Package ‘DESeq2’

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Type Package

Title Differential gene expression analysis based on the negative binomial distribution

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Description Estimate variance-mean dependence in count data from high-throughput sequencing assays and test for differential expression based on a model using the negative binomial distribution.

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DESeq2-package

DESeq2 package for differential analysis of count data

Description

The main functions for differential analysis are DESeq and results. See the examples at DESeq for basic analysis steps. Two transformations offered for count data are the "regularized logarithm", rlog, and varianceStabilizingTransformation. For more detailed information on usage, see the package vignette, by typing vignette("DESeq2"), or the workflow linked to on the first page of the vignette. All support questions should be posted to the Bioconductor support site: http://support.bioconductor.org.

Author(s)

Michael Love, Wolfgang Huber, Simon Anders
References

DESeq2 reference:

DESeq reference:

coef

Extract a matrix of model coefficients/standard errors

Description

Note: results tables with log2 fold change, p-values, adjusted p-values, etc. for each gene are best generated using the results function. The coef function is designed for advanced users who wish to inspect all model coefficients at once.

Usage

## S3 method for class 'DESeqDataSet'
coef(object, SE = FALSE, ...)

Arguments

object       a DESeqDataSet returned by DESeq, nbinomWaldTest, or nbinomLRT.
SE           whether to give the standard errors instead of coefficients. defaults to FALSE so
             that the coefficients are given.
...          additional arguments

Details

Estimated model coefficients or estimated standard errors are provided in a matrix form, number of genes by number of parameters, on the log2 scale. The columns correspond to columns of the model matrix for final GLM fitting, i.e., attr(dds, “modelMatrix”).

Author(s)

Michael Love

Examples

dds <- makeExampleDESeqDataSet(m=4)
dds <- DESeq(dd)
coef(dd)[1,]
coef(dd, SE=TRUE)[1,]
collapseReplicates  
Collapse technical replicates in a RangedSummarizedExperiment or DESeqDataSet

Description
Collapses the columns in object by summing within levels of a grouping factor groupby. The purpose of this function is to sum up read counts from technical replicates to create an object with a single column of read counts for each sample. Note: by "technical replicates", we mean multiple sequencing runs of the same library, in contrast to "biological replicates" in which multiple libraries are prepared from separate biological units. Optionally renames the columns of returned object with the levels of the grouping factor. Note: this function is written very simply and can be easily altered to produce other behavior by examining the source code.

Usage
```
collapseReplicates(object, groupby, run, renameCols = TRUE)
```

Arguments
- **object**: A RangedSummarizedExperiment or DESeqDataSet
- **groupby**: a grouping factor, as long as the columns of object
- **run**: optional, the names of each unique column in object. if provided, a new column runsCollapsed will be added to the colData which pastes together the names of run
- **renameCols**: whether to rename the columns of the returned object using the levels of the grouping factor

Value
the object with as many columns as levels in groupby. This object has assay/count data which is summed from the various columns which are grouped together, and the colData is subset using the first column for each group in groupby.

Examples
```
dds <- makeExampleDESeqDataSet(m=12)
# make data with two technical replicates for three samples
dds$sample <- factor(sample(paste0("sample",rep(1:9, c(2,1,1,2,1,2,1,1,1)))))
dds$run <- paste0("run",1:12)
ddsColl <- collapseReplicates(dds, dds$sample, dds$run)
# examine the colData and column names of the collapsed data
colData(ddsColl)
colnames(ddsColl)
# check that the sum of the counts for "sample1" is the same
# as the counts in the "sample1" column in ddsColl
matchFirstLevel <- dds$sample == levels(dds$sample)[1]
```
counts

stopifnot(all(rowSums(counts(dds[,matchFirstLevel])) == counts(ddsColl[,1])))

counts

Accessors for the 'counts' slot of a DESeqDataSet 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

## S4 method for signature 'DESeqDataSet'
counts(object, normalized = FALSE, replaced = FALSE)

## S4 replacement method for signature 'DESeqDataSet,matrix'
counts(object) <- value

Arguments

object a DESeqDataSet object.

normalized logical indicating whether or not to divide the counts by the size factors or normalization factors before returning (normalization factors always preempt size factors).

replaced after a DESeq call, this argument will return the counts with outliers replaced instead of the original counts, and optionally normalized. The replaced counts are stored by DESeq in assays(object)[['replaceCounts']].

value an integer matrix

Author(s)

Simon Anders

See Also

sizeFactors, normalizationFactors

Examples

dds <- makeExampleDESeqDataSet(m=4)
head(counts(dds))

dds <- estimateSizeFactors(dds) # run this or DESeq() first
head(counts(dds, normalized=TRUE))
DESeq

Differential expression analysis based on the Negative Binomial (a.k.a. Gamma-Poisson) distribution

Description

This function performs a default analysis through the steps:

1. estimation of size factors: estimateSizeFactors
2. estimation of dispersion: estimateDispersions
3. Negative Binomial GLM fitting and Wald statistics: nbinomWaldTest

For complete details on each step, see the manual pages of the respective functions. After the DESeq function returns a DESeqDataSet object, results tables (log2 fold changes and p-values) can be generated using the results function. See the manual page for results for information on independent filtering and p-value adjustment for multiple test correction.

Usage

DESeq(object, test = c("Wald", "LRT"), fitType = c("parametric", "local", "mean"), betaPrior, full = design(object), reduced, quiet = FALSE, minReplicatesForReplace = 7, modelMatrixType, parallel = FALSE, BPPARAM = bpparam())

Arguments

object a DESeqDataSet object, see the constructor functions DESeqDataSet, DESeqDataSetFromMatrix, DESeqDataSetFromHTSeqCount.

test either "Wald" or "LRT", which will then use either Wald significance tests (defined by nbinomWaldTest), or the likelihood ratio test on the difference in deviance between a full and reduced model formula (defined by nbinomLRT)

fitType either "parametric", "local", or "mean" for the type of fitting of dispersions to the mean intensity. See estimateDispersions for description.

betaPrior whether or not to put a zero-mean normal prior on the non-intercept coefficients. See nbinomWaldTest for description of the calculation of the beta prior. In versions >=1.16, the default is set to FALSE, and shrunken LFCs are obtained afterwards using lfcShrink.

full for test="LRT", the full model formula, which is restricted to the formula in design(object). alternatively, it can be a model matrix constructed by the user. advanced use: specifying a model matrix for full and test="Wald" is possible if betaPrior=FALSE

reduced for test="LRT", a reduced formula to compare against, i.e., the full formula with the term(s) of interest removed. alternatively, it can be a model matrix constructed by the user

quiet whether to print messages at each step

minReplicatesForReplace the minimum number of replicates required in order to use replaceOutliers on a sample. If there are samples with so many replicates, the model will be refit after these replacing outliers, flagged by Cook’s distance. Set to Inf in order to never replace outliers.
either "standard" or "expanded", which describe how the model matrix, X of
the GLM formula is formed. "standard" is as created by model.matrix using
the design formula. "expanded" includes an indicator variable for each level of
factors in addition to an intercept. for more information see the Description of
nbinomWaldTest. betaPrior must be set to TRUE in order for expanded model
matrices to be fit.

if FALSE, no parallelization. if TRUE, parallel execution using BiocParallel,
see next argument BPPARAM. A note on running in parallel using BiocParallel:
it may be advantageous to remove large, unneeded objects from your current
R environment before calling DESeq, as it is possible that R’s internal garbage
collection will copy these files while running on worker nodes.

an optional parameter object passed internally to bplapply when parallel=TRUE.
If not specified, the parameters last registered with register will be used.

The differential expression analysis uses a generalized linear model of the form:

\[ K_{ij} \sim \text{NB}(\mu_{ij}, \alpha_i) \]
\[ \mu_{ij} = s_j q_{ij} \]
\[ \log_2(q_{ij}) = x_j \beta_i \]

where counts \( K_{ij} \) for gene i, sample j are modeled using a Negative Binomial distribution with
fitted mean \( \mu_{ij} \) and a gene-specific dispersion parameter \( \alpha_i \). The fitted mean is composed of a
sample-specific size factor \( s_j \) and a parameter \( q_{ij} \) proportional to the expected true concentration
of fragments for sample j. The coefficients \( \beta_i \) give the log2 fold changes for gene i for each col-
umn of the model matrix X. The sample-specific size factors can be replaced by gene-specific
normalization factors for each sample using normalizationFactors.

For details on the fitting of the log2 fold changes and calculation of p-values, see nbinomWaldTest
if using test="Wald", or nbinomLRT if using test="LRT".

Experiments without replicates do not allow for estimation of the dispersion of counts around the
expected value for each group, which is critical for differential expression analysis. If an experi-
mental design is supplied which does not contain the necessary degrees of freedom for differential
analysis, DESeq will provide a message to the user and follow the strategy outlined in Anders and
Huber (2010) under the section ‘Working without replicates’, wherein all the samples are consid-
ered as replicates of a single group for the estimation of dispersion. As noted in the reference above:
"Some overestimation of the variance may be expected, which will make that approach conserva-
tive." Furthermore, "while one may not want to draw strong conclusions from such an analysis, it
may still be useful for exploration and hypothesis generation."

The argument minReplicatesForRepLace is used to decide which samples are eligible for auto-
matic replacement in the case of extreme Cook’s distance. By default, DESeq will replace outliers
if the Cook’s distance is large for a sample which has 7 or more replicates (including itself). This
replacement is performed by the replaceOutliers function. This default behavior helps to prevent
filtering genes based on Cook’s distance when there are many degrees of freedom. See results
for more information about filtering using Cook’s distance, and the ‘Dealing with outliers’ section
of the vignette. Unlike the behavior of replaceOutliers, here original counts are kept in the ma-
trix returned by counts, original Cook’s distances are kept in assays(dds)[["cooks"]], and the
replacement counts used for fitting are kept in assays(dds)[["replaceCounts"]].
Note that if a log2 fold change prior is used (betaPrior=TRUE) then expanded model matrices will be used in fitting. These are described in `nbinomWaldTest` and in the vignette. The contrast argument of `results` should be used for generating results tables.

**Value**

A `DESeqDataSet` object with results stored as metadata columns. These results should be accessed by calling the `results` function. By default this will return the log2 fold changes and p-values for the last variable in the design formula. See `results` for how to access results for other variables.

**Author(s)**

Michael Love

**References**


**See Also**

`nbinomWaldTest`, `nbinomLRT`

**Examples**

```r
# see vignette for suggestions on generating count tables from RNA-Seq data
cnts <- matrix(rnbinom(n=1000, mu=100, size=1/0.5), ncol=10)
cond <- factor(rep(1:2, each=5))

# object construction
dds <- DESeqDataSetFromMatrix(cnts, DataFrame(cond), ~ cond)

# standard analysis
dds <- DESeq(dds)
res <- results(dds)

# moderated log2 fold changes
resultsNames(dds)
resLFC <- lfcShrink(dds, coef=2, res=res)

# an alternate analysis: likelihood ratio test
ddsLRT <- DESeq(dds, test="LRT", reduced=~ 1)
resLRT <- results(ddsLRT)
```
DESeqDataSet-class

DESeqDataSet-class

DESeqDataSet object and constructors

Description

DESeqDataSet is a subclass of RangedSummarizedExperiment, used to store the input values, intermediate calculations and results of an analysis of differential expression. The DESeqDataSet class enforces non-negative integer values in the "counts" matrix stored as the first element in the assay list. In addition, a formula which specifies the design of the experiment must be provided. The constructor functions create a DESeqDataSet object from various types of input: a RangedSummarizedExperiment, a matrix, count files generated by the python package HTSeq, or a list from the tximport function in the tximport package. See the vignette for examples of construction from different types.

Usage

DESeqDataSet(se, design, ignoreRank = FALSE)

DESeqDataSetFromMatrix(countData, colData, design, tidy = FALSE, ignoreRank = FALSE, ...)

DESeqDataSetFromHTSeqCount(sampleTable, directory = ".", design, ignoreRank = FALSE, ...)

DESeqDataSetFromTximport(txi, colData, design, ...)

Arguments

se
a RangedSummarizedExperiment with columns of variables indicating sample information in colData, and the counts as the first element in the assays list, which will be renamed "counts". A RangedSummarizedExperiment object can be generated by the function summarizeOverlaps in the GenomicAlignments package.

design
a formula which expresses how the counts for each gene depend on the variables in colData. Many R formula are valid, including designs with multiple variables, e.g., ~ group + condition, and designs with interactions, e.g., ~ genotype + treatment + genotype:treatment. See results for a variety of designs and how to extract results tables. By default, the functions in this package will use the last variable in the formula for building results tables and plotting. ~ 1 can be used for no design, although users need to remember to switch to another design for differential testing.

ignoreRank
use of this argument is reserved for DEXSeq developers only. Users will immediately encounter an error upon trying to estimate dispersion using a design with a model matrix which is not full rank.

countData
for matrix input: a matrix of non-negative integers

colData
for matrix input: a DataFrame or data.frame with at least a single column. Rows of colData correspond to columns of countData

tidy
for matrix input: whether the first column of countData is the rownames for the count matrix
DESeqResults-class

DESeqResults object and constructor

Description

This constructor function would not typically be used by "end users". This simple class extends the DataFrame class of the IRanges package to allow other packages to write methods for results objects from the DESeq2 package. It is used by results to wrap up the results table.

Usage

DESeqResults(DataFrame)

Arguments

DataFrame a DataFrame of results, standard column names are: baseMean, log2FoldChange, lfcSE, stat, pvalue, padj.
**DESeqTransform-class**

**Value**

a DESeqResults object

---

**DESeqTransform-class  DESeqTransform object and constructor**

**Description**

This constructor function would not typically be used by "end users". This simple class extends the RangedSummarizedExperiment class of the SummarizedExperiment package. It is used by `rlog` and `varianceStabilizingTransformation` to wrap up the results into a class for downstream methods, such as plotPCA.

**Usage**

```r
DESeqTransform(SummarizedExperiment)
```

**Arguments**

- `SummarizedExperiment`  
a RangedSummarizedExperiment

**Value**

a DESeqTransform object

---

**design**

*Accessors for the 'design' slot of a DESeqDataSet object.*

**Description**

The design holds the R formula which expresses how the counts depend on the variables in colData. See DESeqDataSet for details.

**Usage**

```r
## S4 method for signature 'DESeqDataSet'
design(object)
```

```r
## S4 replacement method for signature 'DESeqDataSet,formula'
design(object) <- value
```

**Arguments**

- `object`  
a DESeqDataSet object
- `value`  
a formula used for estimating dispersion and fitting Negative Binomial GLMs
dispersionFunction

Examples

```r
dds <- makeExampleDESeqDataSet(m=4)
design(dds) <- formula(~ 1)

dispersionFunction(dds)
```

dispersionFunction

Accessors for the 'dispersionFunction' slot of a DESeqDataSet object.

Description

The dispersion function is calculated by `estimateDispersions` and used by `varianceStabilizingTransformation`. Parametric dispersion fits store the coefficients of the fit as attributes in this slot.

Usage

```r
dispersionFunction(object, ...)
dispersionFunction(object, ...) <- value

## S4 method for signature 'DESeqDataSet'
dispersionFunction(object)

## S4 replacement method for signature 'DESeqDataSet,Function'
dispersionFunction(object, estimateVar = TRUE) <- value
```

Arguments

- **object**: a DESeqDataSet object.
- **...**: additional arguments
- **value**: a function
- **estimateVar**: whether to estimate the variance of dispersion residuals. Setting to FALSE is needed, e.g. within `estimateDispersionsMAP` when called on a subset of the full dataset in parallel execution.

Details

Setting this will also overwrite `mcols(object)$dispFit` and the estimate the variance of dispersion residuals, see `estimateVar` below.

See Also

- `estimateDispersions`

Examples

```r
dds <- makeExampleDESeqDataSet(m=4)
dds <- estimateSizeFactors(dds)
dds <- estimateDispersions(dds)
dispersionFunction(dds)
```
dispersions

Accessor functions for the dispersion estimates in a DESeqDataSet object.

Description

The dispersions for each row of the DESeqDataSet. Generally, these are set by estimateDispersions.

Usage

dispersions(object, ...)
dispersions(object, ...) <- value

## S4 method for signature 'DESeqDataSet'
dispersions(object)

## S4 replacement method for signature 'DESeqDataSet,numeric'
dispersions(object) <- value

Arguments

object       a DESeqDataSet object.
...
    additional arguments
value       the dispersions to use for the Negative Binomial modeling

Author(s)

Simon Anders

See Also

estimateDispersions

estimateBetaPriorVar    Steps for estimating the beta prior variance

Description

These lower-level functions are called within DESeq or nbinomWaldTest. End users should use those higher-level function instead. NOTE: estimateBetaPriorVar returns a numeric vector, not a DESeqDataSet! For advanced users: to use these functions, first run estimateMLEForBetaPriorVar and then run estimateBetaPriorVar.

Usage

estimateBetaPriorVar(object, betaPriorMethod = c("weighted", "quantile"),
                      upperQuantile = 0.05)

estimateMLEForBetaPriorVar(object, maxit = 100, useOptim = TRUE,
                           useQR = TRUE, modelMatrixType = NULL)
estimateDispersions

Estimate the dispersions for a DESeqDataSet

Arguments

object       a DESeqDataSet
betaPriorMethod
              the method for calculating the beta prior variance, either "quantile" or "weighted": "quantile" matches a normal distribution using the upper quantile of the finite MLE betas. "weighted" matches a normal distribution using the upper quantile, but weighting by the variance of the MLE betas.
upperQuantile
              the upper quantile to be used for the "quantile" or "weighted" method of beta prior variance estimation
maxit
              as defined in link{nbinomWaldTest}
useOptim
              as defined in link{nbinomWaldTest}
useQR
              as defined in link{nbinomWaldTest}
modelMatrixType
              an optional override for the type which is set internally

Value

for estimateMLEForBetaPriorVar, a DESeqDataSet, with the necessary information stored in order to calculate the prior variance. for estimateBetaPriorVar, the vector of variances for the prior on the betas in the DESeq GLM

Description

This function obtains dispersion estimates for Negative Binomial distributed data.

Usage

## S4 method for signature 'DESeqDataSet'
estimateDispersions(object, fitType = c("parametric", "local", "mean"), maxit = 100, quiet = FALSE, modelMatrix = NULL)

Arguments

object       a DESeqDataSet
fitType      either "parametric", "local", or "mean" for the type of fitting of dispersions to the mean intensity.
              • parametric - fit a dispersion-mean relation of the form:

                             dispersion = asymtDisp + extraPois/mean

                            via a robust gamma-family GLM. The coefficients asymtDisp and extraPois are given in the attribute coefficients of the dispersionFunction of the object.
              • local - use the locfit package to fit a local regression of log dispersions over log base mean (normal scale means and dispersions are input and output for dispersionFunction). The points are weighted by normalized mean count in the local regression.
estimateDispersions

- mean - use the mean of gene-wise dispersion estimates.

maxit  control parameter: maximum number of iterations to allow for convergence
quiet  whether to print messages at each step
modelMatrix  an optional matrix which will be used for fitting the expected counts. by default, the model matrix is constructed from design(object)

Details

Typically the function is called with the idiom:

dds <- estimateDispersions(dds)

The fitting proceeds as follows: for each gene, an estimate of the dispersion is found which maximizes the Cox Reid-adjusted profile likelihood (the methods of Cox Reid-adjusted profile likelihood maximization for estimation of dispersion in RNA-Seq data were developed by McCarthy, et al. (2012), first implemented in the edgeR package in 2010); a trend line capturing the dispersion-mean relationship is fit to the maximum likelihood estimates; a normal prior is determined for the log dispersion estimates centered on the predicted value from the trended fit with variance equal to the difference between the observed variance of the log dispersion estimates and the expected sampling variance; finally maximum a posteriori dispersion estimates are returned. This final dispersion parameter is used in subsequent tests. The final dispersion estimates can be accessed from an object using dispersions. The fitted dispersion-mean relationship is also used in varianceStabilizingTransformation. All of the intermediate values (gene-wise dispersion estimates, fitted dispersion estimates from the trended fit, etc.) are stored in mcols(dds), with information about these columns in mcols(mcols(dds)).

The log normal prior on the dispersion parameter has been proposed by Wu, et al. (2012) and is also implemented in the DSS package.

In DESeq2, the dispersion estimation procedure described above replaces the different methods of dispersion from the previous version of the DESeq package.

estimateDispersions checks for the case of an analysis with as many samples as the number of coefficients to fit, and will temporarily substitute a design formula ~ 1 for the purposes of dispersion estimation. This treats the samples as replicates for the purpose of dispersion estimation. As mentioned in the DESeq paper: "While one may not want to draw strong conclusions from such an analysis, it may still be useful for exploration and hypothesis generation."

The lower-level functions called by estimateDispersions are: estimateDispersionsGeneEst, estimateDispersionsFit, and estimateDispersionsMAP.

Value

The DESeqDataSet passed as parameters, with the dispersion information filled in as metadata columns, accessible via mcols, or the final dispersions accessible via dispersions.

References

estimateDispersionsGeneEst

_Examples_

```r
dds <- makeExampleDESeqDataSet()
dds <- estimateSizeFactors(dds)
dds <- estimateDispersions(dds)
head(dispersions(dds))
```

estimateDispersionsGeneEst

*Low-level functions to fit dispersion estimates*

*Description*

Normal users should instead use `estimateDispersions`. These low-level functions are called by `estimateDispersions`, but are exported and documented for non-standard usage. For instance, it is possible to replace fitted values with a custom fit and continue with the maximum a posteriori dispersion estimation, as demonstrated in the examples below.

*Usage*

```r
estimateDispersionsGeneEst(object, minDisp = 1e-08, kappa_0 = 1,
dispTol = 1e-06, maxit = 100, quiet = FALSE, modelMatrix = NULL,
niter = 1, linearMu = NULL)
```

```r
estimateDispersionsFit(object, fitType = c("parametric", "local", "mean"),
minDisp = 1e-08, quiet = FALSE)
```

```r
estimateDispersionsMAP(object, outlierSD = 2, dispPriorVar, minDisp = 1e-08,
kappa_0 = 1, dispTol = 1e-06, maxit = 100, modelMatrix = NULL,
quiet = FALSE)
```

```r
estimateDispersionsPriorVar(object, minDisp = 1e-08, modelMatrix = NULL)
```

*Arguments*

- **object**: a DESeqDataSet
- **minDisp**: small value for the minimum dispersion, to allow for calculations in log scale, one order of magnitude above this value is used as a test for inclusion in mean-dispersion fitting
- **kappa_0**: control parameter used in setting the initial proposal in backtracking search, higher kappa_0 results in larger steps
- **dispTol**: control parameter to test for convergence of log dispersion, stop when increase in log posterior is less than dispTol
- **maxit**: control parameter: maximum number of iterations to allow for convergence
- **quiet**: whether to print messages at each step
- **modelMatrix**: for advanced use only, a substitute model matrix for gene-wise and MAP dispersion estimation
**estimateSizeFactors**

Estimate the size factors for a DESeqDataSet

---

### Description

This function estimates the size factors using the "median ratio method" described by Equation 5 in Anders and Huber (2010). The estimated size factors can be accessed using the accessor function `sizeFactors`. Alternative library size estimators can also be supplied using the assignment function `sizeFactors<-`.

---

**niter**

Number of times to iterate between estimation of means and estimation of dispersion

**linearMu**

Estimate the expected counts matrix using a linear model, default is NULL, in which case a linear model is used if the number of groups defined by the model matrix is equal to the number of columns of the model matrix

**fitType**

Either "parametric", "local", or "mean" for the type of fitting of dispersions to the mean intensity. See `estimateDispersions` for description.

**outlierSD**

The number of standard deviations of log gene-wise estimates above the prior mean (fitted value), above which dispersion estimates will be labelled outliers. Outliers will keep their original value and not be shrunk using the prior.

**dispPriorVar**

The variance of the normal prior on the log dispersions. If not supplied, this is calculated as the difference between the mean squared residuals of gene-wise estimates to the fitted dispersion and the expected sampling variance of the log dispersion.

---

### Value

A DESeqDataSet with gene-wise, fitted, or final MAP dispersion estimates in the metadata columns of the object.

`estimateDispersionsPriorVar` is called inside of `estimateDispersionsMAP` and stores the dispersion prior variance as an attribute of `dispersionFunction(dds)`, which can be manually provided to `estimateDispersionsMAP` for parallel execution.

---

### See Also

`estimateDispersions`

---

### Examples

```r
dds <- makeExampleDESeqDataSet()
dds <- estimateSizeFactors(dds)
dds <- estimateDispersionsGeneEst(dds)
dds <- estimateDispersionsFit(dds)
dds <- estimateDispersionsMAP(dds)
plotDispEsts(dds)

# after having run estimateDispersionsFit()
# the dispersion prior variance over all genes
# can be obtained like so:

dispPriorVar <- estimateDispersionsPriorVar(dds)
```
Usage

```r
## S4 method for signature 'DESeqDataSet'
estimateSizeFactors(object, type = c("ratio", "poscounts", "iterate"), locfunc = stats::median, geoMeans, controlGenes, normMatrix)
```

Arguments

- `object`: a DESeqDataSet
- `type`: Method for estimation: either "ratio", "poscounts", or "iterate". "ratio" uses the standard median ratio method introduced in DESeq. The size factor is the median ratio of the sample over a "pseudosample": for each gene, the geometric mean of all samples. "poscounts" and "iterate" offer alternative estimators, which can be used even when all genes contain a sample with a zero (a problem for the default method, as the geometric mean becomes zero, and the ratio undefined). The "poscounts" estimator deals with a gene with some zeros, by calculating a modified geometric mean by taking the n-th root of the product of the non-zero counts. This evolved out of use cases with Paul McMurdie’s phyloseq package for metagenomic samples. The "iterate" estimator iterates between estimating the dispersion with a design of ~1, and finding a size factor vector by numerically optimizing the likelihood of the ~1 model.
- `locfunc`: a function to compute a location for a sample. By default, the median is used. However, especially for low counts, the `shorth` function from the genefilter package may give better results.
- `geoMeans`: by default this is not provided and the geometric means of the counts are calculated within the function. A vector of geometric means from another count matrix can be provided for a "frozen" size factor calculation.
- `controlGenes`: optional, numeric or logical index vector specifying those genes to use for size factor estimation (e.g. housekeeping or spike-in genes)
- `normMatrix`: optional, a matrix of normalization factors which do not yet control for library size. Note that this argument should not be used (and will be ignored) if the dds object was created using tximport. In this case, the information in `assays(dds)[["avgTxLength"]]' is automatically used to create appropriate normalization factors. Providing `normMatrix` will estimate size factors on the count matrix divided by `normMatrix` and store the product of the size factors and `normMatrix` as `normalizationFactors`. It is recommended to divide out the row-wise geometric mean of `normMatrix` so the rows roughly are centered on 1.

Details

Typically, the function is called with the idiom:

```r
dds <- estimateSizeFactors(dds)
```

See `DESeq` for a description of the use of size factors in the GLM. One should call this function after `DESeqDataSet` unless size factors are manually specified with `sizeFactors`. Alternatively, gene-specific normalization factors for each sample can be provided using `normalizationFactors` which will always preempt `sizeFactors` in calculations.

Internally, the function calls `estimateSizeFactorsForMatrix`, which provides more details on the calculation.
Value

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

Author(s)

Simon Anders

References

Reference for the median ratio method:

See Also

estimateSizeFactorsForMatrix

Examples

dds <- makeExampleDESeqDataSet(n=1000, m=4)
dds <- estimateSizeFactors(dds)
sizeFactors(dds)

dds <- estimateSizeFactors(dds, controlGenes=1:200)

m <- matrix(runif(1000 * 4, .5, 1.5), ncol=4)
dds <- estimateSizeFactors(dds, normMatrix=m)
normalizationFactors(dds)[1:3,]

geoMeans <- exp(rowMeans(log(counts(dds))))
dds <- estimateSizeFactors(dds, geoMeans=geoMeans)
sizeFactors(dds)

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, one will not call this function directly, but use estimateSizeFactors.

Usage

estimateSizeFactorsForMatrix(counts, locfunc = stats::median, geoMeans, controlGenes)
fpkm

Arguments

- **counts**: a matrix or data frame of counts, i.e., non-negative integer values
- **loccfunc**: a function to compute a location for a sample. By default, the median is used. However, especially for low counts, the `shorth` function from genefilter may give better results.
- **geoMeans**: by default this is not provided, and the geometric means of the counts are calculated within the function. A vector of geometric means from another count matrix can be provided for a "frozen" size factor calculation
- **controlGenes**: optional, numeric or logical index vector specifying those genes to use for size factor estimation (e.g. housekeeping or spike-in genes)

Value

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

Author(s)

Simon Anders

See Also

- `estimateSizeFactors`

Examples

```r
dds <- makeExampleDESeqDataSet()
estimateSizeFactorsForMatrix(counts(dds))
geoMeans <- exp(rowMeans(log(counts(dds))))
estimateSizeFactorsForMatrix(counts(dds), geoMeans=geoMeans)
```

---

FPKM: fragments per kilobase per million mapped fragments

Description

The following function returns fragment counts normalized per kilobase of feature length per million mapped fragments (by default using a robust estimate of the library size, as in `estimateSizeFactors`).

Usage

```r
fpkm(object, robust = TRUE)
```

Arguments

- **object**: a DESeqDataSet
- **robust**: whether to use size factors to normalize rather than taking the column sums of the raw counts, using the `fpkm` function.
The length of the features (e.g. genes) is calculated one of two ways: (1) If there is a matrix named "avgTxLength" in assays(dds), this will take precedence in the length normalization. This occurs when using the tximport-DESeq2 pipeline. (2) Otherwise, feature length is calculated from the rowRanges of the dds object, if a column basepairs is not present in mcols(dds). The calculated length is the number of basepairs in the union of all GRanges assigned to a given row of object, e.g., the union of all basepairs of exons of a given gene. Note that the second approach over-estimates the gene length (average transcript length, weighted by abundance is a more appropriate normalization for gene counts), and so the FPKM will be an underestimate of the true value.

Note that, when the read/fragment counting has inter-feature dependencies, a strict normalization would not incorporate the basepairs of a feature which overlap another feature. This inter-feature dependence is not taken into consideration in the internal union basepair calculation.

a matrix which is normalized per kilobase of the union of basepairs in the GRangesList or GRanges of the mcols(object), and per million of mapped fragments, either using the robust median ratio method (robust=TRUE, default) or using raw counts (robust=FALSE). Defining a column mcols(object)$basepairs takes precedence over internal calculation of the kilobases for each row.

Examples

# create a matrix with 1 million counts for the # 2nd and 3rd column, the 1st and 4th have # half and double the counts, respectively.
m <- matrix(1e6 * rep(c(.125, .25, .25, .5), each=4), ncol=4, dimnames=list(1:4,1:4))
mode(m) <- "integer"
se <- SummarizedExperiment(list(counts=m), colData=DataFrame(sample=1:4))
dds <- DESeqDataSet(se, ~ 1)
# create 4 GRanges with lengths: 1, 1, 2, 2.5 Kb
gr1 <- GRanges("chr1",IRanges(1,1000)) # 1kb
gr2 <- GRanges("chr1",IRanges(c(1,1001),c(500,1500))) # 1kb
gr3 <- GRanges("chr1",IRanges(c(1,1001),c(1000,2000))) # 2kb
gr4 <- GRanges("chr1",IRanges(c(1,1001),c(200,1300))) # 500bp
rowRanges(dds) <- GRangesList(gr1,gr2,gr3,gr4)

# the raw counts
counts(dds)

# the FPM values
fpm(dds)

# the FPKM values
fpkm(dds)
**Description**

Calculates either a robust version (default) or the traditional matrix of fragments/counts per million mapped fragments (FPM/CPM). Note: this function is written very simply and can be easily altered to produce other behavior by examining the source code.

**Usage**

```r
fpm(object, robust = TRUE)
```

**Arguments**

- `object` a `DESeqDataSet`
- `robust` whether to use size factors to normalize rather than taking the column sums of the raw counts. If TRUE, the size factors and the geometric mean of column sums are multiplied to create a robust library size estimate. Robust normalization is not used if average transcript lengths are present.

**Value**

a matrix which is normalized per million of mapped fragments, either using the robust median ratio method (robust=TRUE, default) or using raw counts (robust=FALSE).

**See Also**

- `fpkm`

**Examples**

```r
# generate a dataset with size factors: .5, 1, 1, 2
dds <- makeExampleDESeqDataSet(m = 4, n = 1000,
    interceptMean=log2(1e3),
    interceptSD=0,
    sizeFactors=c(.5,1,1,2),
    dispMeanRel=function(x) .01)

# add a few rows with very high count
counts(dds)[4:10,] <- 2e5L

# in this robust version, the counts are comparable across samples
round(head(fpm(dds), 3))

# in this column sum version, the counts are still skewed:
# sample1 < sample2 & 3 < sample 4
round(head(fpm(dds, robust=FALSE), 3))

# the column sums of the robust version
# are not equal to 1e6, but the
# column sums of the non-robust version
```
**lfcShrink**

# are equal to 1e6 by definition

colSums(fpm(dds))/1e6
colSums(fpm(dds, robust=FALSE))/1e6

---

<table>
<thead>
<tr>
<th>lfcShrink</th>
<th>Shrink log2 fold changes</th>
</tr>
</thead>
</table>

**Description**

This function adds shrunk log2 fold changes (LFC) to a results table which was run without LFC moderation. Note: this function is still being prototyped.

**Usage**

`lfcShrink(dds, coef, contrast, res, type = "normal")`

**Arguments**

- **dds**: a DESeqDataSet object, which has been run through DESeq, or at the least, estimateDispersions
- **coef**: the number of the coefficient (LFC) to shrink, consult resultsNames(dds) after running DESeq(dds, betaPrior=FALSE). only coef or contrast can be specified, not both
- **contrast**: see argument description in results. only coef or contrast can be specified, not both
- **res**: a DESeqResults object (can be missing)
- **type**: at this time, ignored argument, because only one shrinkage estimator, but more to come!

**Value**

if res is not missing, a DESeqResults object with the log2FoldChange column replaced with a shrunk LFC. If res is missing, just the shrunk LFC vector.

**Examples**

```r
dds <- makeExampleDESeqDataSet(betaSD=1)
dds <- DESeq(dds, betaPrior=FALSE)
res <- results(dds)
res.shr <- lfcShrink(dds=dds, coef=2, res=res)
res.shr <- lfcShrink(dds=dds, contrast=c("condition","B","A"), res=res)
```
**makeExampleDESeqDataSet**

*Make a simulated DESeqDataSet*

**Description**

Constructs a simulated dataset of Negative Binomial data from two conditions. By default, there are no fold changes between the two conditions, but this can be adjusted with the betaSD argument.

**Usage**

```
makeExampleDESeqDataSet(n = 1000, m = 12, betaSD = 0, interceptMean = 4,
                          interceptSD = 2, dispMeanRel = function(x) 4/x + 0.1,
                          sizeFactors = rep(1, m))
```

**Arguments**

- **n**: number of rows
- **m**: number of columns
- **betaSD**: the standard deviation for non-intercept betas, i.e. beta ~ N(0,betaSD)
- **interceptMean**: the mean of the intercept betas (log2 scale)
- **interceptSD**: the standard deviation of the intercept betas (log2 scale)
- **dispMeanRel**: a function specifying the relationship of the dispersions on 2^trueIntercept
- **sizeFactors**: multiplicative factors for each sample

**Value**

A `DESeqDataSet` with true dispersion, intercept and beta values in the metadata columns. Note that the true betas are provided on the log2 scale.

**Examples**

```
dds <- makeExampleDESeqDataSet()
```  

**nbinomLRT**

*Likelihood ratio test (chi-squared) for GLMs*

**Description**

This function tests for significance of change in deviance between a full and reduced model which are provided as formula. Fitting uses previously calculated `sizeFactors` (or `normalizationFactors`) and dispersion estimates.
Usage

nbinomLRT(object, full = design(object), reduced, betaTol = 1e-08, maxit = 100, useOptim = TRUE, quiet = FALSE, useQR = TRUE)

Arguments

- **object**: a DESeqDataSet
- **full**: the full model formula, this should be the formula in `design(object)`. alternatively, can be a matrix
- **reduced**: a reduced formula to compare against, e.g. the full model with a term or terms of interest removed. alternatively, can be a matrix
- **betaTol**: control parameter defining convergence
- **maxit**: the maximum number of iterations to allow for convergence of the coefficient vector
- **useOptim**: whether to use the native optim function on rows which do not converge within `maxit`
- **quiet**: whether to print messages at each step
- **useQR**: whether to use the QR decomposition on the design matrix X while fitting the GLM

Details

The difference in deviance is compared to a chi-squared distribution with \( \text{df} = \text{(reduced residual degrees of freedom - full residual degrees of freedom)} \). This function is comparable to the `nbinomGLMTest` of the previous version of DESeq and an alternative to the default `nbinomWaldTest`.

Value

a DESeqDataSet with new results columns accessible with the `results` function. The coefficients and standard errors are reported on a log2 scale.

See Also

- **DESeq**, `nbinomWaldTest`

Examples

```r
dds <- makeExampleDESeqDataSet()
dds <- estimateSizeFactors(dds)
dds <- estimateDispersions(dds)
dds <- nbinomLRT(dds, reduced = ~ 1)
res <- results(dds)
```
nbinomWaldTest

Wald test for the GLM coefficients

Description

This function tests for significance of coefficients in a Negative Binomial GLM, using previously calculated sizeFactors (or normalizationFactors) and dispersion estimates. See DESeq for the GLM formula.

Usage

nbinomWaldTest(object, betaPrior = FALSE, betaPriorVar, modelMatrix = NULL, modelMatrixType, betaTol = 1e-08, maxit = 100, useOptim = TRUE, quiet = FALSE, useT = FALSE, df, useQR = TRUE)

Arguments

object          a DESeqDataSet
betaPrior       whether or not to put a zero-mean normal prior on the non-intercept coefficients
betaPriorVar    a vector with length equal to the number of model terms including the intercept. betaPriorVar gives the variance of the prior on the sample betas on the log2 scale. if missing (default) this is estimated from the data
modelMatrix     an optional matrix, typically this is set to NULL and created within the function. only can be supplied if betaPrior=FALSE
modelMatrixType either "standard" or "expanded", which describe how the model matrix, X of the formula in DESeq, is formed. "standard" is as created by model.matrix using the design formula. "expanded" includes an indicator variable for each level of factors in addition to an intercept. betaPrior must be set to TRUE in order for expanded model matrices to be fit.
betaTol         control parameter defining convergence
maxit           the maximum number of iterations to allow for convergence of the coefficient vector
useOptim        whether to use the native optim function on rows which do not converge within maxit
quiet           whether to print messages at each step
useT            whether to use a t-distribution as a null distribution, for significance testing of the Wald statistics. If FALSE, a standard normal null distribution is used.
df              the degrees of freedom for the t-distribution
useQR           whether to use the QR decomposition on the design matrix X while fitting the GLM

Details

The fitting proceeds as follows: standard maximum likelihood estimates for GLM coefficients (synonymous with "beta", "log2 fold change", "effect size") are calculated. Then, optionally, a zero-centered Normal prior distribution (betaPrior) is assumed for the coefficients other than the intercept.
Note that this posterior log2 fold change estimation is now not the default setting for nbinomWaldTest, as the standard workflow for coefficient shrinkage has moved to an additional function link(lfcShrink).

For calculating Wald test p-values, the coefficients are scaled by their standard errors and then compared to a standard Normal distribution. The results function without any arguments will automatically perform a contrast of the last level of the last variable in the design formula over the first level. The contrast argument of the results function can be used to generate other comparisons.

The Wald test can be replaced with the nbinomLRT for an alternative test of significance.

Notes on the log2 fold change prior:

The variance of the prior distribution for each non-intercept coefficient is calculated using the observed distribution of the maximum likelihood coefficients. The final coefficients are then maximum a posteriori estimates using this prior (Tikhonov/ridge regularization). See below for details on the prior variance and the Methods section of the DESeq2 manuscript for more detail. The use of a prior has little effect on genes with high counts and helps to moderate the large spread in coefficients for genes with low counts.

The prior variance is calculated by matching the 0.05 upper quantile of the observed MLE coefficients to a zero-centered Normal distribution. In a change of methods since the 2014 paper, the weighted upper quantile is calculated using the wtd.quantile function from the Hmisc package. The weights are the inverse of the expected variance of log counts, so the inverse of $1/\bar{\mu} + \alpha_{tr}$ using the mean of normalized counts and the trended dispersion fit. The weighting ensures that noisy estimates of log fold changes from small count genes do not overly influence the calculation of the prior variance. See estimateBetaPriorVar. The final prior variance for a factor level is the average of the estimated prior variance over all contrasts of all levels of the factor.

When a log2 fold change prior is used (betaPrior=TRUE), then nbinomWaldTest will by default use expanded model matrices, as described in the modelMatrixType argument, unless this argument is used to override the default behavior. This ensures that log2 fold changes will be independent of the choice of reference level. In this case, the beta prior variance for each factor is calculated as the average of the mean squared maximum likelihood estimates for each level and every possible contrast.

Value

a DESeqDataSet with results columns accessible with the results function. The coefficients and standard errors are reported on a log2 scale.

See Also

DESeq, nbinomLRT

Examples

def makeExampleDESeqDataSet()
def estimateSizeFactors(dds)
def estimateDispersions(dds)
def nbinomWaldTest(dds)
def results(dds)
normalizationFactors

Accessor functions for the normalization factors in a DESeqDataSet object.

Description

Gene-specific normalization factors for each sample can be provided as a matrix, which will preempt sizeFactors. In some experiments, counts for each sample have varying dependence on covariates, e.g. on GC-content for sequencing data run on different days, and in this case it makes sense to provide gene-specific factors for each sample rather than a single size factor.

Usage

normalizationFactors(object, ...)

normalizationFactors(object, ...) <- value

## S4 method for signature 'DESeqDataSet'
normalizationFactors(object)

## S4 replacement method for signature 'DESeqDataSet, matrix'
normalizationFactors(object) <- value

Arguments

object a DESeqDataSet object.

... additional arguments

value the matrix of normalization factors

Details

Normalization factors alter the model of DESeq in the following way, for counts $K_{ij}$ and normalization factors $NF_{ij}$ for gene i and sample j:

\[
K_{ij} \sim \text{NB}(\mu_{ij}, \alpha_i)
\]

\[
\mu_{ij} = NF_{ij} q_{ij}
\]

Note

Normalization factors are on the scale of the counts (similar to sizeFactors) and unlike offsets, which are typically on the scale of the predictors (in this case, log counts). Normalization factors should include library size normalization. They should have row-wise geometric mean near 1, as is the case with size factors, such that the mean of normalized counts is close to the mean of unnormalized counts. See example code below.
normalizeGeneLength

Examples

```r
dds <- makeExampleDESeqDataSet(n=100, m=4)

normFactors <- matrix(runif(nrow(dds)*ncol(dds),0.5,1.5),
                       ncol=ncol(dds),nrow=nrow(dds),
                       dimnames=list(1:nrow(dds),1:ncol(dds)))

# the normalization factors matrix should not have 0’s in it
# it should have geometric mean near 1 for each row
normFactors <- normFactors / exp(rowMeans(log(normFactors)))
normalizationFactors(dds) <- normFactors

dds <- DESeq(dds)
```

normalizeGeneLength  Normalize for gene length

Description

Normalize for gene length using the output of transcript abundance estimators

Usage

```r
normalizeGeneLength(...)```

Arguments

... ...

Details

This function is deprecated and moved to a new general purpose package, tximport, which will be added to Bioconductor.

normTransform  Normalized counts transformation

Description

A simple function for creating a DESeqTransform object after applying: f(count + pc).

Usage

```r
normTransform(object, f = log2, pc = 1)```

Arguments

- **object**: a DESeqDataSet object
- **f**: a function to apply to normalized counts
- **pc**: a pseudocount to add to normalized counts
plotCounts

Description

Normalized counts plus a pseudocount of 0.5 are shown by default.

Usage

plotCounts(dds, gene, intgroup = "condition", normalized = TRUE, transform = TRUE, main, xlab = "group", returnData = FALSE, replaced = FALSE, pc, ...)

Arguments

- **dds**: a DESeqDataSet
- **gene**: a character, specifying the name of the gene to plot
- **intgroup**: interesting groups: a character vector of names in colData(x) to use for grouping
- **normalized**: whether the counts should be normalized by size factor (default is TRUE)
- **transform**: whether to have log scale y-axis or not. defaults to TRUE
- **main**: as in ‘plot’
- **xlab**: as in ‘plot’
- **returnData**: should the function only return the data.frame of counts and covariates for custom plotting (default is FALSE)
- **replaced**: use the outlier-replaced counts if they exist
- **pc**: pseudocount for log transform
- **...**: arguments passed to plot

Examples

```r
dds <- makeExampleDESeqDataSet()
plotCounts(dds, "gene1")
```
plotDispEsts

Plot dispersion estimates

Description

A simple helper function that plots the per-gene dispersion estimates together with the fitted mean-dispersion relationship.

Usage

```r
## S4 method for signature 'DESeqDataSet'
plotDispEsts(object, ymin, CV = FALSE, 
genecol = "black", fitcol = "red", finalcol = "dodgerblue", 
legend = TRUE, xlab, ylab, log = "xy", cex = 0.45, ...)
```

Arguments

- `object`: a DESeqDataSet, with dispersions estimated
- `ymin`: the lower bound for points on the plot, points beyond this are drawn as triangles at `ymin`
- `CV`: logical, whether to plot the asymptotic or biological coefficient of variation (the square root of dispersion) on the y-axis. As the mean grows to infinity, the square root of dispersion gives the coefficient of variation for the counts. Default is FALSE, plotting dispersion.
- `genecol`: the color for gene-wise dispersion estimates
- `fitcol`: the color of the fitted estimates
- `finalcol`: the color of the final estimates used for testing
- `legend`: logical, whether to draw a legend
- `xlab`: `xlab`
- `ylab`: `ylab`
- `log`: `log`
- `cex`: `cex`
- `...`: further arguments to `plot`

Author(s)

Simon Anders

Examples

```r
dds <- makeExampleDESeqDataSet()
dds <- estimateSizeFactors(dds)
dds <- estimateDispersions(dds)
plotDispEsts(dds)
```
plotMA

MA-plot from base means and log fold changes

Description

A simple helper function that makes a so-called "MA-plot", i.e. a scatter plot of log2 fold changes (on the y-axis) versus the mean of normalized counts (on the x-axis).

Usage

## S4 method for signature 'DESeqDataSet'
plotMA(object, alpha = 0.1, main = "",
       xlab = "mean of normalized counts", ylim, MLE = FALSE, ...)

## S4 method for signature 'DESeqResults'
plotMA(object, alpha, main = "",
       xlab = "mean of normalized counts", ylim, MLE = FALSE, ...)

Arguments

object a DESeqResults object produced by results; or a DESeqDataSet processed by DESeq, or the individual functions nbinomWaldTest or nbinomLRT
alpha the significance level for thresholding adjusted p-values
main optional title for the plot
xlab optional defaults to "mean of normalized counts"
ylim optional y limits
MLE if betaPrior=TRUE was used, whether to plot the MLE (unshrunken estimates), defaults to FALSE. Requires that results was run with addMLE=TRUE. Note that the MLE will be plotted regardless of this argument, if DESeq() was run with betaPrior=FALSE. See lfcShrink for examples on how to plot shrunken log2 fold changes.
...
further arguments passed to plotMA if object is DESeqResults or to results if object is DESeqDataSet

Details

This function is essentially two lines of code: building a data.frame and passing this to the plotMA method for data.frame from the geneplotter package. The code of this function can be seen with: getMethod("plotMA", "DESeqDataSet") If users wish to modify the graphical parameters of the plot, it is recommended to build the data.frame in the same manner and call plotMA.

Author(s)

Michael Love
plotPCA

Examples

```r
dds <- makeExampleDESeqDataSet()
dds <- DESeq(dds)
plotMA(dds)
res <- results(dds)
plotMA(res)
```

plotPCA

Sample PCA plot for transformed data

Description

This plot helps to check for batch effects and the like.

Usage

```r
## S4 method for signature 'DESeqTransform'
plotPCA(object, intgroup = "condition",
        ntop = 500, returnData = FALSE)
```

Arguments

- `object`: a `DESeqTransform` object, with data in `assay(x)`, produced for example by either `rlog` or `varianceStabilizingTransformation`.
- `intgroup`: interesting groups: a character vector of names in `colData(x)` to use for grouping
- `ntop`: number of top genes to use for principal components, selected by highest row variance
- `returnData`: should the function only return the data.frame of PC1 and PC2 with intgroup covariates for custom plotting (default is FALSE)

Value

An object created by `ggplot`, which can be assigned and further customized.

Note

See the vignette for an example of variance stabilization and PCA plots. Note that the source code of `plotPCA` is very simple. The source can be found by typing `DESeq2:::plotPCA.DESeqTransform` or `getMethod("plotPCA","DESeqTransform")`, or browsed on github at https://github.com/Bioconductor-mirror/DESeq2/blob/master/R/plots.R Users should find it easy to customize this function.

Author(s)

Wolfgang Huber
Examples

# using rlog transformed data:
dds <- makeExampleDESeqDataSet(betaSD=1)
rld <- rlog(dds)
plotPCA(rld)

# also possible to perform custom transformation:
dds <- estimateSizeFactors(dds)
# shifted log of normalized counts
se <- SummarizedExperiment(log2(counts(dds, normalized=TRUE) + 1),
                           colData=colData(dds))
# the call to DESeqTransform() is needed to
# trigger our plotPCA method.
plotPCA(DESeqTransform(se))

---------------------------------------------------------------------

plotSparsity

Sparsity plot

Description

A simple plot of the concentration of counts in a single sample over the sum of counts per gene. Not technically the same as "sparsity", but this plot is useful diagnostic for datasets which might not fit a negative binomial assumption: genes with many zeros and individual very large counts are difficult to model with the negative binomial distribution.

Usage

plotSparsity(x, normalized = TRUE, ...)

Arguments

x a matrix or DESeqDataSet
normalized whether to normalize the counts from a DESeqDataSEt
... passed to plot

Examples

dds <- makeExampleDESeqDataSet(n=1000,m=4,dispMeanRel=function(x) .5)
dds <- estimateSizeFactors(dds)
plotSparsity(dds)
replaceOutliers  Replace outliers with trimmed mean

Description

Note that this function is called within DESeq, so is not necessary to call on top of a DESeq call. See the minReplicatesForReplace argument documented in link{DESeq}.

Usage

```r
replaceOutliers(object, trim = 0.2, cooksCutoff, minReplicates = 7, whichSamples)
```

```r
replaceOutliersWithTrimmedMean(object, trim = 0.2, cooksCutoff, minReplicates = 7, whichSamples)
```

Arguments

- `object`: a DESeqDataSet object, which has already been processed by either DESeq, nbinomWaldTest or nbinomLRT, and therefore contains a matrix contained in `assays(dds)[["cooks"]]. These are the Cook’s distances which will be used to define outlier counts.
- `trim`: the fraction (0 to 0.5) of observations to be trimmed from each end of the normalized counts for a gene before the mean is computed
- `cooksCutoff`: the threshold for defining an outlier to be replaced. Defaults to the .99 quantile of the F(p, m - p) distribution, where p is the number of parameters and m is the number of samples.
- `minReplicates`: the minimum number of replicate samples necessary to consider a sample eligible for replacement (including itself). Outlier counts will not be replaced if the sample is in a cell which has less than minReplicates replicates.
- `whichSamples`: optional, a numeric or logical index to specify which samples should have outliers replaced. if missing, this is determined using minReplicates.

Details

This function replaces outlier counts flagged by extreme Cook’s distances, as calculated by DESeq, nbinomWaldTest or nbinomLRT, with values predicted by the trimmed mean over all samples (and adjusted by size factor or normalization factor). This function replaces the counts in the matrix returned by `counts(dds)` and the Cook’s distances in `assays(dds)[["cooks"]]. Original counts are preserved in `assays(dds)[["originalCounts"]].

The DESeq function calculates a diagnostic measure called Cook’s distance for every gene and every sample. The results function then sets the p-values to NA for genes which contain an outlying count as defined by a Cook’s distance above a threshold. With many degrees of freedom, i.e. many more samples than number of parameters to be estimated– it might be undesirable to remove entire genes from the analysis just because their data include a single count outlier. An alternate strategy is to replace the outlier counts with the trimmed mean over all samples, adjusted by the size factor or normalization factor for that sample. The following simple function performs this replacement for the user, for samples which have at least minReplicates number of replicates (including that sample). For more information on Cook’s distance, please see the two sections of the vignette: ’Dealing with count outliers’ and ’Count outlier detection’.
Value

a DESeqDataSet with replaced counts in the slot returned by `counts` and the original counts preserved in `assays(dds)[["originalCounts"]]

See Also

DESeq

results

Extract results from a DESeq analysis

Description

`results` extracts a result table from a DESeq analysis giving base means across samples, log2 fold changes, standard errors, test statistics, p-values and adjusted p-values; `resultsNames` returns the names of the estimated effects (coefficients) of the model; `removeResults` returns a DESeqDataSet object with results columns removed.

Usage

```r
results(object, contrast, name, lfcThreshold = 0,
altHypothesis = c("greaterAbs", "lessAbs", "greater", "less"),
listValues = c(1, -1), cooksCutoff, independentFiltering = TRUE,
alpha = 0.1, filter, theta, pAdjustMethod = "BH", filterFun,
format = c("DataFrame", "GRanges", "GRangesList"), test, addMLE = FALSE,
tidy = FALSE, parallel = FALSE, BPPARAM = bpparam(), ...)
```

```r
resultsNames(object)
removeResults(object)
```

Arguments

- **object**: a DESeqDataSet, on which one of the following functions has already been called: `DESeq`, `nbinomWaldTest`, or `nbinomLRT`

- **contrast**: this argument specifies what comparison to extract from the object to build a results table. one of either:
  - a character vector with exactly three elements: the name of a factor in the design formula, the name of the numerator level for the fold change, and the name of the denominator level for the fold change (simplest case)
  - a list of 2 character vectors: the names of the fold changes for the numerator, and the names of the fold changes for the denominator. these names should be elements of `resultsNames(object)`. if the list is length 1, a second element is added which is the empty character vector, character(). (more general case, can be to combine interaction terms and main effects)
  - a numeric contrast vector with one element for each element in `resultsNames(object)` (most general case)

If specified, the name argument is ignored.
name

the name of the individual effect (coefficient) for building a results table. Use this argument rather than contrast for continuous variables, individual effects or for individual interaction terms. The value provided to name must be an element of resultsNames(object).

lfcThreshold

a non-negative value which specifies a log2 fold change threshold. The default value is 0, corresponding to a test that the log2 fold changes are equal to zero. The user can specify the alternative hypothesis using the altHypothesis argument, which defaults to testing for log2 fold changes greater in absolute value than a given threshold. If lfcThreshold is specified, the results are for Wald tests, and LRT p-values will be overwritten.

altHypothesis

character which specifies the alternative hypothesis, i.e. those values of log2 fold change which the user is interested in finding. The complement of this set of values is the null hypothesis which will be tested. If the log2 fold change specified by name or by contrast is written as $\beta$, then the possible values for altHypothesis represent the following alternate hypotheses:

- greaterAbs: $|\beta| > \text{lfcThreshold}$, and p-values are two-tailed
- lessAbs: $|\beta| < \text{lfcThreshold}$, NOTE: this requires that betaPrior=FALSE has been specified in the previous DESeq call. p-values are the maximum of the upper and lower tests.
- greater: $\beta > \text{lfcThreshold}$
- less: $\beta < -\text{lfcThreshold}$

listValues

only used if a list is provided to contrast: a numeric of length two: the log2 fold changes in the list are multiplied by these values. the first number should be positive and the second negative. by default this is c(1,-1)

cooksCutoff

threshold on Cook's distance, such that if one or more samples for a row have a distance higher, the p-value for the row is set to NA. The default cutoff is the .99 quantile of the F(p, m-p) distribution, where p is the number of coefficients being fitted and m is the number of samples. Set to Inf or FALSE to disable the resetting of p-values to NA. Note: this test excludes the Cook's distance of samples belonging to experimental groups with only 2 samples.

independentFiltering

logical, whether independent filtering should be applied automatically

alpha

the significance cutoff used for optimizing the independent filtering (by default 0.1). If the adjusted p-value cutoff (FDR) will be a value other than 0.1, alpha should be set to that value.

filter

the vector of filter statistics over which the independent filtering will be optimized. By default the mean of normalized counts is used.

theta

the quantiles at which to assess the number of rejections from independent filtering

pAdjustMethod

the method to use for adjusting p-values, see ?p.adjust

filterFun

an optional custom function for performing independent filtering and p-value adjustment, with arguments res (a DESeqResults object), filter (the quantity for filtering tests), alpha (the target FDR), pAdjustMethod. This function should return a DESeqResults object with a padj column.

format

character, either "DataFrame", "GRanges", or "GRangesList", whether the results should be printed as a DESeqResults DataFrame, or if the results DataFrame should be attached as metadata columns to the GRanges or GRangesList rowRanges of the DESeqDataSet. If the rowRanges is a GRangesList, and GRanges is requested, the range of each gene will be returned
the one exception is after \texttt{nb1nomLRT} has been run, \texttt{test="Wald"} will generate Wald statistics and Wald test p-values.\\

\texttt{addMLE} \quad \text{if} \ \texttt{betaPrior=TRUE} \ \text{was used, whether the "unshrunken" maximum likelihood estimates (MLE) of log2 fold change should be added as a column to the results table (default is FALSE). This argument is preserved for backward compatibility, as now the recommended pipeline is to generate shrunken MAP estimates using \texttt{lfcShrink}. This argument functionality is only implemented for contrast specified as three element character vectors.\\

\texttt{tidy} \quad \text{whether to output the results table with rownames as a first column \"row\". the table will also be coerced to \texttt{data.frame}}\\

\texttt{parallel} \quad \text{if FALSE, no parallelization. if TRUE, parallel execution using BiocParallel, see next argument \texttt{BPPARAM}.}\\

\texttt{BPPARAM} \quad \text{an optional parameter object passed internally to \texttt{bplapply} when \texttt{parallel=TRUE}. If not specified, the parameters last registered with \texttt{register} will be used.}\\

\texttt{...} \quad \text{optional arguments passed to \texttt{filterFun}}

\textbf{Details}\\

The results table when printed will provide the information about the comparison, e.g. "log2 fold change (MAP): condition treated vs untreated", meaning that the estimates are of log2(treated / untreated), as would be returned by \texttt{contrast=c("condition","treated","untreated"). Multiple results can be returned for analyses beyond a simple two group comparison, so \texttt{results} takes arguments \texttt{contrast} and \texttt{name} to help the user pick out the comparisons of interest for printing a results table. The use of the \texttt{contrast} argument is recommended for exact specification of the levels which should be compared and their order.\\

If \texttt{results} is run without specifying \texttt{contrast} or \texttt{name}, it will return the comparison of the last level of the last variable in the design formula over the first level of this variable. For example, for a simple two-group comparison, this would return the log2 fold changes of the second group over the first group (the reference level). Please see examples below and in the vignette.\\

The argument \texttt{contrast} can be used to generate results tables for any comparison of interest, for example, the log2 fold change between two levels of a factor, and its usage is described below. It can also accommodate more complicated numeric comparisons. The test statistic used for a contrast is:

\[
c^T \beta / \sqrt{c^T \Sigma c}
\]

The argument \texttt{name} can be used to generate results tables for individual effects, which must be individual elements of \texttt{resultsNames(object). These individual effects could represent continuous covariates, effects for individual levels, or individual interaction effects.}\\

Information on the comparison which was used to build the results table, and the statistical test which was used for p-values (Wald test or likelihood ratio test) is stored within the object returned by \texttt{results}. This information is in the metadata columns of the results table, which is accessible by calling \texttt{mcols} on the \texttt{DESeqResults} object returned by \texttt{results}.\\

On p-values:\\

By default, independent filtering is performed to select a set of genes for multiple test correction which maximizes the number of adjusted p-values less than a given critical value \texttt{alpha} (by default 0.1). See the reference in this man page for details on independent filtering. The filter used for maximizing the number of rejections is the mean of normalized counts for all samples in the
results

dataset. Several arguments from the filtered_p function of the genefilter package (used within the results function) are provided here to control the independent filtering behavior. In DESeq2 version >= 1.10, the threshold that is chosen is the lowest quantile of the filter for which the number of rejections is close to the peak of a curve fit to the number of rejections over the filter quantiles. 'Close to' is defined as within 1 residual standard deviation. The adjusted p-values for the genes which do not pass the filter threshold are set to NA.

By default, results assigns a p-value of NA to genes containing count outliers, as identified using Cook’s distance. See the cooksCutoff argument for control of this behavior. Cook’s distances for each sample are accessible as a matrix "cooks" stored in the assays() list. This measure is useful for identifying rows where the observed counts might not fit to a Negative Binomial distribution.

For analyses using the likelihood ratio test (using nbinomLRT), the p-values are determined solely by the difference in deviance between the full and reduced model formula. A single log2 fold change is printed in the results table for consistency with other results table outputs, however the test statistic and p-values may nevertheless involve the testing of one or more log2 fold changes. Which log2 fold change is printed in the results table can be controlled using the name argument, or by default this will be the estimated coefficient for the last element of resultsNames(object).

Value

For results: a DESeqResults object, which is a simple subclass of DataFrame. This object contains the results columns: baseMean, log2FoldChange, lfcSE, stat, pvalue and padj, and also includes metadata columns of variable information. The lfcSE gives the standard error of the log2FoldChange. For the Wald test, stat is the Wald statistic: the log2FoldChange divided by lfcSE, which is compared to a standard Normal distribution to generate a two-tailed pvalue. For the likelihood ratio test (LRT), stat is the difference in deviance between the reduced model and the full model, which is compared to a chi-squared distribution to generate a pvalue.

For resultsNames: the names of the columns available as results, usually a combination of the variable name and a level

For removeResults: the original DESeqDataSet with results metadata columns removed

References

Richard Bourgon, Robert Gentleman, Wolfgang Huber: Independent filtering increases detection power for high-throughput experiments. PNAS (2010), http://dx.doi.org/10.1073/pnas.0914005107

See Also

DESeq, filtered_R

Examples

```r
## Example 1: two-group comparison
dds <- makeExampleDESeqDataSet(m=4)
dds <- DESeq(dds)
res <- results(dds, contrast=c("condition","B","A"))
# with more than two groups, the call would look similar, e.g.:
# results(dds, contrast=c("condition","C","A"))
# etc.
```
## Example 2: two conditions, two genotypes, with an interaction term

```r
dds <- makeExampleDESeqDataSet(n=100,m=12)
dds$genotype <- factor(rep(rep(c("I","II"),each=3),2))
design(dds) <- ~ genotype + condition + genotype:condition
dds <- DESeq(dds)
resultsNames(dds)
```

# Note: design with interactions terms by default have betaPrior=FALSE

# the condition effect for genotype I (the main effect)
results(dds, contrast=c("condition","B","A"))

# the condition effect for genotype II
# this is, by definition, the main effect *plus* the interaction term
# (the extra condition effect in genotype II compared to genotype I).
results(dds, list( c("condition_B_vs_A","genotypeII.conditionB") ))

# the interaction term, answering: is the condition effect *different* across genotypes?
results(dds, name="genotypeII.conditionB")

## Example 3: two conditions, three genotypes

# --- Using interaction terms ---

```r
dds <- makeExampleDESeqDataSet(n=100,m=18)
dds$genotype <- factor(rep(rep(c("I","II","III"),each=3),2))
design(dds) <- ~ genotype + condition + genotype:condition
dds <- DESeq(dds)
resultsNames(dds)
```

# the condition effect for genotype I (the main effect)
results(dds, contrast=c("condition","B","A"))

# the condition effect for genotype III.
# this is the main effect *plus* the interaction term
# (the extra condition effect in genotype III compared to genotype I).
results(dds, contrast=list( c("condition_B_vs_A","genotypeIII.conditionB") ))

# the interaction term for condition effect in genotype III vs genotype I.
# this tests if the condition effect is different in III compared to I
results(dds, name="genotypeIII.conditionB")

# the interaction term for condition effect in genotype III vs genotype II.
# this tests if the condition effect is different in III compared to II
results(dds, contrast=list("genotypeIII.conditionB", "genotypeII.conditionB"))

# Note that a likelihood ratio could be used to test if there are any
# differences in the condition effect between the three genotypes.

# --- Using a grouping variable ---

# This is a useful construction when users just want to compare
# specific groups which are combinations of variables.

```r
dds$group <- factor(paste0(dds$genotype, dds$condition))
```
design(dds) <- ~ group
dds <- DESeq(dds)
resultsNames(dds)

# the condition effect for genotypeIII
results(dds, contrast=c("group", "IIIB", "IIIA"))

---

**rlog**

*Apply a 'regularized log' transformation*

**Description**

This function transforms the count data to the log2 scale in a way which minimizes differences between samples for rows with small counts, and which normalizes with respect to library size. The rlog transformation produces a similar variance stabilizing effect as `varianceStabilizingTransformation`, though rlog is more robust in the case when the size factors vary widely. The transformation is useful when checking for outliers or as input for machine learning techniques such as clustering or linear discriminant analysis. rlog takes as input a `DESeqDataSet` and returns a `RangedSummarizedExperiment` object.

**Usage**

```r
rlog(object, blind = TRUE, intercept, betaPriorVar, fitType = "parametric")
rlogTransformation(object, blind = TRUE, intercept, betaPriorVar, fitType = "parametric")
```

**Arguments**

- `object` a `DESeqDataSet`, or matrix of counts
- `blind` logical, whether to blind the transformation to the experimental design. blind=TRUE should be used for comparing samples in an manner unbiased by prior information on samples, for example to perform sample QA (quality assurance). blind=FALSE should be used for transforming data for downstream analysis, where the full use of the design information should be made. blind=FALSE will skip re-estimation of the dispersion trend, if this has already been calculated. If many of genes have large differences in counts due to the experimental design, it is important to set blind=FALSE for downstream analysis.
- `intercept` by default, this is not provided and calculated automatically. if provided, this should be a vector as long as the number of rows of object, which is log2 of the mean normalized counts from a previous dataset. this will enforce the intercept for the GLM, allowing for a "frozen" rlog transformation based on a previous dataset. You will also need to provide `mcols(object)$dispFit`.
- `betaPriorVar` a single value, the variance of the prior on the sample betas, which if missing is estimated from the data
- `fitType` in case dispersions have not yet been estimated for object, this parameter is passed on to `estimateDispersions` (options described there).
Details

Note that neither rlog transformation nor the VST are used by the differential expression estimation in DESeq, which always occurs on the raw count data, through generalized linear modeling which incorporates knowledge of the variance-mean dependence. The rlog transformation and VST are offered as separate functionality which can be used for visualization, clustering or other machine learning tasks. See the transformation section of the vignette for more details.

The transformation does not require that one has already estimated size factors and dispersions.

The regularization is on the log fold changes of the count for each sample over an intercept, for each gene. As nearby count values for low counts genes are almost as likely as the observed count, the rlog shrinkage is greater for low counts. For high counts, the rlog shrinkage has a much weaker effect. The fitted dispersions are used rather than the MAP dispersions (so similar to the varianceStabilizingTransformation).

The prior variance for the shrinkage of log fold changes is calculated as follows: a matrix is constructed of the logarithm of the counts plus a pseudocount of 0.5, the log of the row means is then subtracted, leaving an estimate of the log fold changes per sample over the fitted value using only an intercept. The prior variance is then calculated by matching the upper quantiles of the observed log fold change estimates with an upper quantile of the normal distribution. A GLM fit is then calculated using this prior. It is also possible to supply the variance of the prior. See the vignette for an example of the use and a comparison with varianceStabilizingTransformation.

The transformed values, rlog(K), are equal to \( rlog(K_{ij}) = \log_2(q_{ij}) = \beta_{i0} + \beta_{ij} \), with formula terms defined in DESeq.

The parameters of the rlog transformation from a previous dataset can be frozen and reapplied to new samples. See the 'Data quality assessment' section of the vignette for strategies to see if new samples are sufficiently similar to previous datasets. The frozen rlog is accomplished by saving the dispersion function, beta prior variance and the intercept from a previous dataset, and running rlog with 'blind' set to FALSE (see example below).

Value

a DESeqTransform if a DESeqDataSet was provided, or a matrix if a count matrix was provided as input. Note that for DESeqTransform output, the matrix of transformed values is stored in assay(rld). To avoid returning matrices with NA values, in the case of a row of all zeros, the rlog transformation returns zeros (essentially adding a pseudocount of 1 only to these rows).

References

Reference for regularized logarithm (rlog):


See Also

plotPCA, varianceStabilizingTransformation, normTransform

Examples

dds <- makeExampleDESeqDataSet(m=6,betaSD=1)
rld <- rlog(dds)
dists <- dist(t(assay(rld)))
plot(hclust(dists))

# run the rlog transformation on one dataset
design(dds) <- ~ 1
dds <- estimateSizeFactors(dds)
dds <- estimateDispersions(dds)
rld <- rlog(dds, blind=FALSE)

# apply the parameters to a new sample

ddsNew <- makeExampleDESeqDataSet(m=1)
mcols(ddsNew)$dispFit <- mcols(dds)$dispFit
betaPriorVar <- attr(rld,"betaPriorVar")
intercept <- mcols(rld)$rlog Intercept
rldNew <- rlog(ddsNew, blind=FALSE,
intercept=intercept,
betaPriorVar=betaPriorVar)

show method for DESeqResults objects

Description
Prints out the information from the metadata columns of the results object regarding the log2 fold changes and p-values, then shows the DataFrame using the standard method.

Usage
## S4 method for signature 'DESeqResults'
show(object)

Arguments

object a DESeqResults object

Author(s)
Michael Love

sizeFactors Accessor functions for the 'sizeFactors' information in a DESeq-DataSet object.

Description
The sizeFactors vector assigns to each column of the count matrix 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 (as performed by counts(dds, normalized=TRUE)). See DESeq for a description of the use of size factors. If gene-specific normalization is desired for each sample, use normalizationFactors.
Usage

```r
## S4 method for signature 'DESeqDataSet'
sizeFactors(object)
```

```r
## S4 replacement method for signature 'DESeqDataSet,numeric'
sizeFactors(object) <- value
```

**Arguments**

- `object`: a `DESeqDataSet` object.
- `value`: a numeric vector, one size factor for each column in the count data.

**Author(s)**

Simon Anders

**See Also**

- `estimateSizeFactors`

---

### summary

**Summarize DESeq results**

**Description**

Print a summary of the results from a DESeq analysis.

**Usage**

```r
## S3 method for class 'DESeqResults'
summary(object, alpha, ...)
```

**Arguments**

- `object`: a `DESeqResults` object
- `alpha`: the adjusted p-value cutoff. If not set, this defaults to the `alpha` argument which was used in `results` to set the target FDR for independent filtering, or if independent filtering was not performed, to 0.1.
- `...`: additional arguments

**Author(s)**

Michael Love

**Examples**

```r
dds <- makeExampleDESeqDataSet(m=4)
dds <- DESeq(dds)
res <- results(dds)
summary(res)
```
**unmix**

**Unmix samples using loss in a variance stabilized space**

**Description**

Unmixes samples in x according to pure components, using numerical optimization. The components in pure are added on the scale of gene expression (either normalized counts, or TPMs). The loss function when comparing fitted expression to the samples in x occurs in a variance stabilized space. This task is sometimes referred to as "deconvolution", and can be used, for example, to identify contributions from various tissues. Note: if the pbapply package is installed a progress bar will be displayed while mixing components are fit.

**Usage**

```r
unmix(x, pure, alpha, shift, loss = 1, quiet = FALSE)
```

**Arguments**

- **x**: normalized counts or TPMs of the samples to be unmixed
- **pure**: normalized counts or TPMs of the "pure" samples
- **alpha**: for normalized counts, the dispersion of the data when a negative binomial model is fit. this can be found by examining the asymptotic value of dispersionFunction(dds), when using fitType="parametric" or the mean value when using fitType="mean".
- **shift**: for TPMs, the shift which approximately stabilizes the variance of log shifted TPMs. Can be assessed with vsn::meanSdPlot.
- **loss**: either 1 (for L1) or 2 (for squared) loss function. Default is 1.
- **quiet**: suppress progress bar. default is FALSE, show progress bar if pbapply is installed.

**Value**

mixture components for each sample (rows), which sum to 1.

---

**varianceStabilizingTransformation**

*Apply a variance stabilizing transformation (VST) to the count data*

**Description**

This function calculates a variance stabilizing transformation (VST) from the fitted dispersion-mean relation(s) and then transforms the count data (normalized by division by the size factors or normalization factors), yielding a matrix of values which are now approximately homoskedastic (having constant variance along the range of mean values). The transformation also normalizes with respect to library size. The rlog is less sensitive to size factors, which can be an issue when size factors vary widely. These transformations are useful when checking for outliers or as input for machine learning techniques such as clustering or linear discriminant analysis.
Usage

varianceStabilizingTransformation(object, blind = TRUE,
    fitType = "parametric")

getVarianceStabilizedData(object)

Arguments

object
    a DESeqDataSet or matrix of counts

blind
    logical, whether to blind the transformation to the experimental design. blind=TRUE
    should be used for comparing samples in an manner unbiased by prior information on
    samples, for example to perform sample QA (quality assurance). blind=FALSE should be
    used for transforming data for downstream analysis, where the full use of the design
    information should be made. blind=FALSE will skip re-estimation of the dispersion
    trend, if this has already been calculated. If many of genes have large differences
    in counts due to the experimental design, it is important to set blind=FALSE for
    downstream analysis.

fitType
    in case dispersions have not yet been estimated for object, this parameter is
    passed on to estimateDispersions (options described there).

Details

For each sample (i.e., column of counts(dds)), the full variance function is calculated from the raw
variance (by scaling according to the size factor and adding the shot noise). We recommend a blind
estimation of the variance function, i.e., one ignoring conditions. This is performed by default, and
and can be modified using the 'blind' argument.

Note that neither rlog transformation nor the VST are used by the differential expression estimation
in DESeq, which always occurs on the raw count data, through generalized linear modeling which
incorporates knowledge of the variance-mean dependence. The rlog transformation and VST are
offered as separate functionality which can be used for visualization, clustering or other machine
learning tasks. See the transformation section of the vignette for more details.

The transformation does not require that one has already estimated size factors and dispersions.
A typical workflow is shown in Section Variance stabilizing transformation in the package vignette.

If estimateDispersions was called with:

fitType="parametric", a closed-form expression for the variance stabilizing transformation is
used on the normalized count data. The expression can be found in the file ‘vst.pdf’ which is
distributed with the vignette.

fitType="local", the reciprocal of the square root of the variance of the normalized counts, as
derived from the dispersion fit, is then numerically integrated, and the integral (approximated by a
spline function) is evaluated for each count value in the column, yielding a transformed value.

fitType="mean", a VST is applied for Negative Binomial distributed counts, ‘k’, with a fixed
dispersion, ’a’: ( 2 asinh(sqrt(a k)) - log(a) - log(4) )/log(2).

In all cases, the transformation is scaled such that for large counts, it becomes asymptotically (for
large values) equal to the logarithm to base 2 of normalized counts.

The variance stabilizing transformation from a previous dataset can be frozen and reapplied to new
samples. See the 'Data quality assessment' section of the vignette for strategies to see if new
samples are sufficiently similar to previous datasets. The frozen VST is accomplished by saving the
dispersion function accessible with dispersionFunction, assigning this to the DESeqDataSet with
the new samples, and running varianceStabilizingTransformation with 'blind' set to FALSE (see
example below). Then the dispersion function from the previous dataset will be used to transform the new sample(s).

Limitations: In order to preserve normalization, the same transformation has to be used for all samples. This results in the variance stabilizition to be only approximate. The more the size factors differ, the more residual dependence of the variance on the mean will be found in the transformed data. rlog is a transformation which can perform better in these cases. As shown in the vignette, the function meanSdPlot from the package vsn can be used to see whether this is a problem.

Value

varianceStabilizingTransformation returns a DESeqTransform if a DESeqDataSet was provided, or returns a a matrix if a count matrix was provided. Note that for DESeqTransform output, the matrix of transformed values is stored in assay(vsd). getVarianceStabilizedData also returns a matrix.

Author(s)

Simon Anders

References

Reference for the variance stabilizing transformation for counts with a dispersion trend:

See Also

plotPCA, rlog, normTransform

Examples

```r
dds <- makeExampleDESeqDataSet(m=6)
vsd <- varianceStabilizingTransformation(dds)
dists <- dist(t(assay(vsd)))
plot(hclust(dists))

# learn the dispersion function of a dataset
design(dds) <- ~ 1
dds <- estimateSizeFactors(dds)
dds <- estimateDispersions(dds)

# use the previous dispersion function for a new sample
ddsNew <- makeExampleDESeqDataSet(m=1)
ddsNew <- estimateSizeFactors(ddsNew)
dispersionFunction(ddsNew) <- dispersionFunction(dds)
vsdNew <- varianceStabilizingTransformation(ddsNew, blind=FALSE)
```
vst  

*Quickly estimate dispersion trend and apply a variance stabilizing transformation*

**Description**

This is a wrapper for the `varianceStabilizingTransformation` (VST) that provides much faster estimation of the dispersion trend used to determine the formula for the VST. The speed-up is accomplished by subsetting to a smaller number of genes in order to estimate this dispersion trend. The subset of genes is chosen deterministically, to span the range of genes’ mean normalized count. This wrapper for the VST is not blind to the experimental design: the sample covariate information is used to estimate the global trend of genes’ dispersion values over the genes’ mean normalized count. It can be made strictly blind to experimental design by first assigning a design of ~1 before running this function, or by avoiding subsetting and using `varianceStabilizingTransformation`.

**Usage**

```r
vst(object, blind = TRUE, nsub = 1000, fitType = "parametric")
```

**Arguments**

- `object` a DESeqDataSet or a matrix of counts
- `blind` logical, whether to blind the transformation to the experimental design (see `varianceStabilizingTransformation`)
- `nsub` the number of genes to subset to (default 1000)
- `fitType` for estimation of dispersions: this parameter is passed on to `estimateDispersions` (options described there)

**Value**

a DESeqTransform object or a matrix of transformed, normalized counts

**Examples**

```r
dds <- makeExampleDESeqDataSet(n=20000, m=20)
vsd <- vst(dds)
```
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