative-Binomial distribution. In this case, a Likelihood Ratio Test is performed. When the given value for 'a' is different from 0, a Wald test is performed. This function calls [testShapePT](#).

**Value**

a vector of statistics that follows a  $\chi^2$  distribution with one degree of freedom under the null hypothesis.

**References**

Esnaola M, Puig P, Gonzalez D, Castelo R and Gonzalez JR (2013). A flexible count data model to fit the wide diversity of expression profiles arising from extensively replicated RNA-seq experiments. *BMC Bioinformatics* 14: 254

A.H. El-Shaarawi, R. Zhu, H. Joe (2010). Modelling species abundance using the Poisson-Tweedie family. *Environmetrics* 22, pages 152-164.

P. Hougaard, M.L. Ting Lee, and G.A. Whitmore (1997). Analysis of overdispersed count data by mixtures of poisson variables and poisson processes. *Biometrics* 53, pages 1225-1238.

**See Also**

[testShapePT](#)



**Examples**

```
## Generate a random matrix of counts
counts <- matrix(rPT(n=2000, a=0.5, mu=10, D=5), nrow=20)

## Perform the goodness-of-fit tests for every row in the matrix
chi2gof <- gofTest(counts)

## Calculate and sort the corresponding P-values for the
## null hypothesis that counts follow a negative binomial distribution
sort(pchisq(chi2gof, df=1, lower.tail=FALSE))
```

---

Methods for objects of class 'mlePT'

*Methods for objects of class 'mlePT'*

---

**Description**

print, extract loglikelihood or compute confidence interval for an object of class 'mlePT'.

**Usage**

```
## S3 method for class 'mlePT'
print(x, digits = 3, ...)
## S3 method for class 'mlePT'
logLik(object, ...)
## S3 method for class 'mlePT'
confint(object, parm, level = 0.95, ...)
```

**Arguments**

x	object of class 'mlePT'.
object	object of class 'mlePT'.
digits	integer scalar giving the number of digits to be rounded the solution.
parm	a specification of which parameters are to be given confidence intervals, either a vector of numbers or a vector of names. If missing, all parameters are considered.
level	the confidence level required (default is 0.95).
...	additional arguments.

**Value**

'logLik' returns the loglikelihood of the selected model.

'confint' returns a matrix (or vector) with columns giving lower and upper confidence limits for each parameter.

**See Also**

[mlePoissonTweedie](#)

**Examples**

```
# Load and aggregate the 'seizure' database
data(seizure)
aggCounts <- aggregate(x = cbind(seizure$count, seizure$trx), by =
  list(seizure$id), FUN = sum)

# Estimate the parameters
mleSeizure <- mlePoissonTweedie(x = aggCounts[,2], a.ini = 0, D.ini =
  10)

# Print
mleSeizure

# Extract loglikelihood
logLik(mleSeizure)

# Compute confidence interval
confint(mleSeizure)
```

---

mlePoissonTweedie	<i>Maximum likelihood estimation of the Poisson-Tweedie parameters</i>
-------------------	--

---

**Description**

Maximum likelihood estimation of the Poisson-Tweedie parameters using L-BFGS-B quasi-Newton method.

**Usage**

```
mlePoissonTweedie(x, a, D.ini, a.ini, maxit = 100, loglik=TRUE,
  maxCount=20000, w = NULL, ...)
getParam(object)
```

**Arguments**

x	numeric vector containing the read counts.
a	numeric scalar smaller than 1, if specified the PT shape parameter will be fixed.
D.ini	numeric positive scalar giving the initial value for the dispersion.
a.ini	numeric scalar smaller than 1 giving the initial value for the shape parameter (ignored if 'a' is specified).
maxit	numeric scalar providing the maximum number of 'L-BFGS-B' iterations to be performed (default is '100').
loglik	is log-likelihood computed? The default is TRUE

object	an object of class 'mlePT'.
maxCount	if $\max(x) > \text{maxCount}$ , then moment method is used to estimate model parameters to reduce computation time. The default is 20000.
w	vector of weights with length equal to the length of 'x'.
...	additional arguments to be passed to the 'optim' 'control' options.

### Details

The L-BFGS-B quasi-Newton method is used to calculate iteratively the maximum likelihood estimates of the three Poisson-Tweedie parameters. If 'a' argument is specified, this parameter will be fixed and the method will only estimate the other two.

### Value

An object of class 'mlePT' containing the following information:

par: numeric vector giving the estimated mean ('mu'), dispersion ('D') and shape parameter 'a'.

se: numeric vector containing the standard errors of the estimated parameters 'mu', 'D' and 'a'.

loglik: numeric scalar providing the value of the loglikelihood for the estimated parameters.

iter: numeric scalar giving the number of performed iterations.

paramZhu: numeric vector giving the values of the estimated parameters in the Zhu parameterization 'a', 'b' and 'c'.

paramHou: numeric vector giving the values of the estimated parameters in the Hougaard parameterization 'alpha', 'delta' and 'theta'.

skewness: numeric scalar providing the estimate of the skewness given the estimated parameters.

x: numeric vector containing the count data introduced as the 'x' argument by the user.

convergence: A character string giving any additional information returned by the optimizer, or 'NULL'.

### References

Esnaola M, Puig P, Gonzalez D, Castelo R and Gonzalez JR (2013). A flexible count data model to fit the wide diversity of expression profiles arising from extensively replicated RNA-seq experiments. BMC Bioinformatics 14: 254

A.H. El-Shaarawi, R. Zhu, H. Joe (2010). Modelling species abundance using the Poisson-Tweedie family. Environmetrics 22, pages 152-164.

P. Hougaard, M.L. Ting Lee, and G.A. Whitmore (1997). Analysis of overdispersed count data by mixtures of poisson variables and poisson processes. Biometrics 53, pages 1225-1238.

### See Also

[testShapePT](#) [print.mlePT](#)

## Examples

```
# Generate 500 random counts following a Poisson Inverse Gaussian
# distribution with mean = 20 and dispersion = 5
randomCounts <- rPT(n = 500, mu = 20, D = 5, a = 0.5)

# Estimate all three parameters
res1 <- mlePoissonTweedie(x = randomCounts, a.ini = 0, D.ini
= 10)
res1
getParam(res1)

#Fix 'a = 0.5' and estimate the other two parameters
res2 <- mlePoissonTweedie(x = randomCounts, a = 0.5, D.ini
= 10)
res2
getParam(res2)
```

---

normalizeCounts	<i>Count data normalization</i>
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---

## Description

Normalize count data to remove systematic technical effects.

## Usage

```
normalizeCounts(counts, group=rep.int(1,ncol(counts)), method=c("TMM", "cqn"),
common.disp = FALSE, prior.df=8, annot=NULL, lib.sizes=NULL, verbose=TRUE)
```

## Arguments

counts	numeric data.frame or matrix containing the count data.
group	vector giving the experimental group/condition for each sample/library. This argument is only relevant when method="cqn".
method	specific method to use in order to normalize the input matrix of counts. By default this is set to TMM (Robinson and Oshlack, 2010) using the implementation available in the edgeR package. The other option is cqn (Hansen, Irizarry and Wu, 2012).
common.disp	logical indicating whether a common or tagwise (default) dispersions should be estimated and employed when adjusting counts. This argument is only relevant when method="TMM".
prior.df	argument provided to the call of <a href="#">estimateTagwiseDisp</a> which defines the prior degrees of freedom. It is used in calculating 'prior.n' which, in turn, defines the amount of shrinkage of the estimated tagwise dispersions to the common one. By default prior.df=8 thus assuming no shrinkage toward that common dispersion. This argument is not used if common.disp=TRUE. This argument is only relevant when method="TMM".

annot	matrix or data frame with row names matching at least part of the row names in the counts input matrix, containing feature/tag/gene lengths in bp on its first column, and a second covariate, such as G+C content, on its second column. These two pieces of information are provided to arguments lengths and x when calling <code>cqn</code> . This argument is only relevant when <code>method="TMM"</code> .
lib.sizes	vector of the total number of reads to be considered per sample/library. If <code>lib.sizes=NULL</code> (default) then these quantities are estimated as the column sums in the input matrix of counts.
verbose	logical indicating whether progress should be reported.

### Details

This function encapsulates calls to RNA-seq normalization procedures available in the [edgeR](#) and [cqn](#) packages in order to try to remove systematic technical effects from raw counts. By default, the TMM method described in Robinson and Oshlack (2010) is employed to calculate normalization factors which are applied to estimate effective library sizes, then common and tagwise (only when the argument `common.disp=TRUE`) dispersions are calculated (Robinson and Smyth, Bioinformatics 2007) and finally counts are adjusted so that library sizes are approximately equal for the given dispersion values (Robinson and Smyth, Biostatistics 2008). Setting the argument `method="cqn"`, conditional quantile normalization (Hansen, Irizarry and Wu, 2012) is applied which aims at adjusting for tag/feature/gene length and other covariate such as G+C content. This information should be provided through the `annot` argument. This procedure calculates, for every gene and every sample, an offset to apply to the log<sub>2</sub> reads per million (RPM) and the function `normalizeCounts()` adds this offset to the the log<sub>2</sub> RPM values calculated from the input count data matrix, unlogs them and rolls back these normalized RPM values into integer counts. Details on these two normalization procedures are given in the documentation of the `edgeR` and `cqn` Bioconductor packages.

### Value

A matrix of normalized counts.

### Author(s)

J.R. Gonzalez and R. Castelo

### References

- K.D. Hansen, R.A. Irizarry and Z. Wu. Removing technical variability in RNA-seq data using conditional quantile normalization. *Biostatistics*, 2012.
- M.D. Robinson and A. Oshlack. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol*, 11:R25, 2010.
- Robinson MD and Smyth GK (2007). Moderated statistical tests for assessing differences in tag abundance. *Bioinformatics* 23, 2881-2887
- Robinson MD and Smyth GK (2008). Small-sample estimation of negative binomial dispersion, with applications to SAGE data. *Biostatistics*, 9, 321-332

### See Also

[filterCounts](#)

**Examples**

```
# Generate a random matrix of counts
counts <- matrix(rPT(n=1000, a=0.5, mu=10, D=5), ncol = 40)

colSums(counts)
counts[1:5, 1:5]

# Normalize counts
normCounts <- normalizeCounts(counts, rep(c(1,2), 20))

colSums(normCounts)
normCounts[1:5, 1:5]
```

---

qqchisq

*Chi-square quantile-quantile plot*


---

**Description**

Make a chi-square quantile-quantile plot.

**Usage**

```
qqchisq(stat, df=1, normal=FALSE, rangeExpected=FALSE,
obsQuantiles=c(0.50, 0.75, 0.95), ylim = NULL, ...)
```

**Arguments**

stat	vector of $\chi^2$ statistics.
df	degrees of freedom of stat.
normal	logical; set to TRUE if the $\chi^2$ statistics in stat should be transform into normal z-scores in order to improve the display of lower quantiles. For this purpose, this function uses the <a href="#">zscoreGamma</a> function from the limma package. Default is set to FALSE.
rangeExpected	logical; set to TRUE if the displayed range of the observed $\chi^2$ statistics is restricted to the range of their expected values. Default is set to FALSE.
obsQuantiles	observed quantiles to indicate by horizontal dash lines. By default, these are set to 50%, 75% and 95%.
ylim	they y limits of the plot. If 'NULL' (default), these will be obtained from the data.
...	further arguments to pass to the <a href="#">plot</a> function.

**Details**

The main purpose of this function in the tweedEseq package is to provide means to assess the goodness of fit of count data to the negative binomial distribution. The main input argument stats should be the output of [gofTest](#).

**Value**

it returns invisibly a list with two components x and y corresponding to the coordinates of the plotted statistics.

**References**

Esnaola M, Puig P, Gonzalez D, Castelo R and Gonzalez JR (2013). A flexible count data model to fit the wide diversity of expression profiles arising from extensively replicated RNA-seq experiments. BMC Bioinformatics 14: 254

**See Also**

[compareCountDist](#) [testShapePT](#)

**Examples**

```
## Generate a random matrix of counts
counts <- matrix(rPT(n=2000, a=0.5, mu=10, D=5), nrow=20)

## Perform the goodness-of-fit tests for every row in the matrix
chi2gof <- gofTest(counts)

qqchisq(chi2gof)
```

---

seizure

*Epileptic seizure counts*

---

**Description**

Data on seizure counts for 59 epileptics.

**Usage**

```
data(seizure)
```

**Format**

A data frame with 236 observations on the following 6 variables.

`id` a numeric vector, identification number for each patient

`count` a numeric vector, seizure counts

`visit` a numeric vector, visit number

`trx` a numeric vector, treatment: progabide (1) or placebo (0)

`baseline` a numeric vector, baseline 8 week seizure count

`age` a numeric vector, age of patient

**Details**

The data are from a placebo-controlled clinical trial of 59 epileptics. Patients with partial seizures were enrolled in a randomized clinical trial of the anti-epileptic drug, progabide. Participants in the study were randomized to either progabide or a placebo, as an adjuvant to the standard anti-epileptic chemotherapy. Progabide is an anti-epileptic drug whose primary mechanism of action is to enhance gamma-aminobutyric acid (GABA) content; GABA is the primary inhibitory neurotransmitter in the brain. Prior to receiving treatment, baseline data on the number of epileptic seizures during the preceding 8-week interval were recorded. Counts of epileptic seizures during 2-week intervals before each of four successive post-randomization clinic visits were recorded.

**Value**

void

**Source**

P.F Thall, and S.C. Vail (1990). Some covariance models for longitudinal count data with overdispersion. *Biometrics*, 46, 657-671,

**References**

P. Hougaard, M.L. Ting Lee, and G.A. Whitmore (1997): Analysis of overdispersed count data by mixtures of poisson variables and poisson processes. *Biometrics* 53, pages 1225-1238.

**See Also**

[testPoissonTweedie](#) [mlePoissonTweedie](#)

**Examples**

```
# Although this is not a differential expression dataset, it is appropriate
# to illustrate the application of the Poisson-Tweedie in
# epidemiological studies

data(seizure)
summary(seizure)

# Aggregate
aggCounts <- aggregate(x = cbind(seizure$count, seizure$trx), by =
list(seizure$id), FUN = sum)

# Estimation of the three parameters for all individuals
mleSeizure <- mlePoissonTweedie(x = aggCounts[,2], a.ini = 0, D.ini = 10)
mleSeizure

#Poisson-Tweedie test
testPoissonTweedie(x = aggCounts[,2], group = aggCounts[,3])
```



---

testShapePT	<i>Test shape parameter of PT</i>
-------------	-----------------------------------

---

**Description**

Function to test whether the shape parameter is equal to a given value.

**Usage**

```
testShapePT(x, a = 0)
```

**Arguments**

x	object of class 'mlePT'.
a	numeric scalar smaller than 1. The function will test whether the shape parameter is equal to the introduced 'a' (default is 0).

**Details**

By default  $a = 0$ , and therefore the function tests whether the count data follows a Negative-Binomial distribution or not. In this case, a Likelihood Ratio Test is performed. When the given value for 'a' is different from 0, a Wald test is performed.

If  $a = 1$ , the function tests whether the count data follows a Poisson distribution or not.

If  $a = 0.5$ , the function tests whether the count data follows a Poisson-inverse Gaussian distribution or not.

If  $a = -1$ , the function tests whether the count data follows a Polya-Aeppli distribution or not.

**Value**

numeric p-value of the test.

**References**

Esnaola M, Puig P, Gonzalez D, Castelo R and Gonzalez JR (2013). A flexible count data model to fit the wide diversity of expression profiles arising from extensively replicated RNA-seq experiments. BMC Bioinformatics 14: 254

A.H. El-Shaarawi, R. Zhu, H. Joe (2010). Modelling species abundance using the Poisson-Tweedie family. Environmetrics 22, pages 152-164.

P. Hougaard, M.L. Ting Lee, and G.A. Whitmore (1997). Analysis of overdispersed count data by mixtures of poisson variables and poisson processes. Biometrics 53, pages 1225-1238.

**See Also**

[gofTest mlePoissonTweedie compareCountDist](#)

**Examples**

```
# Generate a random matrix of counts
counts <- rPT(n=1000, a=0.5, mu=10, D=5)

# Maximum likelihood estimation of the Poisson-Tweedie parameters
mleEstimate <- mlePoissonTweedie(x = counts, a.ini = 0, D.ini
= 10)

# Test whether data comes from Negative-Binomial distribution
testShapePT(mleEstimate)

# Test whether data comes from Poisson-inverse Gaussian
testShapePT(mleEstimate, a = 0.5)
```

---

tweeDE

*Score test for differences between two Poisson-Tweedie groups*


---

**Description**

Carry out a score test for differences between two Poisson-Tweedie groups.

**Usage**

```
tweeDE(object, group, mc.cores = 1, pair = NULL, a = NULL, ...)
testPoissonTweedie(x, group, saveModel = FALSE, a = NULL, log = FALSE, ...)
MAplot(x, ...)
Vplot(x, ...)
## S3 method for class 'tweeDE'
print(x, n=6L, sort.by="pval",
      log2fc.cutoff=0,
      pval.adjust.cutoff=1,
      print=TRUE, ...)
## S3 method for class 'tweeDE'
MAplot(x, log2fc.cutoff=0, highlight=NULL, ...)
## S3 method for class 'tweeDE'
Vplot(x, log2fc.cutoff=0, pval.adjust.cutoff=1, highlight=NULL,
      ylab=expression(paste(-log[10], " Raw P-value")), ...)
```

**Arguments**

object	a data.frame or a matrix of RNA-seq counts.
group	vector giving the experimental group/condition for each sample/library.
mc.cores	number of cpu cores to be used. This option is only available when the 'multi-core' package is installed and loaded first. In such a case, if the default value of mc.cores=1 is not changed, all available cores will be used.
pair	vector of two elements containing the representants of each of the two groups (default is 'NULL').

<code>a</code>	for <code>'tweeDE'</code> function, numerical vector with values strictly less than 1. It allows to fix the shape parameter <code>'a'</code> during differential expression tests for each of the genes (rows of <code>'object'</code> ). for <code>'testPoissonTweedie'</code> function, numeral value strictly less than 1 which fixes the shape parameter <code>'a'</code> for the test.
<code>n</code>	maximum number of genes printed.
<code>sort.by</code>	character string, indicating whether genes should be ranked by their P-value ( <code>pval</code> ), which is the default setting, or by absolute log <sub>2</sub> fold-change ( <code>log2fc</code> ).
<code>log2fc.cutoff</code>	cutoff on the minimum value of the log <sub>2</sub> fold change.
<code>pval.adjust.cutoff</code>	cutoff on the maximum adjusted P-value (FDR).
<code>print</code>	logical; it indicates whether the output should be printed on the terminal.
<code>highlight</code>	list of arguments to the <code>points()</code> plotting function in order to highlight genes in the MA or volcano plots. A component called <code>genes</code> is expected to have the identifiers of the genes to be highlighted.
<code>ylab</code>	label on the y-axis of the volcano plot set by default to <code>-log10</code> of the raw P-value which is what this plot displays on that axis.
<code>x</code>	object returned by the function <code>tweeDE</code> in the case of <code>print</code> and vector of count data in the case of <code>testPoissonTweedie</code> .
<code>saveModel</code>	logical indicating whether the results of fitting the model should be saved or not (default is <code>'FALSE'</code> ).
<code>log</code>	logical (default is <code>FALSE</code> ). If <code>FALSE</code> , the tested Null Hypothesis states that the difference between the means is 0 while, if <code>TRUE</code> , it states that the quotient between the logarithm of means is equal to 1. For this last case, the standard error is computed using the Delta Method.
<code>...</code>	additional arguments.

## Details

`'testPoissonTweedie'` performs the test for a vector of counts.

`'tweeDE'` performs the test for a whole `'data.frame'`. The P-values are then corrected using the Benjamini and Hochberg method.

## Value

`'testPoissonTweedie'` returns a list with:

`'mean'`: means for each group `'pvalue'`: p-value for the test

`'tweeDE'` returns a `'data.frame'` with columns

`'overallMean'`: overall mean counts `'meanA'`: mean counts of the first group `'meanB'`: mean counts of the second group `'log2fc'`: logarithm (base 2) of the fold-change (second group vs. first group) `'pval'`: p-value for the test `'pval.adjust'`: adjusted p-value using Benjamini-Hochberg method

**References**

Esnaola M, Puig P, Gonzalez D, Castelo R and Gonzalez JR (2013). A flexible count data model to fit the wide diversity of expression profiles arising from extensively replicated RNA-seq experiments. *BMC Bioinformatics* 14: 254

A.H. El-Shaarawi, R. Zhu, H. Joe (2010). Modelling species abundance using the Poisson-Tweedie family. *Environmetrics* 22, pages 152-164.

P. Hougaard, M.L. Ting Lee, and G.A. Whitmore (1997). Analysis of overdispersed count data by mixtures of poisson variables and poisson processes. *Biometrics* 53, pages 1225-1238.

**See Also**

[normalizeCounts](#) [mlePoissonTweedie](#)

**Examples**

```
# Generate a random matrix of counts
counts <- matrix(rPT(n = 1000, a = 0.5, mu = 10, D = 5), ncol = 40)

# Test for differences between the two groups
tweeDE(counts, group = rep(c(1,2),20))
```

---

tweeDEseq-internal      *Internal 'tweeDEseq' functions*

---

**Description**

Internal tweeDEseq functions

**Usage**

```
loglikPoissonTweedie(p, x, mu, verbose = FALSE, tol = 1e-15, probs =
FALSE, w = NULL)
loglikPoissonTweedie2(p, a, x, mu, verbose = FALSE, tol = 1e-15, probs =
FALSE, w = NULL)
momentEstimates(x, w)
shapeTrend(x)
loglikGlmPT(par, X, Y, offset = NULL, allFactors=FALSE, a=NULL,
tol=1e-300, maxCount, verbose = FALSE)
glmPT.fit(X, Y, offset=NULL, allFactors=FALSE, a = NULL, maxCount =
2000, ...)
```

**Details**

These are not to be called by the user

**Value**

void

**Examples**

```
message("These are not to be called by the user")
```

---

tweeDExact

*Exact test for differences between two Poisson-Tweedie groups*


---

**Description**

Carry out an exact test for differences between two Poisson-Tweedie populations.

**Usage**

```
tweeDExact(counts, group, tol = 1e-15, mc.cores = 1)
exactTestPT(counts, group, tol = 1e-15, threshold = 150e3)
```

**Arguments**

counts	The RNA-seq counts. An object of type 'matrix' or 'data.frame' for 'tweeDExact', or an object of type 'vector' for 'exactTest'.
group	vector giving the experimental group/condition for each sample/library.
tol	Tolerance for the Poisson-Tweedie probability computations. The probabilities under the 'tol' value will automatically considered as 0.
threshold	an integer (default is 50e3). If the sum of all counts in a certain gene exceeds this value 'testPoissonTweedie' will be called instead of 'exactTest'. Larger values will result in a longer computing time.
mc.cores	number of cpu cores to be used. This option is only available when the 'multicore' package is installed and loaded first. In such a case, if the default value of 'mc.cores=1' is not changed, all available cores will be used.

**Details**

'exactTest' performs the exact test for a vector of counts.

'tweeDExact' performs the test for a whole 'data.frame'. The P-values are then corrected using the Benjamini and Hochberg method.

**Value**

'exactTest' returns the p-value resulting from the exact test between two different Poisson-Tweedie populations, as well as the method that was used to compute it.

'tweeDExact' returns a 'data.frame'. Each row corresponds to a gene and it contains the following information:

- In the first columns the mean of counts in each of the subgroups.
- In the third column the p-value of the test for differential expression between the two subgroups.
- In the fourth column the p-value corrected for multiple comparisons using the Benjamini-Hochberg FDR procedure.
- In the last (fifth) column the method that was used to compute the p-value.

**Author(s)**

Mikel Esnaola

**References**

P. Hougaard, M.L. Ting Lee, and G.A. Whitmore (1997). Analysis of overdispersed count data by mixtures of poisson variables and poisson processes. *Biometrics* 53, pages 1225-1238.

**See Also**

[testPoissonTweedie](#) [tweedExact](#)

**Examples**

```
counts <- matrix(rPT(n = 1000, a = 0.5, mu = 10, D = 5), ncol = 40)

tweedExact(counts, group = rep(c(1,2),20))
```

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