Package 'baySeq'

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Type Package Title Empirical Bayesian analysis of patterns of differential expression in count data Version 2.30.0 Date 2012-12-20 Depends R (>= 2.3.0), methods, GenomicRanges, abind, parallel Imports edgeR Suggests BiocStyle, BiocGenerics Author Thomas J. Hardcastle Maintainer Thomas J. Hardcastle <tjh48@cam.ac.uk> **Description** This package identifies differential expression in high-throughput 'count' data, such as that derived from next-generation sequencing machines, calculating estimated posterior likelihoods of differential expression (or more complex hypotheses) via empirical Bayesian methods. License GPL-3

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baySeq-package *Empirical Bayesian analysis of patterns of differential expression in count data.*

Description

This package is intended to identify differential expression in high-throughput 'count' data, such as that derived from next-generation sequencing machines. We achieve this by empirical bayesian methods, first bootstrapping to estimate prior parameters from the data and then assessing posterior likelihoods of the models proposed.

Details

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To use the package, construct a [countData](#page-4-1) object and use the functions documented in [getPriors](#page-16-1) to empirically determine priors on the data. Then use the functions documented in [getLikelihoods](#page-10-1) to establish posterior likelihoods for the models proposed. A few convenience functions, [getTPs](#page-18-1) and [topCounts](#page-34-1) are also included.

The package (optionally) makes use of the 'snow' package for parallelisation of computationally intensive functions. This is highly recommended for large data sets.

See the vignette for more details.

Author(s)

Thomas J. Hardcastle

Maintainer: Thomas J. Hardcastle <tjh48@cam.ac.uk>

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

Examples

```
# See vignette for more examples.
```
load test data data(simData)

```
# replicate structure of data
replicates <- c("simA", "simA", "simA", "simA", "simA", "simB", "simB", "simB", "simB", "simB")
```

```
# define hypotheses on data
groups <- list(NDE = c(1,1,1,1,1,1,1,1,1,1), DE = c(1,1,1,1,1,2,2,2,2,2))
```

```
# construct 'countData' object
CD <- new("countData", data = simData, replicates = replicates, groups =
groups)
```

```
#estimate library sizes for countData object
libsizes(CD) <- getLibsizes(CD)
```

```
# estimate prior distributions on 'countData' object using negative binomial
# method. Other methods are available - see getPriors
CDPriors <- getPriors.NB(CD, cl = NULL)
```

```
# estimate posterior likelihoods for each row of data belonging to each hypothesis
CDPost <- getLikelihoods(CDPriors, cl = NULL)
```

```
# display the rows of data showing greatest association with the second
# hypothesis (differential expression)
topCounts(CDPost, group = "DE", number = 10)
```
find true positive selection rate

getTPs(CDPost, group = "DE", TPs = 1:100)[1:100]

allModels *Function to generate all possible models for a countData object based on the replicate data.*

Description

This function populates the '@groups' slot of the supplied countData object with all possible models for equivalence/non-equivalence of expression between replicate groups.

Usage

allModels(CD)

Arguments

CD A countData object with a populated '@replicates' slot.

Details

Given a large number of different replicate groups, the total number of possible models listed in the '@groups' slot rises exponentially. This function will attempt to list them all. The use of consensus priors (see [getPriors](#page-16-1)) is recommended if the number of models is high.

Value

A [countData](#page-4-1) with populated '@groups' slot.

Author(s)

Thomas J. Hardcastle

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

See Also

[getPriors](#page-16-1)

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Examples

```
# load test data
data(simData)
# Create a {countData} object from test data, supposing that there are
# multiple experimental groups present.
replicates <- c("simA", "simA", "simB", "simC", "simC", "simD", "simE", "simE", "simF", "simG")
CD <- new("countData", data = simData, replicates = replicates)
CD <- allModels(CD)
# The total number of models generated is high.
length(CD@groups)
```
baySeq-classes *baySeq - classes*

Description

The countData class is used to define summaries of count data and establishing prior and posterior parameters on distributions defined upon the count data.

Slots

Objects of these class contain the following components:

Details

The seglens slot describes, for each row of the data object, the length of the 'segment' that contains the number of counts described by that row. For example, if we are looking at the number of hits matching genes, the seglens object would consist of transcript lengths. Exceptionally, we may want to use different segment lengths for different samples and so the slot takes the form of a matrix. If the matrix has only one column, it is duplicated for all samples. Otherwise, it should have the same number of columns as the '@data' slot. If the slot is the empty matrix, then it is assumed that all segments have the same length.

Methods

The standard methods 'new', 'dim', '[', 'show', 'rbind' and 'c' have been defined for these classes. The methods 'groups', 'groups<-', 'replicates', 'replicates<-', 'libsizes' and 'libsizes<-' have also been defined in order to access and modify these slots, and their use is recommended. The method 'flatten' can be used to produce a data.frame object containing much of the basic data in a member of this class.

Author(s)

Thomas J. Hardcastle

Examples

```
#load test data
data(simData)
# Create a 'countData' object from test data.
replicates <- c("simA", "simA", "simA", "simA", "simA", "simB", "simB", "simB", "simB", "simB")
groups <- list(NDE = c(1,1,1,1,1,1,1,1,1,1), DE = c(1,1,1,1,1,2,2,2,2,2))
CD <- new("countData", data = simData, replicates = replicates, groups = groups)
#estimate library sizes for countData object
libsizes(CD) <- getLibsizes(CD)
CD[1:10,]
dim(CD)
```


Description

This function takes a numeric vector and finds the value which splits the data into two sets of minimal total variance, weighted by the size of subsets (Otsu's method). It is principally intended to be a quick and easy way of separating bimodally distributed data.

Usage

```
bimodalSeparator(x, weights = NULL, minperc = 0.1, elbow = NULL)
```
Arguments

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elbow If set, finds the 'left' or 'right' elbow of variance, instead of the minimum; defaults to NULL. See Details.

Details

This function is intended to give a quick and easy way of splitting bimodally distributed data. Where there are large outliers in the data, it may be that the value which minimises the variance does not split the bimodal data but isolates the outliers. The 'minperc' parameter can be used to ensure that each subset of the split data will be of some minimum size, avoiding the outlier problem.

If 'elbow $=$ NULL' (the default) then the split occurs at the value that minimises the variance, $x0$. If 'elbow = left' then we attempt to find the elbow point to the left of the value that minimises the variance, if 'elbow = right' then we find the elbow point to the right of the value that minimises the variance. Elbow points are found by drawing a line from the first point (for the left elbow) or the last point (for the right elbow) to x0, and finding the location on the curve of summed variances which maximises the distance to that line.

Value

Numeric value which splits the data.

Author(s)

Thomas J. Hardcastle

Examples

bimodalSeparator(c(rnorm(200, mean = $c(5,7)$, sd = 1)))

Description

This 'countData' object is derived from the data set 'simData' and contains the estimated likelihoods of differential expression. This data set is intended to be used to speed the processing of the examples.

Usage

CDPost

Format

A 'countData' object.

Source

Simulation.

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

Description

This 'countData' object is derived from the data set 'simData' and contains the estimated priors. This data set is intended to be used to speed the processing of the examples.

Usage

CDPriors

Format

A 'countData' object.

Source

Simulation.

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

densityFunction-class *Class* "densityFunction"

Description

This function fills the '@densityFunction' slot of a 'countData' object. It defines the distribution used to estimate posterior likelihoods, and associated values used in these calculations.

Objects from the Class

Objects can be created by calls of the form new("densityFunction", ...).

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Slots

description: A description of the distribution defined.

- density: A "function", defining the likelihood of a data array given observed data and hyperparameters.
- initiatingValues: A "list" of functions (may be supplied as numerics) that define initial values of numeric prior discovery.
- equalOverReplicates: A "logical", describing which of the hyperparameters are equally marginally distributed over all groups, and which are not.
- lower: A "function", required to define the lower limit of optimisation in the case where only one hyperparameter is *not* equally marginally distributed over all groups.
- upper: A "function", required to define the upper limit of optimisation, as for 'lower', above.
- stratifyFunction: An optional "function", used to stratify the data for more accurate prior estimation.
- stratifyBreaks: An optional "numeric", used to define the number of strata in a stratification.
- nullFunction: An optional "function" on the hyperparameters, used to generate a one-dimensional distribution which can be partitioned to identify 'null' data.

orderingFunction: An optional "function" for ordering the data between groups of a model.

modifyNullPriors: An optional "function" for modifying the priors for the 'null' data.

Methods

No methods defined with class "densityFunction" in the signature.

Author(s)

Thomas J. Hardcastle

Examples

showClass("densityFunction")

densityFunctions *Lists all currently available densityFunctions.*

Description

The [densityFunction](#page-4-1) objects define the distribution and various other parameters used to analyse the data stored in a [countData](#page-4-1) object.

Usage

densityFunctions()

Character string giving names of available [densityFunction](#page-4-1) objects.

Author(s)

Thomas J. Hardcastle

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

See Also

[densityFunction](#page-4-1)

getLibsizes *Estimates library scaling factors (library sizes) for count data.*

Description

This function estimates the library scaling factors that should be used for either a 'countData', or a matrix of counts and replicate information.

Usage

 $getLib sizes(CD, data, replicates, subset = NULL, estimationType = c("quantile", "total", "edgeR"), quar$

Arguments

Details

This function estimates the library scaling factors (surrogates for library size) in one of several ways, depending on the 'estimationType' argument. 'total' will give the library sizes by summing all counts in each sample. 'quantile' will give a library scaling factor by the method of Bullard et al (Bioinformatics 2010), summing all counts in each sample whose value below the qth quantile of non-zero counts for that sample. 'edgeR' uses the Trimmed Mean of M-vales (TMM) method of Robinson \& Oshlack (Genome Biology, 2010) via the 'edgeR' calcNormFactors function; other options are available through this function.

If a [countData](#page-4-1) object 'cD' is given, the library sizes will be inferred from this. Alternatively, a matrix of count values (columns are libraries) and a replicate structure (a vector defining which samples belong to which replicate group) can be given.

Value

If a \link{countData} object is given, an identical object will be returned with updated library sizes. If only the data and replicate structure are given, a numerical vector of library sizes (scaling factors) for each library in the data will be returned.

Author(s)

Thomas J. Hardcastle

See Also

[countData](#page-4-1)

Examples

```
data(simData)
replicates <- c(1,1,1,1,1,2,2,2,2,2)
groups <- list(c(1,1,1,1,1,1,1,1,1,1), c(1,1,1,1,1,2,2,2,2,2))
CD <- new("countData", data = simData, replicates = replicates, groups = groups)
```
libsizes(CD) <- getLibsizes(CD)

Description

These functions calculate posterior probabilities for each of the rows in a 'countData' object belonging to each of the models specified in the 'groups' slot.

Usage

```
getLikelihoods.NB(cD, prs, pET = "BIC", marginalise = FALSE, subset = NULL,
priorSubset = NULL, bootStraps = 1, conv = 1e-4, nullData = FALSE,
returnAll = FALSE, returnPD = FALSE, verbose = TRUE, discardSampling =
FALSE, cl, \ldots)getLikelihoods.BB(cD, prs, pET = "BIC", marginalise = FALSE, subset =
NULL, priorSubset = NULL, bootStraps = 1, conv = 1e-04, nullData = FALSE,
returnAll = FALSE, returnPD = FALSE, verbose = TRUE, discardSampling =
FALSE, cl, \ldots)getLikelihoods(cD, prs, pET = "BIC", marginalise = FALSE, subset = NULL,
priorSubset = NULL, bootStraps = 1, bsNullOnly = TRUE, conv = 1e-4, nullData = FALSE,
weightByLocLikelihoods = TRUE, modelPriorSets = list(),
modelPriorValues = list(), returnAll = FALSE, returnPD = FALSE, verbose
= TRUE, discardSampling = FALSE, modelLikes = TRUE, cl = NULL, tempFile
= NULL, largeness = 1e+08)
```
Arguments

Details

These functions estimate, under the assumption of various distributions, the (log) posterior likelihoods that each count belongs to a group defined by the @group slot of the input object. The posterior likelihoods are stored on the natural log scale in the @posteriors slot of the [countData](#page-4-1) object generated by this function. This is because the posterior likelihoods are calculated in this form, and ordering of the counts is better done on these log-likelihoods than on the likelihoods.

If 'pET = "none"' then no attempt is made to re-estimate the prior likelihoods given in the 'prs' variable. However, if 'pET = "BIC"', then the function will attempt to estimate the prior likelihoods by using the Bayesian Information Criterion to identify the proportion of the data best explained by each model and taking these proportions as prior. Alternatively, an iterative re-estimation of priors is possible ('pET = "iteratively"'), in which an inital estimate for the prior likelihoods of the models is used to calculated the posteriors and then the priors are updated by taking the mean of the posterior likelihoods for each model across all data. This often works well, particularly if the 'BIC' method is used (see Hardcastle & Kelly 2010 for details). However, if the data are sufficiently non-independent, this approach may substantially mis-estimate the true priors. If it is possible to select a representative subset of the data by setting the variable 'subsetPriors' that is sufficiently independent, then better estimates may be acquired.

In certain circumstances, it may be expected that certain subsets of the data are likely to behave differently to others; for example, if a set of genes are expected in advance to be differentially expressed, while the majority of the data are not. In this case, it may be advantageous (in terms of improving false discovery rates) to specify these different subsets in the modelPriorSets variable. However, care should be taken downstream to avoid confirmation bias.

Filtering the data may be extremely advantageous in reducing run time. This can be done by passing a numeric vector to 'subset' defining a subset of the data for which posterior likelihoods are required.

See Hardcastle & Kelly (2010) for a definition of the negative binomial methods.

A 'cluster' object is strongly recommended in order to parallelise the estimation of posterior likelihoods, particularly for the negative binomial method. However, passing NULL to the cl variable will allow the functions to run in non-parallel mode.

The 'getLikelihoods.NB' and 'getLikelihoods.BB' functions are now deprecated and will soon be removed.

Value

A [countData](#page-4-1) object.

Author(s)

Thomas J. Hardcastle

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

See Also

[countData](#page-4-1), [getPriors](#page-16-1), [topCounts](#page-34-1), [getTPs](#page-18-1)

Examples

See vignette for more examples.

If we do not wish to parallelise the functions we set the cluster # object to NULL.

```
cl <- NULL
```

```
# Alternatively, if we have the 'snow' package installed we
# can parallelise the functions. This will usually (not always) offer
# significant performance gain.
```

```
## Not run: try(library(snow))
## Not run: try(cl <- makeCluster(4, "SOCK"))
```

```
# load test data
data(simData)
```
Create a {countData} object from test data.

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```
replicates <- c("simA", "simA", "simA", "simA", "simA", "simB", "simB", "simB", "simB", "simB")
groups <- list(NDE = c(1,1,1,1,1,1,1,1,1,1), DE = c(1,1,1,1,1,2,2,2,2,2))
CD <- new("countData", data = simData, replicates = replicates, groups = groups)
# set negative binomial density function
densityFunction(CD) <- nbinomDensity
#estimate library sizes for countData object
libsizes(CD) <- getLibsizes(CD)
# Get priors for negative binomial method
## Not run: CDPriors <- getPriors(CD, samplesize = 10^5, estimation = "QL", cl = cl)
# the `CDPriors' object.
# To speed up the processing of this example, we have already created
data(CDPriors)
# Get likelihoods for data with negative binomial method.
CDPost <- getLikelihoods(CDPriors, pET = "BIC", cl = cl)
try(stopCluster(cl))
```


Description

For likelihoods of the data given a set of models, this function calculates the posterior likelihoods of the models given the data. An internal function of baySeq, which should not in general be called by the user.

Usage

```
getPosteriors(ps, prs, pET = "none", marginalise = FALSE, groups, priorSubset = NULL, maxit = 100, accur
1e-5, eqOverRep = NULL, cl = cl)
```
Arguments

Details

An internal function, that will not in general be called by the user. It takes the log-likelihoods of the data given the models being tested and returns the posterior likelihoods of the models.

The function may attempt to estimate the prior likelihoods either by using the Bayesian Information Criterion ('pET = "BIC"') to identify the proportion of the data best explained by each model and taking these proportions as prior. Alternatively, an iterative re-estimation of priors is possible ('pET = "iteratively"', in which an inital estimate for the prior likelihoods of the models is used to calculated the posteriors and then the priors are updated by taking the mean of the posterior likelihoods for each model across all data.

Value

A list containing posteriors: estimated posterior likelihoods of the model for each count (log-scale) priors: estimated (or given) prior probabilities of the model

Author(s)

Thomas J. Hardcastle

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

See Also

[getLikelihoods](#page-10-1)

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Examples

```
# Simulate some log-likeihoods of data given models (each model
# describes one column of the 'ps' object).
ps <- log(rbind(
                cbind(runif(10000, 0, 0.1), runif(10000, 0.3, 0.9)),
                cbind(runif(10000, 0.4, 0.9), runif(1000, 0, 0.2))))
# get posterior log-likelihoods of model, estimating prior likelihoods
# of each model from the data.
pps \leq getPosteriors(ps, prs \leq c(0.5, 0.5), pET = "none", cl =
NULL)
pps$priors
pps$posteriors[1:10,]
```
getPriors *Estimates prior parameters for the underlying distributions of 'count' data.*

Description

These functions estimate, via maximum likelihood methods, the parameters of the underlying distributions specified in the 'densityFunction' slot of the countData object. A special case is maintained for historical reasons; getPriors.NB estimates parameters for a negative binomial distribution using quasi-maximum-likelihood methods.

Usage

```
getPriors(cD, samplesize = 1e5, samplingSubset = NULL,
consensus = FALSE, cl, verbose = TRUE)
getPriors.NB(cD, samplesize = 1e5, samplingSubset = NULL,
equalDispersions = TRUE, estimation = "QL", verbose = TRUE, zeroML =
FALSE, consensus = FALSE, cl, ...)
```
Arguments

Details

These functions empirically estimate prior parameters for the distributions used in estimating posterior likelihoods of each count belonging to a particular group.

For priors estimated for the negative binomial methods, three options are available. Differences in the options focus on the way in which the dispersion is estimated for the data. In simulation studies, quasi-likelihood methods ('estimation = " QL'' ') performed best and so these are used by default. Alternatives are maximum-likelihood methods ('estimation = "ML"'), and the 'edgeR' packages moderated dispersion estimates ('estimation = "edgeR"').

The priors estimated for the negative binomial methods ('getPriors.NB') may assume that the dispersion of data for a given row is identical for all group structures defined in 'cD@groups' ('equalDispersions = TRUE'). Alternatively, the dispersions may be estimated individually for each group structure ('equalDispersions = FALSE'). Unless there is a strong reason for believing that the data are differently dispersed between groups, 'equalDispersions = TRUE' is recommended. If 'estimation = "edgeR"' then this parameter is ignored and dispersion is assumed identical for all group structures.

If all counts in a given row for a given group are zero, then maximum and quasi-likelihood estimation methods will result in a zero parameter for the mean. In analyses where segment length is a factor, this makes it hard to differentiate between (for example) a region which contains no reads but is only ten bases long and one which likewise contains no reads but is ten megabases long. If 'zeroML' is FALSE, therefore, the dispersion is set to 1 and the mean estimated as the value that leaves the likelihood of zero data at fifty percent.

If 'consensus = TRUE', then a consensus distribution is created and used for each group in the 'cD' object. This allows faster computation of the priors and likelihoods, but with some degradation of accuracy.

A 'cluster' object is recommended in order to estimate the priors for the negative binomial distribution. Passing NULL to this variable will cause the function to run in non-parallel mode.

Value

A [countData](#page-4-1) object.

Author(s)

Thomas J. Hardcastle

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References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

See Also

[countData](#page-4-1), [getLikelihoods](#page-10-1)

Examples

```
# See vignette for more examples.
# If we do not wish to parallelise the functions we set the cluster
# object to NULL.
cl <- NULL
# Alternatively, if we have the 'snow' package installed we
# can parallelise the functions. This will usually (not always) offer
# significant performance gain.
## Not run: try(library(snow))
## Not run: try(cl <- makeCluster(4, "SOCK"))
# load test data
data(simData)
# Create a {countData} object from test data.
replicates <- c("simA", "simA", "simA", "simA", "simA", "simB", "simB", "simB", "simB", "simB")
groups <- list(NDE = c(1,1,1,1,1,1,1,1,1,1), DE = c(1,1,1,1,1,2,2,2,2,2))
CD <- new("countData", data = simData, replicates = replicates, groups = groups)
#estimate library sizes for countData object
libsizes(CD) <- getLibsizes(CD)
# Get priors for negative binomial method
CDPriors \leq getPriors.NB(CD, samplesize = 10^5, estimation = "QL", cl = cl)
```
getTPs *Gets the number of true positives in the top n counts selected by ranked posterior likelihoods*

Description

If the true positives are known, this function will return a vector, the ith member of which gives the number of true positives identified if the top i counts, based on estimated posterior likelihoods, are chosen.

Usage

```
getTPs(cD, group, decreasing = TRUE, TPs)
```
Arguments

Details

In the rare (or simulated) cases where the true positives are known, this function will calculate the number of true positives selected at any cutoff.

The 'group' can be defined either as the number of the element in 'cD@groups' or as a string which will be partially matched to the names of the 'cD@groups' elements.If group = NULL, then the function looks at the posterior likelihoods that the data have no true differential expression (if calculated).

Value

A vector, the ith member of which gives the number of true positives identified if the top i counts are chosen.

Author(s)

Thomas J. Hardcastle

See Also

[countData](#page-4-1)

Examples

See vignette for more examples.

```
...<br># See vignette for more examples.<br># We load in a `countData' object containing the estimated posterior
% See vignette for more examples.<br># We load in a `countData' object containing the es<br># likelihoods of expression (see `getLikelihoods').
```
data(CDPost)

```
# If the first hundred rows in the 'simData' matrix are known to be
# truly differentially expressed (the second hypothesis defined in the
# 'groups' list) then we find the number of true positives for the top n
# genes selected as the nth member of
```

```
getTPs(CDPost, group = "DE", decreasing = TRUE, TPs = 1:100)
```
makeOrderings *Construct orderings for count data given a model structure and an ordering function.*

Description

Given a model structure as defined in the '@groups' slot of a [countData](#page-4-1) object containing more than one group, it is often possible to define an ordering on the groups for a given genomic event. To take a simple example, if the average expression of a gene is higher in sample set A then in sample set B, then we might impose an ordering $A > B$.

Usage

makeOrderings(cD, orderingFunction)

Arguments

cD A [countData](#page-4-1) object, or a descendant thereof.

orderingFunction

A function defining the orderings. If not given, will be taken from the '@densityFunction' slot of 'cD'. See Details, and examples below.

Details

The orderingFunction takes 'dat' and 'observables' as arguments. 'dat' is equivalent to the '@data' slot of the 'cD' object, and 'observables' the combined data in the '@sampleObservables', '@rowObservables' and '@cellObservables' slots.

Value

A [countData](#page-4-1) with populated '@orderings' slot.

Author(s)

Thomas J. Hardcastle

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

Examples

load test data data(simData)

Create a countData object from test data

```
replicates <- c("simA", "simA", "simA", "simA", "simA", "simB", "simB", "simB", "simB", "simB")
groups <- list(NDE = c(1,1,1,1,1,1,1,1,1,1), DE = c(1,1,1,1,1,2,2,2,2,2))
CD <- new("countData", data = simData, replicates = replicates, groups = groups)
libsizes(CD) <- getLibsizes(CD)
# order on expression normalised for library size (scaling factor) and gene length
CD <- makeOrderings(CD, orderingFunction = function(dat, observables) dat / observables$libsizes)
# orderings calculated for DE group
head(CD@orderings)
# load test (paired) data
data(pairData)
# create a countData object from paired data
pairCD <- new("countData", data = list(pairData[,1:4], pairData[,5:8]),
                  replicates = c(1,1,2,2),groups = list(NDE = c(1,1,1,1), DE = c(1,1,2,2)),
               densityFunction = bbDensity)
libsizes(pairCD) <- getLibsizes(pairCD)
# order on (log-)ratio of pairs, with fudge-factor on zeros.
pairCD <- makeOrderings(pairCD, orderingFunction = function(dat, observables) {
                                      data <- dat / observables$libsizes
                                      adjmin \leq - min(data[data > 0]) / 10
                                      log(data[,, 1] + adjmin) - log(data[,, 2] + adjmin)})
# orderings calculated for DE group
head(pairCD@orderings)
```

```
marginaliseEqual Computes marginal likelihoods that two replicate groups are equal.
```
Description

In cases where multiple models are simultaneously evaluated in the 'getLikelihoods' function, the posterior likelihoods for each model in which two conditions are equivalent can be summed to give the marginal likelihood of equivalence for all biomolecular events (i.e., data rows).

Usage

```
marginaliseEqual(cD, r1, r2)
```


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Arguments

Value

A vector of marginal posterior likelihoods defining the probability that the two group identifiers are equal for each row of the data.

Author(s)

Thomas J. Hardcastle

See Also

[allModels](#page-3-1) [marginalisePairwise](#page-23-1)

Examples

```
# load test data
data(simData)
# Create a {countData} object from test data, supposing that there are
# multiple experimental groups present.
replicates <- c("simA", "simA", "simB", "simC", "simC", "simD", "simE", "simE", "simF", "simG")
CD <- new("countData", data = simData, replicates = replicates)
CD <- allModels(CD)
# The total number of models generated is high.
length(CD@groups)
# Priors and likelihoods acquired through standard means.
## Not run: CD <- getPriors(CD, cl = cl)
## Not run: CD <- getLikelihoods(CD, cl = cl)
# Marginal likelihood that 'simA' and 'simD' replicate groups are equal
# for each row of the data.
## Not run: marginaliseEqual(CD, "simA", "simD")
```
marginalisePairwise *Computes marginal likelihoods that two replicate groups differ, in a paticular direction.*

Description

In cases where multiple models are simultaneously evaluated in the 'getLikelihoods' function, the posterior likelihoods for each model in which one condition is greater than another can be summed to give the marginal likelihood of (directed) difference for all biomolecular events (i.e., data rows).

Usage

marginalisePairwise(cD, greaterThan, lessThan)

Arguments

Value

A vector of marginal posterior likelihoods defining the probability that the two group identifiers are (directionally) different for each row of the data.

Author(s)

Thomas J. Hardcastle

See Also

[allModels](#page-3-1) [marginaliseEqual](#page-21-1)

Examples

```
# load test data
data(simData)
```
Create a {countData} object from test data, supposing that there are # multiple experimental groups present.

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```
replicates <- c("simA", "simA", "simB", "simC", "simC", "simD", "simE", "simE", "simF", "simG")
CD <- new("countData", data = simData, replicates = replicates)
CD <- allModels(CD)
# The total number of models generated is high.
length(CD@groups)
# Priors and likelihoods acquired through standard means.
## Not run: CD <- getPriors(CD, cl = cl)
## Not run: CD <- getLikelihoods(CD, cl = cl)
# Marginal likelihood that 'simA' condition is greater than 'simD' group
# for each row of the data.
## Not run: marginalisePairwise(CD, "simA", "simD")
```
methObservables *Generation of intermediate values in likelihood estimation for methylation data.*

Description

Estimating prior and posterior values for methylation data that account for non-conversion rates is a time-consuming process. Significant increases in speed can be made by calculating in advance sets of data that will be re-used at several points of these analyses. This function populates the '@cellObservables' slot of a 'countData' object that contains a 'nonconversion' object in the '@sampleObservables' slot.

Usage

```
methObservables(mD, tail = 0.01)
```
Arguments

Details

For loci with large numbers of observed cytosines, the full dataset to be pre-computed will be very large. However, only the pre-computations near the average expression level will contribute significantly to the estimated priors and posteriors. The 'tail' parameter sets the quantile at which the distribution is considered to no longer contribute significantly to the results. Values below 0.1 are probably acceptable under nearly all circumstances.

Value

A [countData](#page-4-1) object with the '@cellObservables' slot populated with temporary values useful in the faster calculation of likelihoods.

Author(s)

Thomas J. Hardcastle

Description

This data set is a data.frame ('mobAnnotation') describing three thousand small RNA loci identified in a set of Arabidopsis grafting experiments.

The data acquired through sequencing for these loci is found in data file 'mobData'.

Usage

mobAnnotation

Format

A data.frame defining chromosome and position of the sRNA loci.

Source

Illumina sequencing.

References

Molnar A. and Melnyk C.W. et al. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science (2010)

See Also

[mobData](#page-26-1)

mobData *Data from a set of small RNA sequencing experiments carried out on grafts of Arabidopsis thaliana intended for differential expression analyses.*

Description

This data set is a matrix ('mobData') of counts acquired for three thousand small RNA loci from a set of Arabidopsis grafting experiments. Three different biological conditions exist within these data; one in which a Dicer 2,3,4 triple mutant shoot is grafted onto a Dicer 2,3,4 triple mutant root (SL236 and SL260), one in which a wild-type shoot is grafted onto a wild-type root (SL239 and SL240), and one in which a wild-type shoot is grafted onto a Dicer 2,3,4 triple mutant root (SL237 and SL238). Dicer 2,3,4 is required for the production of 22nt and 24nt small RNAs, as well as some 21nt ones. Consequently, if we detect differentially expressed sRNA loci in the root stock of the grafts, we can make inferences about the mobility of small RNAs.

The annotation of the loci from which these data derive is in data file 'mobAnnotation'.

Usage

mobData

Format

A matrix of which each of the six columns represents a sample, and each row an sRNA locus (acquired by sequencing).

Source

Illumina sequencing.

References

Molnar A. and Melnyk C.W. et al. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science (2010)

See Also

[mobAnnotation](#page-25-1)

Description

This data set is a matrix ('pairData') of simulated counts from a set of high-throughput sequencing data from a paired experimental design. The first four columns of data are to be paired with the second four columns of data respectively. The first two paired samples form one replicate group, the second two paired samples form another replicate group. The first hundred rows of the data are truly differentially expressed between replicate groups, the second hundred are differentially expressed between pairs, the remainder have no differential expression.

It is simulated according to a set of Poisson distributions whose parameters for each row are determined by a beta distribution on the relative proportions of data in each pairing.

Usage

pairData

Format

A matrix of which each of the eight columns represents a sample, and each row some discrete data (acquired by sequencing).

Source

Simulation.

plotMA.CD *'MA'-plot for count data.*

Description

This function creates an MA-plot from two sets of samples. For those data where the log-ratio is infinite (because in one set of sample data all observed counts are zero), we plot instead the log-values of the other group.

Usage

```
plotMA.CD(cD, samplesA, samplesB, normaliseData = TRUE, scale = NULL,
xlab = "A", ylab = "M", ...)
```
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Arguments

Details

The samples sets can be identified either by a numeric vector which specifies the columns of data from the countData object 'cD', or by a character vector. If a character vector is used, the members of the character vector will first be searched for in the @replicates slot of the 'cD' object. Any members of the vector not found in the replicates slot, will be searched for in the column names of the @data slot of the 'cD' object. Different classes of vector can be used for 'samplesA' and 'samplesB', as shown in the example below.

Value

Plotting function.

Author(s)

Thomas J. Hardcastle

See Also

[countData](#page-4-1)

Examples

data(simData)

```
replicates <- c("simA", "simA", "simA", "simA", "simA", "simB", "simB", "simB", "simB", "simB")
groups <- list(NDE = c(1,1,1,1,1,1,1,1,1,1), DE = c(1,1,1,1,1,2,2,2,2,2))
CD <- new("countData", data = simData, replicates = replicates, groups = groups)
```
#estimate library sizes for countData object libsizes(CD) <- getLibsizes(CD)

```
#MA-plot comparing replicate groups
plotMA.CD(CD, samplesA = "simA", samplesB = 6:10)
```
plotNullPrior *Plots distribution of null function and shows the threshold separator.*

Description

In sequencing expression of various genomic events, it is not uncommon to find a subset of genomic events that are qualitatively different from the remainder of the data. Thus, for some function of the estimated priors, we may observe bimodality or long tails which correlate to this subset.

Usage

plotNullPrior(cD, ...)

Arguments

Value

Invisibly, the numeric value of the threshold.

Author(s)

Thomas J. Hardcastle

Description

This function plots the posterior likelihoods estimated for a 'countData' object against the log-ratios observed between two sets of sample data. For those data where the log-ratio is infinite (because in one set of sample data all observed counts are zero), we plot instead the log-values of the other group.

Usage

```
plotPosteriors(cD, group, samplesA, samplesB, ...)
```


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Arguments

Value

Plotting function.

Author(s)

Thomas J. Hardcastle

See Also

[getPosteriors](#page-14-1)

Examples

```
# We load in a `countData' object containing the estimated posterior
# likelihoods of expression (see `getLikelihoods').
data(CDPost)
plotPosteriors(CDPost, group = "DE", samplesA = 1:5, samplesB = 6:10)
# equivalent to plotPosteriors(CDPost, group = 2, samplesA = 1:5, samplesB = 6:10)
```
plotPriors *Plots the density of the log values estimated for the mean rate in the prior data for the Negative Binomial approach to detecting differential expression*

Description

This function plots the density of the log values estimated for the mean rate in the data used to estimate a prior distribution for data under the assumption of a Negative Binomial distribution. This function is useful for looking for bimodality of the distributions, and thus determining whether we should try and identify data with no true expression.

Usage

plotPriors(cD, group, par = 1)

Arguments

Details

If the plot of the data appears bimodal, then it may be sensible to try and look for data with no true expression by using the option nullPosts = TRUE in [getLikelihoods.NB](#page-10-2).

Value

Plotting function.

Author(s)

Thomas J. Hardcastle

See Also

[getPriors.NB](#page-16-2), [getLikelihoods.NB](#page-10-2)

Examples

```
# We load in a `countData' object containing the estimated priors (see `getPriors').
```
data(CDPriors)

```
plotPriors(CDPriors, group = "NDE", par = 1)
```
selectTop *Selects the top genomic events, based on posterior likelihoods, from a 'countData' object.*

Description

This function subsets a countData object by selecting those events that best (or least) represent a model, based on the posterior likelihoods estimated for that model and some threshold. Selection can be done for a specific model (and ordering of the data under that model) or for all models (and all orderings).

selectTop 33

Usage

```
selectTop(cD, group, ordering, orderings = TRUE, decreasing = TRUE,
number = 10, likelihood, FDR, FWER, posteriors)
```
Arguments

Value

Either a single [countData](#page-4-1) object (if 'group' is specified), or a named list of [countData](#page-4-1) objects.

Author(s)

Thomas J. Hardcastle

See Also

[topCounts](#page-34-1)

Examples

```
which the samples<br># We load in a `countData' object containing the estimated posterior<br># We load in a `countData' object containing the estimated posterior
m<mark>ples</mark><br># We load in a `countData' object containing the es<br># likelihoods of expression (see `getLikelihoods').
```
data(CDPost)

select from all models and orderings with FDR equal to or lower than 0.01.

```
selectTop(CDPost, FDR = 0.01)
```
simData *Simulated data for testing the baySeq package methods*

Description

This data set is a matrix ('simData') of simulated counts from a simple pairwise expression analysis. It is simulated according to a negative binomial distribution with varying parameters for each row. The first hundred rows of the data are truly differentially expressed, the remainder have no differential expression.

Usage

simData

Format

A matrix of which each of the ten columns represents a sample, and each row some discrete data (acquired by sequencing).

Source

Simulation.

References

Hardcastle T.J., and Kelly, K. baySeq: Empirical Bayesian Methods For Identifying Differential Expression In Sequence Count Data. BMC Bioinformatics (2010)

summarisePosteriors *Summarises expected number of genomic events given the calculated posterior likelihoods of a countData object.*

Description

Given posterior likelihoods for each model, we can calculate the expected number of genomic events corresponding to each model (and to each ordering within each model) by summing the posterior likelihoods.

Usage

```
summarisePosteriors(cD, orderings = TRUE)
```


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Arguments

Value

Numeric vector of expected number of genomic events belonging to each model (optionally, split by orderings).

Author(s)

Thomas J. Hardcastle

See Also

[topCounts](#page-34-1), [selectTop](#page-31-1)

Examples

We load in a `countData' object containing the estimated posterior # likelihoods of expression (see `getLikelihoods').

data(CDPost)

```
# summarise the expected number of genomic events in each category
summarisePosteriors(CDPost)
```


Get the top counts corresponding to some group from a 'countData' *object*

Description

Takes posterior likelihoods and returns the counts with highest (or lowest) likelihood of association with a given group.

Usage

```
topCounts(cD, group, ordering, decreasing = TRUE, number = 10, likelihood, FDR,
FWER, normaliseData = FALSE, posteriors)
```
Arguments

Details

The argument 'group' can be specified either as a number, giving the index of an element in the cD@groups list, or as a character string identifying an element by name. Partial matching is allowed. If group = NULL, then the function looks at the posterior likelihoods that the data have no true differential expression (if calculated).

If a [countData](#page-4-1) object is given, the returned dataframe will contain either the raw counts for that object, or (if 'normaliseData = TRUE' the counts normalised by library size.

Value

A dataframe of the top counts associated with some model (group), described by annotation drawn from the '@annotation' slot of the 'cD' object and the raw data from the '@data' slot, together with the posterior likelihoods and false discovery rates.

Author(s)

Thomas J. Hardcastle

See Also

[countData](#page-4-1)

Examples

```
# We load in a `countData' object containing the estimated posterior
# likelihoods of expression (see `getLikelihoods').
```
data(CDPost)

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```
# Report the top ten rows of data that have highest likelihood of belonging to
# group 2 of the data (i.e., differentially expressed)
topCounts(CDPost, group = "DE", number = 10)
# equivalently...
topCounts(CDPost, group = 2, number = 10)
# Report the top ten rows of data that have highest likelihood of belonging to
# group 2 of the data (i.e., differentially expressed), with group 1
# being overexpressed compared to group 2.
topCounts(CDPost, group = "DE", ordering = "1>2", number = 10)
```
zimData *Simulated data for testing the baySeq package methods*

Description

This data set is a matrix ('zimData') of zero-inflated simulated counts from a simple pairwise expression analysis. It is simulated according to a negative binomial distribution with varying parameters for each row, and with zero-inflation applied to each row. The first hundred rows of the data are truly differentially expressed, the remainder have no differential expression.

Usage

zimData

Format

A matrix of which each of the ten columns represents a sample, and each row some discrete data (acquired by sequencing).

Source

Simulation.

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