

DESeq

October 25, 2011

CountDataSet-class *Class "CountDataSet" - a container for count data from HTS experiments*

Description

This is the main class for the present package.

Objects from the Class

Objects should be created with calls to `newCountDataSet` (q.v.).

Extends

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

Note

Note: This is a summary for reference. For an explanation of the actual usage, see the vignette.

A `CountDataSet` object stores counts from an HTS data set and offers further slots which are populated during the analysis.

After creation with `newCountDataSet`, a `CountDataSet` typically contains a count table, i.e., a matrix of integer data, that is accessible with the accessor function `counts`. Each row of the matrix corresponds to a gene (or binding region, or the like), and each column to an experimental sample. The experimental conditions of the samples are stored in a factor (with one element for each row of the counts matrix), which can be read with the accessor function `conditions`.

In the following analysis steps, further data slots are populated. First, the size factors can be estimated with `estimateSizeFactors`, which are afterwards accessible via `sizeFactors`. Then, the variance functions are estimated with `estimateVarianceFunctions`. The resulting estimates are accessible via the function `rawVarFunc`.

Internally, the mentioned data is stored in slots as follows:

As `CountDataSet` is derived from `eSet`, it has a `phenoData` slot which allows to store sample annotation. This is used to store the factor with the conditions, as a data frame column named `condition`, and to store the size factors, as a numeric data frame column named `sizeFactor`. The user may add further columns to the `phenoData` `AnnotatedDataFrame`.

The counts table is stored in the `eSet`'s `assayData` locked environment with the name `counts`.

The fits calculated by `estimateVarianceFunctions` are stored as closures in an environment, which is part of the object as slot `rawVarFuncs`. The assignment of the closures' names in the environment to the condition names is stored in as the first column (named "funcName") of the data frame in the slot `rawVarFuncTable`. The second column ("varAdjFactor") contains the variance adjustment factors. For further details on these two slots, see `estimateVarianceFunctions` and `varAdjFactors`.

Examples

```
# See the vignette
```

```
adjustScvForBias Adjust an SCV value for the bias arising when it is calculated from
```

Description

Assume that a small sample of i.i.d. random variables from a negative binomial distribution is given, and you have obtained unbiased estimates of mean and raw variance. Then, a new bias is introduced when the squared coefficient of variation (SCV) is calculated from these unbiased estimates by dividing the raw variance by the square of the mean. This bias can be calculated by numerical simulation and a pre-calculated adjustment table (or rather a fit through tabulated values) is supplied with the package. The present function uses this to remove the bias from a raw SCV estimate.

This function is used internally in `nbinomTest`. You will rarely need to call it directly.

Usage

```
adjustScvForBias(scv, nsamples)
```

Arguments

<code>scv</code>	An estimate for the raw squared coefficient of variation (SCV) for negative binomially distributed data, which has been obtained by dividing an unbiased estimate of the raw variance by the square of an unbiased estimate of the mean.
<code>nsamples</code>	The size of the sample used in the estimation.

Value

an unbiased estimate of the raw SCV

Author(s)

Simon Anders

Examples

```
true_mean <- 100
true_scv <- .1
nsamples <- 3
res <- replicate( 1000, {
  mySample <- rnbinom( nsamples, mu=true_mean, size=1/true_scv )
  mu_est <- mean( mySample )
})
```

```

raw_var_est <- var( mySample ) - mean( mySample )
raw_scv_est <- raw_var_est / mu_est^2
unbiased_raw_scv_est <- adjustScvForBias( raw_scv_est, 4 )
c( raw_scv_est = raw_scv_est, unbiased_raw_scv_est = unbiased_raw_scv_est ) } )
rowMeans( res )

```

conditions

Accessor function for the conditions information in a CountDataSet

Description

The conditions vector is a factor that assigns to each column of the count data a condition (or treatment, or phenotype, or the like). This information is stored in the CountDataSet's "phenoData" slot as a row named "condition".

Usage

```
conditions(cds)
```

Arguments

cds a CountDataSet

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```

cds <- makeExampleCountDataSet()
conditions( cds )

```

counts

Accessor for the 'counts' slot of a CountDataSet object.

Description

The counts slot holds the count data as a matrix of non-negative integer count values, one row for each observational unit (gene or the like), and one column for each sample.

Usage

```
counts(cds)
```

Arguments

cds a CountDataSet object

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```
cds <- makeExampleCountDataSet()  
head( counts( cds ) )
```

```
estimateSizeFactors
```

Estimate the size factors for a CountDataSet

Description

Estimate the size factors for a CountDataSet

Usage

```
estimateSizeFactors( cds, locfunc=median )
```

Arguments

<code>cds</code>	a CountDataSet
<code>locfunc</code>	a function to compute a location for a sample. By default, the median is used. However, especially for low counts, the shorth may give better results.

Details

You need to call this function right after [newCountDataSet](#) unless you have manually specified size factors.

Typically, the function is called with the idiom

```
cds <- estimateSizeFactors( cds )
```

This estimates the size factors and stores the information in the object.

Internally, the function calls [estimateSizeFactorsForMatrix](#). See there for more details on the calculation.

Value

The CountDataSet passed as parameters, with the size factors filled in.

Author(s)

Simon Anders, sanders@fs.tum.de

See Also

[estimateSizeFactorsForMatrix](#)

Examples

```
cds <- makeExampleCountDataSet()  
cds <- estimateSizeFactors( cds )  
sizeFactors( cds )
```

`estimateSizeFactorsForMatrix`*Low-level function to estimate size factors with robust regression.*

Description

Given a matrix or data frame of count data, this function estimates the size factors as follows: Each column is divided by the geometric means of the rows. The median (or, if requested, another location estimator) of these ratios (skipping the genes with a geometric mean of zero) is used as the size factor for this column.

Typically, you will not call this function directly, but use [estimateSizeFactors](#).

Usage

```
estimateSizeFactorsForMatrix( counts, locfunc=median)
```

Arguments

<code>counts</code>	a matrix or data frame of counts, i.e., non-negative integer values
<code>locfunc</code>	a function to compute a location for a sample. By default, the median is used. However, especially for low counts, the shorth may give better results.

Value

a vector with the estimates size factors, one element per column

Author(s)

Simon Anders, sanders@fs.tum.de

See Also

[estimateSizeFactors](#)

Examples

```
cds <- makeExampleCountDataSet()
estimateSizeFactorsForMatrix( counts(cds) )
```

`estimateVarianceFunctionForMatrix`*Lower-level functions to estimate a raw variance function.*

Description

Usually, you should call [estimateVarianceFunctions](#) for a `CountDataSet` instead of calling this function directly. However, if you do not have your data in the form of a `CountDataSet` object, you may want to call this function directly.

Usage

```
estimateVarianceFunctionForMatrix(counts, sizeFactors,
  locfit_extra_args = list(), lp_extra_args = list())

estimatePooledVarianceFunctionForMatrix(counts, sizeFactors,
  conditions, locfit_extra_args = list(), lp_extra_args = list())
```

Arguments

`counts` a matrix of data frame of count data. All the columns of this matrix will be considered as replicates of the same condition.

`sizeFactors` the size factors of the columns, as estimated e.g. with [estimateSizeFactorsForMatrix](#)

`conditions` `estimateVarianceFunctionForMatrix` assumes that all the columns of 'counts' are replicates. If you wish to perform a pooled variance estimation ("pooled" in the sense as, e.g., the variance estimation is done in Student's t test), use `estimatePooledVarianceFunctionForMatrix` and pass it a factor 'conditions' indicating which samples are replicates.

`locfit_extra_args, lp_extra_args` Options to be passed to the `locfit` and to the `lp` function of the `locfit` package. Use this to adjust the local fitting. For example, you may pass a value for `nn` different from the default (0.7) if the fit seems too smooth or too rough by setting `lp_extra_agrs=list(nn=0.9)` or you can set `locfit_extra_args=list(maxk=200)` if you get the error that `locfit` ran out of nodes. See the documentation of the `locfit` package for details. Usually, you will not need to adjust the fitting parameters, as the defaults seem to work quite fine.

Value

a raw variance function. This is a function that, given a base mean (i.e., the mean of counts divided by size factors), returns a raw variance estimate (which needs to be multiplied with the square of a size factor to get the biological variance on the count scale; to get the full variance, add the shot noise as well). For compatibility with [rawVarFunc](#), the function comes with an attribute, named `varAdjFactor`, which is always 1.

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors(cds)
vf <- estimateVarianceFunctionForMatrix(counts(cds), sizeFactors(cds))
vf( head( counts(cds)[,1] / sizeFactors(cds)[1] ) )
attr( "varAdjFactor" )
```

```
estimateVarianceFunctions
```

Estimate the variance functions for a CountDataSet.

Description

This function calls, for each condition that has replicates, the lower-level function `estimateVarianceFunctionForCondition` to estimate the raw variance function for this condition.

Usage

```
estimateVarianceFunctions(cds, method = c("normal", "blind", "pooled"),
  pool = NULL, locfit_extra_args = list(), lp_extra_args = list(),
  modelFrame = NULL)
```

Arguments

<code>cds</code>	a <code>CountDataSet</code> with size factors
<code>method</code>	There are three ways how the variance functions can be estimated: <ul style="list-style-type: none"> • <code>normal</code> For each condition with replicates, estimate a variance function by considering the data from samples for this condition. Then, construct a variance function <code>'_max'</code> that takes the maximum over all other variance functions and assign this one to all samples of unreplicated conditions. • <code>blind</code> Ignore the sample labels and pretend that all samples are replicates of a single condition. This allows to get a variance estimate even if one does not have any biological replicates. However, this can lead to drastic loss of power; see the vignette for details. The single estimated variance condition is the called <code>"_blind"</code> and assigned to all samples. • <code>pooled</code> Use the samples from all conditions with replicates to estimate a single pooled variance function, to be called <code>"_pool"</code> and assign it to all samples.
<code>pool</code>	This argument is deprecated; do not use it. It is (for now) retained for compatibility with code written for DESeq versions 1.1.6 or earlier. Setting <code>'pool=FALSE'</code> is the same as <code>'method="normal"'</code> and setting <code>'pool=TRUE'</code> is the same as <code>'method="blind"'</code> (not <code>'method="pool"'</code>). Note that the deprecation also resolved the issue that calling the <code>'blind'</code> estimation a <code>'pooled'</code> was incorrect use of terminology.
<code>locfit_extra_args, lp_extra_args</code>	Options to be passed to the <code>locfit</code> and to the <code>lp</code> function of the <code>locfit</code> package. Use this to adjust the local fitting. For example, you may pass a value for <code>nn</code> different from the default (0.7) if the fit seems too smooth or too rough by setting <code>lp_extra_args=list(nn=0.9)</code> or you can set <code>locfit_extra_args=list(maxk=200)</code> if you get the error that <code>locfit</code> ran out of nodes. See the documentation of the <code>locfit</code> package for details. Usually, you will not need to adjust the fitting parameters, as the defaults seem to work quite fine.
<code>modelFrame</code>	By default, the information in <code>conditions(cds)</code> or <code>pData(cds)</code> are used to determine, which samples are replicates (see <code>newCountDataSet</code>). Alternatively (and only for method <code>"pooled"</code>), a data frame can be passed here, and all rows that are identical in this data frame are considered replicated.

Details

Behaviour for `pooled=FALSE`: The estimated raw variance functions are placed in the environment `rawVarFuncs`, which is a slot in `CountDataSet`, using the condition labels as names. A further function, named `"_max"`, is placed there as well, which always return the maximum of all the other functions.

Then, the `rawVarFuncTable` (q.v.) is filled to assign to each replicated condition the raw variance function estimated for it, and to each condition without replicates, the `"_max"` function.

Behaviour for `pooled=TRUE`: A single raw variance function is estimated from all the count data, ignoring the condition labels. It is stored in the `rawVarFuncs` slot under the name `"_pooled"`. In the `rawVarFuncTable`, `"_pooled"` is assigned to all conditions.

In either case, all the variance adjustment factors (see `varAdjFactors`) are set to 1.

It is advisable to always call `residualsEcdfPlot` afterwards to verify the fit.

Value

The `CountDataSet` `cds`, with the slots `rawVarFuncs` and `rawVarFuncTable` updated.

Author(s)

Simon Anders, sanders@fs.tum.de

See Also

`scvPlot` to visualize the result and `varianceFitDiagnostics` and `residualsEcdfPlot` to check the fit.

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
vf <- rawVarFunc( cds, "A" )
vf( head( counts( cds )[,1] / sizeFactors( cds ) [1] ) )
```

`getBaseMeansAndVariances`

Perform row-wise estimates of base-level means and variances for count

Description

This function is called internally by a number of other functions. You will need to call it directly only in very special cases.

Usage

```
getBaseMeansAndVariances( counts, sizeFactors )
```


Arguments

<code>counts</code>	a matrix of data frame of count data. All the columns of this matrix will be considered as replicates of the same condition.
<code>sizeFactors</code>	the size factors of the columns, as estimated e.g. with estimateSizeFactorsForMatrix

Value

A data frame with one row for each row in 'counts' and two columns:

<code>baseMean</code>	The base mean for each row. This is the mean of the counts after they have been divided by the size factors
<code>comp2</code>	The base variance for each row. This is the variance of the counts after they have been divided by the size factors

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors(cds)
head( getBaseMeansAndVariances( counts(cds), sizeFactors(cds) ) )
```

```
getRawScvDistanceMatrix
```

Calculates a matrix of "distances" between samples

Description

In order to judge the similarity of samples, a distance matrix can be handy. This function calls [getRawScvForSamplePair](#) for each pair of samples and returns the a symmetric distance matrix.

Usage

```
getRawScvDistanceMatrix(cds)
```

Arguments

<code>cds</code>	a <code>CountDataSet</code> with estimates size factors
------------------	---

Details

See [getRawScvForSamplePair](#) for details on the metric.

Value

A symmetric matrix, with one row and one column for each sample.

Author(s)

Simon Anders

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

```

cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
dm <- getRawScvDistanceMatrix( cds )
heatmap( dm, symm=TRUE )

```

```
getRawScvForSamplePair
```

for a pair of samples, calculate the average raw SCV between them, as a

Description

The average raw SCV between two samples is a good measure of the sample-to-sample variability between two samples that does not include the shot noise, i.e., only reflects the “distance” between the samples without being affected by sequencing depth.

Usage

```
getRawScvForSamplePair(cds, sample1, sample2)
```

Arguments

cds	A CountDataSet with estimated size factors.
sample1	An index or column name identifying a sample.
sample2	Ditto.

Details

This function calls [estimateVarianceFunctionForMatrix](#) with the data for just the two specified columns and so obtains a raw variance function, which is used to calculate the raw variance (i.e., the variance without shot noise) for each gene. This is converted by dividing by the square of the size-factors-normalized count for the gene, to get a raw squared coefficient of variance (raw SCV), which is finally adjusted for the bias due to the division with [adjustScvForBias](#). The mean of the rawSCV values for all genes is returned.

Interpretation: The square root of the mean rawSCV is a measure of the typical variation of the concentration of a gene’s transcript between the two samples. So, if you get, say, a mean raw SCV of 0.1 between two samples, it means that, typically, the concentration of a genes transcript varies by 31 (because $\sqrt{0.1}$ is .31) from one sample to the other.

Check whether the value obtained between biological replicates seems acceptable in the light of this interpretation. For technical replicates, the raw SCV value should be very low, maybe below 0.01, and large values indicate problems with the library preparation.

Value

A single number, the mean raw SCV, as described above.

Author(s)

Simon Anders

See Also

[getRawScvDistanceMatrix](#)

Examples

```
cds <- makeExampleCountDataSet()
cgs <- estimateSizeFactors( cds )
getRawScvForSamplePair( cds, "A1", "A2" )
```

getVarianceStabilizedData

Perform a variance stabilising transformation (VST) on the count data

Description

This function calculates a variance stabilising transformations (VST) from the raw variance functions and then transforms the count data (after normalization by division by the size factor), yielding a matrix of values which are now approximately homoskedastic. This is useful as input to statistical analyses requiring homoskedasticity.

Usage

```
getVarianceStabilizedData(cds)
```

Arguments

`cds` a `CountDataSet` with estimated variance functions

Details

For each sample (i.e., column of `counts(cds)`), the full variance function is calculated from the raw variance (by scaling according to the size factor and adding the shot noise). The function always uses a pooled estimate of the variance function, i.e., one ignoring conditions. The reciprocal of the square root of the base variance (i.e., the full variance divided by the size factor) is then numerically integrated up, and the integral (approximated by a spline function) evaluated for each count value in the column, yielding a transformed value.

Note: This function calls internally [estimateVarianceFunctions](#) with `pooled=TRUE` and bases its calculations on the result. Any condition labels or variance adjustment factors are hence ignored.

Limitations: In order to preserve normalization, the same transformation has to be used for all samples. This results in the variance stabilization to be only approximate. The more the size factors differ, the more residual dependence of the variance on the mean you will find in the transformed data. (Compare the variance of the upper half of your transformed data with the lower half to see whether this is a problem in your case.)

Value

A matrix of the same dimension as the count data, containing the transformed data.

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```
cds <- makeExampleCountDataSet()
cfs <- estimateSizeFactors( cds )
cvs <- estimateVarianceFunctions( cds )
vds <- getVarianceStabilizedData( cds )
colsA <- conditions(cds) == "A"
plot( rank( rowMeans( vds[,colsA] ) ), genefilter::rowVars( vds[,colsA] ) )
```

makeExampleCountDataSet

make a simple example CountDataSet with random data

Description

This function returns an example CountDataSet. It is used for the examples in the package help pages.

Usage

```
makeExampleCountDataSet()
```

Value

a CountDataSet that has been constructed as follows: First, true base mean values for 10,000 genes are drawn from an exponential distribution with rate 1/250. Then, certain genes are declared (with probability 0.3 per gene) as truly differentially expressed (tDE). For these genes, the true base mean is split into two values, one for condition "A" and one for condition "B", such that the log₂ fold change from "A" to "B" follows a zero-centred normal distribution with standard deviation 2. Then, counts are drawn for each gene for 5 samples, the first three corresponding to condition "A" and the remaining two for condition "B". The counts are drawn from a negative binomial with the specified mean, multiplied by the size factor for the sample, with a constant raw SCV of 0.2 (i.e., a 'size' parameter of 1/0.2). The true size factors are fixed to c(1., 1.3, .7, .9, 1.6).

All these values were chosen to give data that at least somewhat resembles what one might encounter in an actual experiment. Note that this function is not meant to verify the package by simulation. For this purpose the parameters and distribution choices should be more varied.

Author(s)

Simon Anders, anders@embl.de

Examples

```
cds <- makeExampleCountDataSet()
```

`nbinomFitGLM`*Fit a generalized linear model (GLM) for each gene.*

Description

Use this function to estimate coefficients and calculate deviance from a GLM for each gene. The GLM uses the `nbkd.sf` family, with the dispersion estimate according to `getVarianceFunction(cds)`. Note that this requires that the variance functions were estimated with method "pooled" or "blind".

Usage

```
nbinomFitGLM( cds, modelFormula, glmControl=list() )
```

Arguments

`cds` a `CountDataSet`

`modelFormula` a formula. The left hand side must be 'count', the right hand side can involve any column of `pData(cds)`, i.e., `pData(cds)` is used as the model frame. If you have passed just a single factor to the 'conditions' argument of `newCountDataSet`, it can be referred to as 'condition' in the formula. If you have passed a data frame to 'conditions', all columns of this data frame will be available.

`glmControl` list of additional parameters to be passed to `glm.control`

Value

A data frame with one row for each gene and columns as follows:

- one column for each estimated coefficient, on a log2 scale (i.e., the natural log reported by `glm` is rescaled to base 2)
- a column 'deviance', with the deviance of the fit
- a boolean column 'converged', indicating whether the fit converged

Furthermore, the data frame has a scalar attribute 'df.residual' that contains the number of residual degrees of freedom.

Author(s)

Simon Anders (sanders@fs.tum.de)

See Also

[newCountDataSet](#), [nbinomGLMTest](#), [nbkd.sf](#)

Examples

```
# see nbinomGLMTest for an example
```

nbinomGLMTest *Perform chi-squared tests comparing two sets of GLM fits*

Description

For each gene, the function calculates a chi-square p value by simply calculating: $1 - \text{pchisq}(\text{resReduced}\$deviance - \text{resFull}\$deviance, \text{attr}(\text{resReduced}, "df.residual") - \text{attr}(\text{resFull}, "df.residual"))$

Usage

```
nbinomGLMTest(resFull, resReduced)
```

Arguments

resFull, resReduced
 GLM fit data frames, as returned by [nbinomFitGLM](#), first the full, then the reduced model.

Value

a vector of p values

Author(s)

Simon Anders, anders@embl.de

See Also

[nbinomFitGLM](#)

Examples

```
cds <- makeExampleCountDataSet()[ 1:100, ]
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds, "pooled" )
fit1 <- nbinomFitGLM( cds, count ~ condition )
fit0 <- nbinomFitGLM( cds, count ~ 1 )
nbinomGLMTest( fit1, fit0 )
```

nbinomGLMsForMatrix

Fit negative binomial GLMs to a count matrix.

Description

This is a low-level function that is wrapped by [nbinomGLMTest](#).

Usage

```
nbinomGLMsForMatrix(counts, sizeFactors, rawScv, modelFormula,
  modelFrame, quiet = FALSE, reportLog2 = TRUE, glmControl = list() )
```

Arguments

counts	a matrix of integer counts. Rows for genes, Columns for samples.
sizeFactors	a vector with a size factor for each column in 'counts'.
rawScv	a vector with a raw SCV (i.e., a dispersion) for each row in 'counts'.
modelFormula	a model formula. The left hand side should read 'count ~'.
modelFrame	a model frame (with one row for each column in 'counts')
quiet	whether to not print dots
reportLog2	whether to convert reported coefficients from natural log to log2 scale
glmControl	list of additional parameters to be passed to <code>glm.control</code>

Value

A data frame with one row for each gene and columns as follows:

- one column for each estimated coefficient, on a log2 scale (i.e., the natural log reported by `glm` is rescaled to base 2)
- a column 'deviance', with the deviance of the fit
- a boolean column 'converged', indicating whether the fit converged

Furthermore, the data frame has a scalar attribute 'df.residual' that contains the number of residual degrees of freedom.

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```
# See the code of nbinomFitGLM for an example.
```

nbinomTest	<i>Test for differences between the base means for two conditions</i>
------------	---

Description

This function tests for differences between the base means of two conditions (i.e., for differential expression in the case of RNA-Seq).

Usage

```
nbinomTest(cds, condA, condB, pvals_only = FALSE, eps=1e-4)
```

Arguments

cds	a <code>CountDataSet</code> with size factors and raw variance functions
condA	one of the conditions in 'cds'
condB	another one of the conditions in 'cds'
pvals_only	return only a vector of (unadjusted) p values instead of the data frame described below.
eps	Precision goal for the p value. This is only a rough guidance with no guarantee of adherence.

Details

See [nbinomTestForMatrices](#) for more technical informations

Value

A data frame with the following columns:

id	The ID of the observable, taken from the row names of the counts slots.
baseMean	The base mean (i.e., mean of the counts divided by the size factors) for the counts for both conditions
baseMeanA	The base mean (i.e., mean of the counts divided by the size factors) for the counts for condition A
baseMeanB	The base mean for condition B
foldChange	The ratio meanB/meanA
log2FoldChange	The log2 of the fold change
pval	The p value for rejecting the null hypothesis 'meanA==meanB'
padj	The adjusted p values (adjusted with 'p.adjust(pval, method="BH")')
resVarA	The ratio of the row-wise estimate of the base variance of the counts for condition A, divided by the value predicted with the base variance function from the base mean. If this number is very high, the hit seems to be a variance outlier and might be false.
resVarB	The same for condition B.

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
head( nbinomTest( cds, "A", "B" ) )
```

nbinomTestForMatrices

Perform row-wise tests for differences between the base means of two

Description

This function is called by [nbinomTest](#). Call it directly only if the S4 interface is unsuitable for your task.

Usage

```
nbinomTestForMatrices(countsA, countsB, sizeFactorsA, sizeFactorsB, rowScvA, rowScvB)
```


Arguments

countsA	A matrix of counts, where each column is a replicate
countsB	Another matrix of counts, where each column is a replicate
sizeFactorsA	Size factors for the columns of the matrix 'countsA'
sizeFactorsB	Size factors for the columns of the matrix 'countsB'
rawScvA	Raw squared coefficient of variation (SCV) for 'countsA', a vector with one value per gene
rawScvB	The same for 'countsB'
eps	Precision goal for the p value. This is only a rough guidance with no guarantee of adherence.

Details

See the paper for an exact description of the null hypothesis tested.

Value

A vector of unadjusted p values, one for each row in the counts matrices.

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```

cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
colsA <- conditions(cds) == "A"
colsB <- conditions(cds) == "B"
bmvA <- getBaseMeansAndVariances( counts(cds)[,colsA], sizeFactors(cds)[colsA] )
bmvB <- getBaseMeansAndVariances( counts(cds)[,colsB], sizeFactors(cds)[colsB] )
pvals <- nbinomTestForMatrices(
  counts(cds)[,colsA],
  counts(cds)[,colsB],
  sizeFactors(cds)[colsA],
  sizeFactors(cds)[colsB],
  adjustScvForBias(
    rawVarFunc( cds, "A" )( bmvA$baseMean ) / bmvA$baseMean^2,
    length( colsA ) ),
  adjustScvForBias(
    rawVarFunc( cds, "B" )( bmvB$baseMean ) / bmvB$baseMean^2,
    length( colsB ) ) )
names( pvals ) <- row.names( counts(cds) )
head( pvals )

```

<code>nbkd.sf</code>	<i>GLM family for a negative binomial with known dispersion and log link</i>
----------------------	--

Description

A distribution family for use with `glm`. It describes a negative binomial (as `negative.binomial` in the MASS package), but with a special link function, namely $\eta[i] = \log(\mu[i] / sf[i])$, i.e., each count value is divided by its size factor before the log is taken. This is used internally by `nbinomFitGLM`.

Usage

```
nbkd.sf(r, sf)
```

Arguments

<code>r</code>	The 'size' argument (see <code>dnbinom</code>), i.e., the reciprocal of the dispersion.
<code>sf</code>	A vector of size factors.

Value

A GLM family object.

Author(s)

Simon Anders, anders@embl.de

<code>newCountDataSet</code>	<i>Create a CountDataSet object</i>
------------------------------	-------------------------------------

Description

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

Usage

```
newCountDataSet(countData, conditions, sizeFactors = NULL, phenoData = NULL, fea
```

Arguments

<code>countData</code>	A matrix or data frame of count data, i.e., of non-negative integer values. The rows correspond to observations (e.g., number of reads that were assigned to a gene), the columns correspond to samples (or experiments). Note that biological replicates should each get their own column, while the counts of technical replicates (i.e., several sequencing runs/lanes from the same sample) have to be summed up into a single column.
------------------------	--

conditions	<p>A factor of experimental conditions (or treatments, or tissue types, or phenotypes, or the like). The length of the factor has to be equal to the number of columns of the countData matrix, assigning a condition to each sample. If 'conditions' is not a factor, it will be converted to one.</p> <p>Alternatively, you may pass a data frame, that will be placed in <code>pData(cds)</code> as is and can then be used with the modes "pooled" and "blind" in <code>estimateVarianceFunctions</code> and its columns can be referred to in a model formula provided to <code>nbinomFitGLM</code>.</p>
sizeFactors	<p>The size factors (see <code>sizeFactors</code>). If you have estimated the size factors beforehand, you can pass your estimate here as a numerical vector with as many elements as there are columns in the count data. Usually, however, this field is left blank. Then, you must call function <code>estimateSizeFactors</code> afterwards, which will estimate the size factors and fill in the information.</p>
phenoData	<p>You may pass an <code>AnnotatedDataFrame</code> here to describe the columns of the count matrix. Note that the package always adds two rows (or creates a new <code>AnnotatedDataFrame</code> with only these two rows in case you do not supply one) with names "condition" and "sizeFactor" to store this information.</p>
featureData	<p>You may pass an <code>AnnotatedDataFrame</code> here to describe the rows of the count matrix. The package will just pass through this information without using it.</p>

Details

See also the documentation of `eSet` (package `Biobase`) for the meaning of the other slots, which `CountDataSet` inherits from `eSet` (but which the present package does not use).

Value

an object of class `CountDataSet`

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```
countsTable <- counts( makeExampleCountDataSet() )
cds <- newCountDataSet( countsTable, c( "A", "A", "A", "B", "B" ) )
```

rawVarFunc

Accessor for raw variance functions

Description

Given the name of a raw variance function or the name of a condition, get the function from the environment `rawVarFuncs` (which is a slot of `CountDataSet`).

Usage

```
rawVarFunc(cds, condOrName=NULL, byName=FALSE)
```

Arguments

<code>cds</code>	a <code>CountDataSet</code> with variance functions (i.e., <code>estimateVarianceFunctions</code> has already been called)
<code>condOrName</code>	For <code>byName==FALSE</code> , this argument is taken to be the name of a condition. <code>rawVarFunc</code> then looks up in <code>rawVarFuncTable(cds)</code> which raw variance function should be used for the given condition and returns this function. For <code>byName==TRUE</code> , the look-up is skipped, i.e., the argument is taken directly as the name under which the raw variance function is stored in the <code>cds</code> 's <code>rawVarFuncs</code> environment. If only one variance function exists (e.g., because <code>estimateVarianceFunctions</code> has been called with <code>method="pooled"</code> or <code>method="blind"</code>), the argument may be left at its default <code>NULL</code> , and the only variance function is returned.
<code>byName</code>	how to interpret the <code>condByName</code> argument, see above

Value

a raw variance function. This is a function that, given a base mean (i.e., the mean of counts divided by size factors), returns a raw variance estimate. To get the full variance, the raw variance needs to be scaled up by multiplying it with the square of the size factor, and the shot noise has to be added. The function comes with an attribute, named `varAdjFactor`, which contains (for `byName==FALSE`) the variance adjustment factor (see `varAdjFactors`) set for this condition. For `byName==TRUE`, the variance adjustment factor is always given as 1. Note that the raw variance function does not multiply its result with this factor; you have to do this yourself if needed.

Author(s)

Simon Anders, sanders@fs.tum.de

See Also

[estimateSizeFactors](#)

Examples

```
# See example for estimateVarianceFunctions
```

`rawVarFuncTable` *Accessor functions for the slot `rawVarFuncTable` of the class*

Description

Accesses the `rawVarFuncTable`.

Usage

```
rawVarFuncTable(cds)
rawVarFuncTable(cds) <- value
```

Arguments

<code>cds</code>	a <code>CountDataSet</code> object
<code>value</code>	The <code>rawVarFuncTable</code> is a named character vector with one element for each condition, and the condition labels used as names. The value of the element is the name, under which the raw variance function to be used for the corresponding condition can be found in the environment <code>rawVarFuncs</code> (which is a slot of <code>CountDataSet</code>). The function <code>estimateVarianceFunctions</code> fills in this table. The function <code>rawVarFunc</code> , and through it several other functions working on <code>CountDataSets</code> , use it to look up which raw variance function to use for which condition.

Author(s)

Simon Anders, sanders@fs.tum.de

See Also

[estimateSizeFactors](#)

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
rawVarFuncTable( cds )
```

`residualsEcdfPlot` *Produce a diagnostic plot to check the fit of a raw variance function.*

Description

The residuals of the fit should follow a scaled chi-squared distribution. This function calls `varianceFitDiagnostic` to get the cumulative chi-squared probabilities of the residuals and plots their ECDFs, stratified by base means.

Usage

```
residualsEcdfPlot( cds, condition, ncuts = 7 )
```

Arguments

<code>cds</code>	a <code>CountDataSet</code> with raw variance functions
<code>condition</code>	the name of a condition
<code>ncuts</code>	the number of base mean strata (i.e. of curves)

Details

As the cumulative chi-square probabilities should be uniform, the ECDF curves should roughly follow the main diagonal (indicated by a green line). It is acceptable if the strata for very low counts deviate from this.

If the ECDF curves deviate too strongly from the green line, your replicates are most likely heterogeneous (i.e., their some are more similar than others), and the variance estimation procedure gets misled. If the ECDF curves are below the green line, variance is underestimated, which leads to too low p values (and hence, too many false positives). If the ECDF curves are above the green line, variance is overestimated, which leads to too high p values (and hence, DESeq might be more conservative in its test when necessary). You can adjust this by setting variance adjustment factors with `varAdjFactors`.

Value

None, but a plot is produced.

See Also

[residualsEcdfPlotFromDiagnostics](#), [varianceFitDiagnostics](#)

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
residualsEcdfPlot( cds, "A" )
```

```
residualsEcdfPlotFromDiagnostics
```

Produce a diagnostic plot to check the fit of a raw variance function.

Description

This function produces the same plot as [residualsEcdfPlot](#). While [residualsEcdfPlot](#) takes a `CountDataSet` as argument and the calls [varianceFitDiagnostics](#), the present function expects the output of a call to [varianceFitDiagnostics](#) (or [varianceFitDiagnosticsForMatrix](#)) as input.

Usage

```
residualsEcdfPlotFromDiagnostics(fitdiag, ncuts = 7, plotTitle = "Residuals ECDF")
```

Arguments

<code>fitdiag</code>	a data frame as returned by varianceFitDiagnostics or varianceFitDiagnosticsForMatrix
<code>ncuts</code>	the number of baseMean strata
<code>plotTitle</code>	the main title for the plot

Details

See [residualsEcdfPlot](#).

Value

None, but a plot is produced.

See Also

[residualsEcdfPlot](#), [varianceFitDiagnostics](#), [varianceFitDiagnosticsForMatrix](#)

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
vfd <- varianceFitDiagnostics( cds, "A" )
residualsEcdfPlotFromDiagnostics( vfd )
```

scvPlot

Produces a diagnostic plot with the variance estimates, given as

Description

The produced plot shows the estimated variance functions of the given `CountDataSet`, in the form of the squared coefficient of variation (SCV), i.e., the variance divided by the squared mean. The solid lines are the raw SCV estimates, one per condition. The dashed lines are the full variance estimates for each sample, i.e., the vertical distance between a dashed line and its corresponding solid line (of the same colour) is the shot noise. As the x axis is scaled as base mean (size-adjusted mean), the amount of shot noise depends on the size factor. The solid black line is a density estimate of the base means. Only where a sufficient density of counts is present can a good estimate be expected.

Usage

```
scvPlot( cds, xlim = NULL, ylim = c(0, 0.8), skipBiasCorrection = FALSE )
```

Arguments

`cds` a `CountDataSet` with estimated variance functions
`xlim, ylim` the plot limits
`skipBiasCorrection`
if set to true, the plotted SCVs are calculated directly from the variance fit, skipping the call to [adjustScvForBias](#) that is done by default.

Value

None; but a plot is produced.

Author(s)

Simon Anders, sanders@fs.tum.de

Examples

```

cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
scvPlot( cds )

```

sizeFactors

Accessor functions for the sizeFactors information in a CountDataSet

Description

The sizeFactors vector assigns to each column of the count data a value, the size factor, such that count values in the columns can be brought to a common scale by dividing by the corresponding size factor.

Usage

```

sizeFactors(cds)
sizeFactors(cds) <- value

```

Arguments

cds	a CountDataSet
value	a vector of number, one size factor for each column in the count data

Author(s)

Simon Anders, sanders@fs.tum.de

See Also

[estimateSizeFactors](#)

Examples

```

cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
sizeFactors(cds)

```

varAdjFactors

Defunct function. Do not use.

Description

For a few version (which were only available in the devel branch), the possibility was given to scale the variance estimates if desired. As this turned out to cause more design issues than it solved, the functionality was removed again.

Author(s)

Simon Anders, sanders@fs.tum.de

```
varianceFitDiagnostics
```

Generate a data frame with diagnostic information on the fit of a base

Description

After fitting base variance function with [estimateVarianceFunctions](#), it is prudent to check the quality of the fit for all or at least a few conditions. This function produces a data frame with useful data for this purpose.

Usage

```
varianceFitDiagnostics(cds, cond = NULL, modelFrame = NULL)
```

Arguments

<code>cds</code>	a <code>CountDataSet</code> with base variance functions
<code>cond</code>	the name of a condition. If only one variance function was estimated for 'cds' (e.g., if the modes "pooled" or "blind" were used in estimateVarianceFunctions), leave this parameter as <code>NULL</code> .
<code>modelFrame</code>	If a model frame was specified in estimateVarianceFunctions , it should be passed here again.

Value

A data frame with these columns:

<code>baseMean</code>	The base mean for the condition (see getBaseMeansAndVariances for details).
<code>baseVar</code>	The base variance for the condition as estimated from the counts of the respective row only (see getBaseMeansAndVariances for details).
<code>fittedRawVar</code>	The raw variance as predicted by the raw variance function when given the base-Mean.
<code>fittedBaseVar</code>	The base variance, found by adding the effective base-level shot noise to the raw variance.
<code>pchisq</code>	The cumulative chi-square probability of the residual.

Note

To check the fit, you might want to do one of the following:

Make a doubly-logarithmic scatter plot of `baseVar` against the `baseMean` and then add a line with the `fittedBaseVar` values. The line should follow the points.

- Calculate the squared coefficient of variance (SCV), i.e., $\text{baseVar}/\text{baseMean}^2$. Make a scatter plot of the SCV against the (log of the) `baseMean` and then add a line of the fitted SCV. The line should follow the points. You can also plot $1/\text{baseMean}$ against `baseMean` as another line to see the shot noise.

- The residuals, i.e., `baseVar/fittedBaseVar`, should follow a chi-square distribution with `k` degrees of freedom, after scaling by `k` (where `k` is the number of replicates minus 1). (See paper for details). The `'pchisq'` column contains the cumulative probabilities (i.e., the result of `'pchisq'`) of the residuals, and these values should be uniformly distributed. You can check this with a histogram, or more conveniently, with ECDF plot generated by the function [residualsEcdfPlot](#).

Author(s)

Simon Anders, sanders@fs.tum.de

See Also

[residualsEcdfPlot](#)

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
vfd <- varianceFitDiagnostics( cds, "A" )
head( vfd )
```

`varianceFitDiagnosticsForMatrix`

A lower-level function to get fit diagnostics

Description

Usually, you call `varianceFitDiagnostics`, which in turn counts `varianceFitDiagnosticsForMatrix`. If you do not have your data in a `CountDataSet` object, you may want to call this function directly.

Usage

```
varianceFitDiagnosticsForMatrix(counts, sizeFactors, rawVarFunc, poolingConditions)
```

Arguments

<code>counts</code>	a matrix of counts, with columns corresponding to replicates
<code>sizeFactors</code>	the size factors for the columns of the counts matrix
<code>rawVarFunc</code>	the raw variance function estimated from the counts data
<code>poolingConditions</code>	by default, all columns in <code>'counts'</code> are considered as replicates. For a pooled variance estimate (in the sense of e.g. the way variance is estimated in Student's t test), pass a factor as <code>'poolingConditions'</code> that indicates which samples are replicates of each other.

Details

See `varianceFitDiagnostics` for more explanation

Value

The same return value as described for [varianceFitDiagnostics](#).

Author(s)

Simon Anders, sanders@fs.tum.de

See Also

[varianceFitDiagnostics](#), [residualsEcdfPlotFromDiagnostics](#)

Examples

```
cds <- makeExampleCountDataSet()
cds <- estimateSizeFactors( cds )
cds <- estimateVarianceFunctions( cds )
colsA <- conditions(cds) == "A"
vfd <- varianceFitDiagnosticsForMatrix(
  counts(cds)[,colsA],
  sizeFactors(cds)[colsA],
  rawVarFunc( cds, "A" ) )
head( vfd )
```

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