Package 'scde'

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Type Package

Title Single Cell Differential Expression

Version 2.40.0

Description The scde package implements a set of statistical methods for analyzing single-cell RNA-seq data. scde fits individual error models for single-cell RNA-seq measurements. These models can then be used for assessment of differential expression between groups of cells, as well as other types of analysis. The scde package also contains the pagoda framework which applies pathway and gene set overdispersion analysis to identify and characterize putative cell subpopulations based on transcriptional signatures. The overall approach to the differential expression analysis is detailed in the following publication: ``Bayesian approach to single-cell differential expression analysis'' (Kharchenko PV, Silberstein L, Scadden DT, Nature Methods, doi: 10.1038/nmeth.2967). The overall approach to subpopulation identification and characterization is detailed in the following pre-print: ``Characterizing transcriptional heterogeneity through pathway and gene set overdispersion analysis'' (Fan J, Salathia N, Liu R, Kaeser G, Yung Y, Herman J, Kaper F, Fan JB, Zhang K, Chun J, and Kharchenko PV, Nature Methods, doi:10.1038/nmeth.3734).

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`bwpca` *Determine principal components of a matrix using per-observation/per-variable weights*

Description

Implements a weighted PCA

Usage

```
bwpca(mat, matw = NULL, npcs = 2, nstarts = 1, smooth = 0,
  em.tol = 1e-06, em.maxiter = 25, seed = 1, center = TRUE,
  n.shuffles = 0)
```

Arguments

`mat`	matrix of variables (columns) and observations (rows)
`matw`	corresponding weights
`npcs`	number of principal components to extract
`nstarts`	number of random starts to use
`smooth`	smoothing span
`em.tol`	desired EM algorithm tolerance
`em.maxiter`	maximum number of EM iterations
`seed`	random seed
`center`	whether mat should be centered (weighted centering)
`n.shuffles`	optional number of per-observation randomizations that should be performed in addition to the main calculations to determine the lambda1 (PC1 eigenvalue) magnitude under such randomizations (returned in $randvar)

Value

a list containing eigenvector matrix ($rotation), projections ($scores), variance (weighted) explained by each component ($var), total (weighted) variance of the dataset ($totalvar)

Examples

```
set.seed(0)
mat <- matrix( c(rnorm(5*10,mean=0,sd=1), rnorm(5*10,mean=5,sd=1)), 10, 10)  # random matrix
base.pca <- bwpca(mat)  # non-weighted pca, equal weights set automatically
matw <- matrix( c(rnorm(5*10,mean=0,sd=1), rnorm(5*10,mean=5,sd=1)), 10, 10)  # random weight matrix
matw <- abs(matw)/max(matw)
base.pca.weighted <- bwpca(mat, matw)  # weighted pca
```

---

clean.counts *Filter counts matrix*

Description

Filter counts matrix based on gene and cell requirements

Usage

```
clean.counts(counts, min.lib.size = 1800, min.reads = 10,
  min.detected = 5)
```

Arguments

counts	read count matrix. The rows correspond to genes, columns correspond to individual cells
min.lib.size	Minimum number of genes detected in a cell. Cells with fewer genes will be removed (default: 1.8e3)
min.reads	Minimum number of reads per gene. Genes with fewer reads will be removed (default: 10)
min.detected	Minimum number of cells a gene must be seen in. Genes not seen in a sufficient number of cells will be removed (default: 5)

Value

a filtered read count matrix

Examples

```
data(pollen)
dim(pollen)
cd <- clean.counts(pollen)
dim(cd)
```

clean.gos *Filter GOs list*

Description

Filter GOs list and append GO names when appropriate

Usage

```
clean.gos(go.env, min.size = 5, max.size = 5000, annot = FALSE)
```

Arguments

go.env	GO or gene set list
min.size	Minimum size for number of genes in a gene set (default: 5)
max.size	Maximum size for number of genes in a gene set (default: 5000)
annot	Whether to append GO annotations for easier interpretation (default: FALSE)

Value

a filtered GO list

Examples

```
# 10 sample GOs
library(org.Hs.eg.db)
go.env <- mget(ls(org.Hs.egGO2ALLEGS)[1:10], org.Hs.egGO2ALLEGS)
# Filter this list and append names for easier interpretation
go.env <- clean.gos(go.env)
```

es.mef.small *Sample data*

Description

A subset of Saiful et al. 2011 dataset containing first 20 ES and 20 MEF cells.

References

http://www.ncbi.nlm.nih.gov/pubmed/21543516

knn *Sample error model*

Description

SCDE error model generated from the Pollen et al. 2014 dataset.

References

www.ncbi.nlm.nih.gov/pubmed/25086649

knn.error.models *Build error models for heterogeneous cell populations, based on K-nearest neighbor cells.*

Description

Builds cell-specific error models assuming that there are multiple subpopulations present among the measured cells. The models for each cell are based on average expression estimates obtained from K closest cells within a given group (if groups = NULL, then within the entire set of measured cells). The method implements fitting of both the original log-fit models (when linear.fit = FALSE), or newer linear-fit models (linear.fit = TRUE, default) with locally fit overdispersion coefficient (local.theta.fit = TRUE, default).

Usage

```
knn.error.models(counts, groups = NULL, k = round(ncol(counts)/2),
  min.nonfailed = 5, min.count.threshold = 1, save.model.plots = TRUE,
  max.model.plots = 50, n.cores = parallel::detectCores(),
  min.size.entries = 2000, min.fpm = 0, cor.method = "pearson",
  verbose = 0, fpm.estimate.trim = 0.25, linear.fit = TRUE,
  local.theta.fit = linear.fit, theta.fit.range = c(0.01, 100),
  alpha.weight.power = 1/2)
```

Arguments

counts	count matrix (integer matrix, rows- genes, columns- cells)
groups	optional groups partitioning known subpopulations
k	number of nearest neighbor cells to use during fitting. If k is set sufficiently high, all of the cells within a given group will be used.
min.nonfailed	minimum number of non-failed measurements (within the k nearest neighbor cells) required for a gene to be taken into account during error fitting procedure
min.count.threshold	minimum number of reads required for a measurement to be considered non-failed

`save.model.plots`
: whether model plots should be saved (file names are (group).models.pdf, or cell.models.pdf if no group was supplied)

`max.model.plots`
: maximum number of models to save plots for (saves time when there are too many cells)

`n.cores`
: number of cores to use through the calculations

`min.size.entries`
: minimum number of genes to use for model fitting

`min.fpm`
: optional parameter to restrict model fitting to genes with group-average expression magnitude above a given value

`cor.method`
: correlation measure to be used in determining k nearest cells

`verbose`
: level of verbosity

`fpm.estimate.trim`
: trim fraction to be used in estimating group-average gene expression magnitude for model fitting (0.5 would be median, 0 would turn off trimming)

`linear.fit`
: whether newer linear model fit with zero intercept should be used (T), or the log-fit model published originally (F)

`local.theta.fit`
: whether local theta fitting should be used (only available for the linear fit models)

`theta.fit.range`
: allowed range of the theta values

`alpha.weight.power`
: 1/theta weight power used in fitting theta dependency on the expression magnitude

Value

a data frame with parameters of the fit error models (rows- cells, columns- fitted parameters)

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
```

make.pagoda.app *Make the PAGODA app*

Description

Create an interactive user interface to explore output of PAGODA.

Usage

```
make.pagoda.app(tamr, tam, varinfo, env, pwpca, clpca = NULL,
  col.cols = NULL, cell.clustering = NULL, row.clustering = NULL,
  title = "pathway clustering", zlim = c(-1, 1) * quantile(tamr$xv, p =
  0.95))
```

Arguments

tamr	Combined pathways that show similar expression patterns. Output of pagoda.reduce.redundancy
tam	Combined pathways that are driven by the same gene sets. Output of pagoda.reduce.loading.redundar
varinfo	Variance information. Output of pagoda.varnorm
env	Gene sets as an environment variable.
pwpca	Weighted PC magnitudes for each gene set provided in the env. Output of pagoda.pathway.wPCA
clpca	Weighted PC magnitudes for de novo gene sets identified by clustering on expression. Output of pagoda.gene.clusters
col.cols	Matrix of column colors. Useful for visualizing cell annotations such as batch labels. Default NULL.
cell.clustering	Dendrogram of cell clustering. Output of pagoda.cluster.cells . Default NULL.
row.clustering	Dendrogram of combined pathways clustering. Default NULL.
title	Title text to be used in the browser label for the app. Default, set as 'pathway clustering'
zlim	Range of the normalized gene expression levels, inputted as a list: c(lower_bound, upper_bound). Values outside this range will be Winsorized. Useful for increasing the contrast of the heatmap visualizations. Default, set to the 5th and 95th percentiles.

Value

PAGODA app

o.ifm *Sample error model*

Description

SCDE error model generated from a subset of Saiful et al. 2011 dataset containing first 20 ES and 20 MEF cells.

References

http://www.ncbi.nlm.nih.gov/pubmed/21543516

pagoda.cluster.cells *Determine optimal cell clustering based on the genes driving the significant aspects*

Description

Determines cell clustering (hclust result) based on a weighted correlation of genes underlying the top aspects of transcriptional heterogeneity. Branch orientation is optimized if 'cba' package is installed.

Usage

```
pagoda.cluster.cells(tam, varinfo, method = "ward.D",
  include.aspects = FALSE, verbose = 0, return.details = FALSE)
```

Arguments

tam	result of pagoda.top.aspects() call
varinfo	result of pagoda.varnorm() call
method	clustering method ('ward.D' by default)
include.aspects	whether the aspect patterns themselves should be included alongside with the individual genes in calculating cell distance
verbose	0 or 1 depending on level of desired verbosity
return.details	Boolean of whether to return just the hclust result or a list containing the hclust result plus the distance matrix and gene values

Value

hclust result

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
pwpca <- pagoda.pathway.wPCA(varinfo, go.env, n.components=1, n.cores=10, n.internal.shuffles=50)
tam <- pagoda.top.aspects(pwpca, return.table = TRUE, plot=FALSE, z.score=1.96) # top aspects based on GO only
hc <- pagoda.cluster.cells(tam, varinfo)
plot(hc)
```

---

pagoda.effective.cells

Estimate effective number of cells based on lambda1 of random gene sets

---

Description

Examines the dependency between the amount of variance explained by the first principal component of a gene set and the number of genes in a gene set to determine the effective number of cells for the Tracy-Widom distribution

Usage

```
pagoda.effective.cells(pwpca, start = NULL)
```

Arguments

pwpca	result of the pagoda.pathway.wPCA() call with n.randomizations > 1
start	optional starting value for the optimization (if the NLS breaks, trying high starting values usually fixed the local gradient problem)

Value

effective number of cells

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
pwpca <- pagoda.pathway.wPCA(varinfo, go.env, n.components=1, n.cores=10, n.internal.shuffles=50)
pagoda.effective.cells(pwpca)
```

pagoda.gene.clusters *Determine de-novo gene clusters and associated overdispersion info*

Description

Determine de-novo gene clusters, their weighted PCA lambda1 values, and random matrix expectation.

Usage

```
pagoda.gene.clusters(varinfo, trim = 3.1/ncol(varinfo$mat),
  n.clusters = 150, n.samples = 60, cor.method = "p",
  n.internal.shuffles = 0, n.starts = 10, n.cores = detectCores(),
  verbose = 0, plot = FALSE, show.random = FALSE, n.components = 1,
  method = "ward.D", secondary.correlation = FALSE,
  n.cells = ncol(varinfo$mat), old.results = NULL)
```

Arguments

varinfo	varinfo adjusted variance info from pagoda.varinfo() (or pagoda.subtract.aspect())
trim	additional Winsorization trim value to be used in determining clusters (to remove clusters that group outliers occurring in a given cell). Use higher values (5-15) if the resulting clusters group outlier patterns
n.clusters	number of clusters to be determined (recommended range is 100-200)
n.samples	number of randomly generated matrix samples to test the background distribution of lambda1 on
cor.method	correlation method ("pearson", "spearman") to be used as a distance measure for clustering
n.internal.shuffles	number of internal shuffles to perform (only if interested in set coherence, which is quite high for clusters by definition, disabled by default; set to 10-30 shuffles to estimate)
n.starts	number of wPCA EM algorithm starts at each iteration
n.cores	number of cores to use
verbose	verbosity level
plot	whether a plot showing distribution of random lambda1 values should be shown (along with the extreme value distribution fit)
show.random	whether the empirical random gene set values should be shown in addition to the Tracy-Widom analytical approximation
n.components	number of PC to calculate (can be increased if the number of clusters is small and some contain strong secondary patterns - rarely the case)
method	clustering method to be used in determining gene clusters

secondary.correlation
: whether clustering should be performed on the correlation of the correlation matrix instead

n.cells
: number of cells to use for the randomly generated cluster lambda1 model

old.results
: optionally, pass old results just to plot the model without recalculating the stats

Value

a list containing the following fields:

- clusters a list of genes in each cluster values
- xf extreme value distribution fit for the standardized lambda1 of a randomly generated pattern
- tci index of a top cluster in each random iteration
- cl.goc weighted PCA info for each real gene cluster
- varm standardized lambda1 values for each randomly generated matrix cluster
- clvlm a linear model describing dependency of the cluster lambda1 on a Tracy-Widom lambda1 expectation

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
clpca <- pagoda.gene.clusters(varinfo, trim=7.1/ncol(varinfo$mat), n.clusters=150, n.cores=10, plot=FALSE)
```

---

pagoda.pathway.wPCA *Run weighted PCA analysis on pre-annotated gene sets*

Description

For each valid gene set (having appropriate number of genes) in the provided environment (setenv), the method will run weighted PCA analysis, along with analogous analyses of random gene sets of the same size, or shuffled expression magnitudes for the same gene set.

Usage

```
pagoda.pathway.wPCA(varinfo, setenv, n.components = 2,
  n.cores = detectCores(), min.pathway.size = 10, max.pathway.size = 1000,
  n.randomizations = 10, n.internal.shuffles = 0, n.starts = 10,
  center = TRUE, batch.center = TRUE, proper.gene.names = NULL,
  verbose = 0)
```

Arguments

varinfo	adjusted variance info from pagoda.varinfo() (or pagoda.subtract.aspect())
setenv	environment listing gene sets (contains variables with names corresponding to gene set name, and values being vectors of gene names within each gene set)
n.components	number of principal components to determine for each gene set
n.cores	number of cores to use
min.pathway.size	minimum number of observed genes that should be contained in a valid gene set
max.pathway.size	maximum number of observed genes in a valid gene set
n.randomizations	number of random gene sets (of the same size) to be evaluated in parallel with each gene set (can be kept at 5 or 10, but should be increased to 50-100 if the significance of pathway overdispersion will be determined relative to random gene set models)
n.internal.shuffles	number of internal (independent row shuffles) randomizations of expression data that should be evaluated for each gene set (needed only if one is interested in gene set coherence P values, disabled by default; set to 10-30 to estimate)
n.starts	number of random starts for the EM method in each evaluation
center	whether the expression matrix should be recentered
batch.center	whether batch-specific centering should be used
proper.gene.names	alternative vector of gene names (replacing rownames(varinfo$mat)) to be used in cases when the provided setenv uses different gene names
verbose	verbosity level

Value

a list of weighted PCA info for each valid gene set

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
# create go environment
library(org.Hs.eg.db)
# translate gene names to ids
ids <- unlist(lapply(mget(rownames(cd), org.Hs.egALIAS2EG, ifnotfound = NA), function(x) x[1]))
rids <- names(ids); names(rids) <- ids
go.env <- lapply(mget(ls(org.Hs.egGO2ALLEGS), org.Hs.egGO2ALLEGS), function(x) as.character(na.omit(rids[x])))
# clean GOs
go.env <- clean.gos(go.env)
# convert to an environment
```

```
go.env <- list2env(go.env)
pwpca <- pagoda.pathway.wPCA(varinfo, go.env, n.components=1, n.cores=10, n.internal.shuffles=50)
```

`pagoda.reduce.loading.redundancy` *Collapse aspects driven by the same combinations of genes*

Description

Examines PC loading vectors underlying the identified aspects and clusters aspects based on a product of loading and score correlation (raised to corr.power). Clusters of aspects driven by the same genes are determined based on the distance.threshold and collapsed.

Usage

```
pagoda.reduce.loading.redundancy(tam, pwpca, clpca = NULL, plot = FALSE,
  cluster.method = "complete", distance.threshold = 0.01, corr.power = 4,
  n.cores = detectCores(), abs = TRUE, ...)
```

Arguments

`tam`	output of pagoda.top.aspects()
`pwpca`	output of pagoda.pathway.wPCA()
`clpca`	output of pagoda.gene.clusters() (optional)
`plot`	whether to plot the resulting clustering
`cluster.method`	one of the standard clustering methods to be used (fastcluster::hclust is used if available or stats::hclust)
`distance.threshold`	similarity threshold for grouping interdependent aspects
`corr.power`	power to which the product of loading and score correlation is raised
`n.cores`	number of cores to use during processing
`abs`	Boolean of whether to use absolute correlation
`...`	additional arguments are passed to the pagoda.view.aspects() method during plotting

Value

a list structure analogous to that returned by pagoda.top.aspects(), but with addition of a $cnam element containing a list of aspects summarized by each row of the new (reduced) $xv and $xvw

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
pwpca <- pagoda.pathway.wPCA(varinfo, go.env, n.components=1, n.cores=10, n.internal.shuffles=50)
tam <- pagoda.top.aspects(pwpca, return.table = TRUE, plot=FALSE, z.score=1.96) # top aspects based on GO only
tamr <- pagoda.reduce.loading.redundancy(tam, pwpca)
```

---

pagoda.reduce.redundancy

Collapse aspects driven by similar patterns (i.e. separate the same sets of cells)

Description

Examines PC loading vectors underlying the identified aspects and clusters aspects based on score correlation. Clusters of aspects driven by the same patterns are determined based on the distance.threshold.

Usage

```
pagoda.reduce.redundancy(tamr, distance.threshold = 0.2,
  cluster.method = "complete", distance = NULL,
  weighted.correlation = TRUE, plot = FALSE, top = Inf, trim = 0,
  abs = FALSE, ...)
```

Arguments

tamr	output of pagoda.reduce.loading.redundancy()
distance.threshold	similarity threshold for grouping interdependent aspects
cluster.method	one of the standard clustering methods to be used (fastcluster::hclust is used if available or stats::hclust)
distance	distance matrix
weighted.correlation	Boolean of whether to use a weighted correlation in determining the similarity of patterns
plot	Boolean of whether to show plot
top	Restrict output to the top n aspects of heterogeneity
trim	Winsorization trim to use prior to determining the top aspects
abs	Boolean of whether to use absolute correlation
...	additional arguments are passed to the pagoda.view.aspects() method during plotting

Value

a list structure analogous to that returned by pagoda.top.aspects(), but with addition of a $cnam element containing a list of aspects summarized by each row of the new (reduced) $xv and $xvw

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
pwpca <- pagoda.pathway.wPCA(varinfo, go.env, n.components=1, n.cores=10, n.internal.shuffles=50)
tam <- pagoda.top.aspects(pwpca, return.table = TRUE, plot=FALSE, z.score=1.96) # top aspects based on GO only
tamr <- pagoda.reduce.loading.redundancy(tam, pwpca)
tamr2 <- pagoda.reduce.redundancy(tamr, distance.threshold = 0.9, plot = TRUE, labRow = NA, labCol = NA, box = TRUE,
```

---

pagoda.show.pathways *View pathway or gene weighted PCA*

Description

Takes in a list of pathways (or a list of genes), runs weighted PCA, optionally showing the result.

Usage

```
pagoda.show.pathways(pathways, varinfo, goenv = NULL, n.genes = 20,
  two.sided = FALSE, n.pc = rep(1, length(pathways)), colcols = NULL,
  zlim = NULL, showRowLabels = FALSE, cexCol = 1, cexRow = 1,
  nstarts = 10, cell.clustering = NULL, show.cell.dendrogram = TRUE,
  plot = TRUE, box = TRUE, trim = 0, return.details = FALSE, ...)
```

Arguments

pathways	character vector of pathway or gene names
varinfo	output of pagoda.varnorm()
goenv	environment mapping pathways to genes
n.genes	number of genes to show
two.sided	whether the set of shown genes should be split among highest and lowest loading (T) or if genes with highest absolute loading (F) should be shown
n.pc	optional integer vector giving the number of principal component to show for each listed pathway
colcols	optional column color matrix
zlim	optional z color limit

showRowLabels	controls whether row labels are shown in the plot
cexCol	column label size (cex)
cexRow	row label size (cex)
nstarts	number of random starts for the wPCA
cell.clustering	cell clustering
show.cell.dendrogram	whether cell dendrogram should be shown
plot	whether the plot should be shown
box	whether to draw a box around the plotted matrix
trim	optional Winsorization trim that should be applied
return.details	whether the function should return the matrix as well as full PCA info instead of just PC1 vector
...	additional arguments are passed to the c.view.pathways

Value

cell scores along the first principal component of shown genes (returned as invisible)

---

pagoda.subtract.aspect

Control for a particular aspect of expression heterogeneity in a given population

---

Description

Similar to subtracting n-th principal component, the current procedure determines (weighted) projection of the expression matrix onto a specified aspect (some pattern across cells, for instance sequencing depth, or PC corresponding to an undesired process such as ribosomal pathway variation) and subtracts it from the data so that it is controlled for in the subsequent weighted PCA analysis.

Usage

```
pagoda.subtract.aspect(varinfo, aspect, center = TRUE)
```

Arguments

varinfo	normalized variance info (from pagoda.varnorm())
aspect	a vector giving a cell-to-cell variation pattern that should be controlled for (length should be corresponding to ncol(varinfo$mat))
center	whether the matrix should be re-centered following pattern subtraction

Value

a modified varinfo object with adjusted expression matrix (varinfo$mat)

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
# create go environment
library(org.Hs.eg.db)
# translate gene names to ids
ids <- unlist(lapply(mget(rownames(cd), org.Hs.egALIAS2EG, ifnotfound = NA), function(x) x[1]))
rids <- names(ids); names(rids) <- ids
go.env <- lapply(mget(ls(org.Hs.egGO2ALLEGS), org.Hs.egGO2ALLEGS), function(x) as.character(na.omit(rids[x])))
# clean GOs
go.env <- clean.gos(go.env)
# convert to an environment
go.env <- list2env(go.env)
# subtract the pattern
cc.pattern <- pagoda.show.pathways(ls(go.env)[1:2], varinfo, go.env, show.cell.dendrogram = TRUE, showRowLabels =
varinfo.cc <- pagoda.subtract.aspect(varinfo, cc.pattern)
```

---

pagoda.top.aspects *Score statistical significance of gene set and cluster overdispersion*

Description

Evaluates statistical significance of the gene set and cluster lambda1 values, returning either a text table of Z scores, etc, a structure containing normalized values of significant aspects, or a set of genes underlying the significant aspects.

Usage

```
pagoda.top.aspects(pwpca, clpca = NULL, n.cells = NULL,
  z.score = qnorm(0.05/2, lower.tail = FALSE), return.table = FALSE,
  return.genes = FALSE, plot = FALSE, adjust.scores = TRUE,
  score.alpha = 0.05, use.oe.scale = FALSE, effective.cells.start = NULL)
```

Arguments

pwpca	output of pagoda.pathway.wPCA()
clpca	output of pagoda.gene.clusters() (optional)
n.cells	effective number of cells (if not provided, will be determined using pagoda.effective.cells())

z.score	Z score to be used as a cutoff for statistically significant patterns (defaults to 0.05 P-value
return.table	whether a text table showing
return.genes	whether a set of genes driving significant aspects should be returned
plot	whether to plot the cv/n vs. dataset size scatter showing significance models
adjust.scores	whether the normalization of the aspect patterns should be based on the adjusted Z scores - qnorm(0.05/2, lower.tail = FALSE)
score.alpha	significance level of the confidence interval for determining upper/lower bounds
use.oe.scale	whether the variance of the returned aspect patterns should be normalized using observed/expected value instead of the default chi-squared derived variance corresponding to overdispersion Z score
effective.cells.start	starting value for the pagoda.effective.cells() call

Value

if return.table = FALSE and return.genes = FALSE (default) returns a list structure containing the following items:

- xv a matrix of normalized aspect patterns (rows- significant aspects, columns- cells
- xvw corresponding weight matrix
- gw set of genes driving the significant aspects
- df text table with the significance testing results

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
pwpca <- pagoda.pathway.wPCA(varinfo, go.env, n.components=1, n.cores=10, n.internal.shuffles=50)
tam <- pagoda.top.aspects(pwpca, return.table = TRUE, plot=FALSE, z.score=1.96)  # top aspects based on GO only
```

---

pagoda.varnorm *Normalize gene expression variance relative to transcriptome-wide expectations*

Description

Normalizes gene expression magnitudes to ensure that the variance follows chi-squared statistics with respect to its ratio to the transcriptome-wide expectation as determined by local regression on expression magnitude (and optionally gene length). Corrects for batch effects.

Usage

```
pagoda.varnorm(models, counts, batch = NULL, trim = 0, prior = NULL,
  fit.genes = NULL, plot = TRUE, minimize.underdispersion = FALSE,
  n.cores = detectCores(), n.randomizations = 100, weight.k = 0.9,
  verbose = 0, weight.df.power = 1, smooth.df = -1, max.adj.var = 10,
  theta.range = c(0.01, 100), gene.length = NULL)
```

Arguments

models	model matrix (select a subset of rows to normalize variance within a subset of cells)
counts	read count matrix
batch	measurement batch (optional)
trim	trim value for Winsorization (optional, can be set to 1-3 to reduce the impact of outliers, can be as large as 5 or 10 for datasets with several thousand cells)
prior	expression magnitude prior
fit.genes	a vector of gene names which should be used to establish the variance fit (default is NULL: use all genes). This can be used to specify, for instance, a set spike-in control transcripts such as ERCC.
plot	whether to plot the results
minimize.underdispersion	whether underdispersion should be minimized (can increase sensitivity in datasets with high complexity of population, however cannot be effectively used in datasets where multiple batches are present)
n.cores	number of cores to use
n.randomizations	number of bootstrap sampling rounds to use in estimating average expression magnitude for each gene within the given set of cells
weight.k	k value to use in the final weight matrix
verbose	verbosity level
weight.df.power	power factor to use in determining effective number of degrees of freedom (can be increased for datasets exhibiting particularly high levels of noise at low expression magnitudes)
smooth.df	degrees of freedom to be used in calculating smoothed local regression between coefficient of variation and expression magnitude (and gene length, if provided). Leave at -1 for automated guess.
max.adj.var	maximum value allowed for the estimated adjusted variance (capping of adjusted variance is recommended when scoring pathway overdispersion relative to randomly sampled gene sets)
theta.range	valid theta range (should be the same as was set in knn.error.models() call
gene.length	optional vector of gene lengths (corresponding to the rows of counts matrix)

Value

a list containing the following fields:

- mat adjusted expression magnitude values
- matw weight matrix corresponding to the expression matrix
- arv a vector giving adjusted variance values for each gene
- avmodes a vector estimated average expression magnitudes for each gene
- modes a list of batch-specific average expression magnitudes for each gene
- prior estimated (or supplied) expression magnitude prior
- edf estimated effective degrees of freedom
- fit.genes fit.genes parameter

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
```

---

pagoda.view.aspects *View PAGODA output*

Description

Create static image of PAGODA output visualizing cell hierarchy and top aspects of transcriptional heterogeneity

Usage

```
pagoda.view.aspects(tamr, row.clustering = hclust(dist(tamr$xv)), top = Inf,
  ...)
```

Arguments

tamr	Combined pathways that show similar expression patterns. Output of pagoda.reduce.redundancy
row.clustering	Dendrogram of combined pathways clustering
top	Restrict output to the top n aspects of heterogeneity
...	additional arguments are passed to the view.aspects method during plotting

Value

PAGODA heatmap

Examples

```
data(pollen)
cd <- clean.counts(pollen)

knn <- knn.error.models(cd, k=ncol(cd)/4, n.cores=10, min.count.threshold=2, min.nonfailed=5, max.model.plots=10)
varinfo <- pagoda.varnorm(knn, counts = cd, trim = 3/ncol(cd), max.adj.var = 5, n.cores = 1, plot = FALSE)
pwpca <- pagoda.pathway.wPCA(varinfo, go.env, n.components=1, n.cores=10, n.internal.shuffles=50)
tam <- pagoda.top.aspects(pwpca, return.table = TRUE, plot=FALSE, z.score=1.96)  # top aspects based on GO only
pagoda.view.aspects(tam)
```

---

papply *wrapper around different mclapply mechanisms*

Description

Abstracts out mclapply implementation, and defaults to lapply when only one core is requested (helps with debugging)

Usage

```
papply(..., n.cores = n)
```

Arguments

`...`	parameters to pass to lapply, mclapply, bplapply, etc.
`n.cores`	number of cores. If 1 core is requested, will default to lapply

---

pollen *Sample data*

Description

Single cell data from Pollen et al. 2014 dataset.

References

www.ncbi.nlm.nih.gov/pubmed/25086649

scde *Single-cell Differential Expression (with Pathway And Gene set Overdispersion Analysis)*

Description

The scde package implements a set of statistical methods for analyzing single-cell RNA-seq data. scde fits individual error models for single-cell RNA-seq measurements. These models can then be used for assessment of differential expression between groups of cells, as well as other types of analysis. The scde package also contains the pagoda framework which applies pathway and gene set overdispersion analysis to identify and characterize putative cell subpopulations based on transcriptional signatures. See vignette("diffexp") for a brief tutorial on differential expression analysis. See vignette("pagoda") for a brief tutorial on pathway and gene set overdispersion analysis to identify and characterize cell subpopulations. More extensive tutorials are available at http://pklab.med.harvard.edu/scde/index.html. (test)

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scde.browse.diffexp *View differential expression results in a browser*

Description

Launches a browser app that shows the differential expression results, allowing to sort, filter, etc. The arguments generally correspond to the scde.expression.difference() call, except that the results of that call are also passed here. Requires Rook and rjson packages to be installed.

Usage

```
scde.browse.diffexp(results, models, counts, prior, groups = NULL,
  batch = NULL, geneLookupURL = NULL, server = NULL, name = "scde",
  port = NULL)
```

Arguments

results	result object returned by scde.expression.difference(). Note to browse group posterior levels, use return.posteriors = TRUE in the scde.expression.difference() call.
models	model matrix
counts	count matrix
prior	prior

groups	group information
batch	batch information
geneLookupURL	The URL that will be used to construct links to view more information on gene names. By default (if can't guess the organism) the links will forward to ENSEMBL site search, using geneLookupURL = "http://useast.ensembl.org/Multi/Search/Results?q = {0}". The "0" in the end will be substituted with the gene name. For instance, to link to GeneCards, use "http://www.genecards.org/cgi-bin/carddisp.pl?gene = {0}".
server	optional previously returned instance of the server, if want to reuse it.
name	app name (needs to be altered only if adding more than one app to the server using server parameter)
port	Interactive browser port

Value

server instance, on which $stop() function can be called to kill the process.

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)
sg <- factor(gsub("(MEF|ESC).*", "\\1", colnames(cd)), levels = c("ESC", "MEF"))
names(sg) <- colnames(cd)

o.ifm <- scde.error.models(counts = cd, groups = sg, n.cores = 10, threshold.segmentation = TRUE)
o.prior <- scde.expression.prior(models = o.ifm, counts = cd, length.out = 400, show.plot = FALSE)
# make sure groups corresponds to the models (o.ifm)
groups <- factor(gsub("(MEF|ESC).*", "\\1", rownames(o.ifm)), levels = c("ESC", "MEF"))
names(groups) <- row.names(o.ifm)
ediff <- scde.expression.difference(o.ifm, cd, o.prior, groups = groups, n.randomizations = 100, n.cores = 10, verb
scde.browse.diffexp(ediff, o.ifm, cd, o.prior, groups = groups, geneLookupURL="http://www.informatics.jax.org/sea
```

---

scde.edff *Internal model data*

Description

Numerically-derived correction for NB->chi squared approximation stored as an local regression model

scde.error.models *Fit single-cell error/regression models*

Description

Fit error models given a set of single-cell data (counts) and an optional grouping factor (groups). The cells (within each group) are first cross-compared to determine a subset of genes showing consistent expression. The set of genes is then used to fit a mixture model (Poisson-NB mixture, with expression-dependent concomitant).

Usage

```
scde.error.models(counts, groups = NULL, min.nonfailed = 3,
  threshold.segmentation = TRUE, min.count.threshold = 4,
  zero.count.threshold = min.count.threshold, zero.lambda = 0.1,
  save.crossfit.plots = FALSE, save.model.plots = TRUE, n.cores = 12,
  min.size.entries = 2000, max.pairs = 5000, min.pairs.per.cell = 10,
  verbose = 0, linear.fit = TRUE, local.theta.fit = linear.fit,
  theta.fit.range = c(0.01, 100))
```

Arguments

counts	read count matrix. The rows correspond to genes (should be named), columns correspond to individual cells. The matrix should contain integer counts
groups	an optional factor describing grouping of different cells. If provided, the cross-fits and the expected expression magnitudes will be determined separately within each group. The factor should have the same length as ncol(counts).
min.nonfailed	minimal number of non-failed observations required for a gene to be used in the final model fitting
threshold.segmentation	use a fast threshold-based segmentation during cross-fit (default: TRUE)
min.count.threshold	the number of reads to use to guess which genes may have "failed" to be detected in a given measurement during cross-cell comparison (default: 4)
zero.count.threshold	threshold to guess the initial value (failed/non-failed) during error model fitting procedure (defaults to the min.count.threshold value)
zero.lambda	the rate of the Poisson (failure) component (default: 0.1)
save.crossfit.plots	whether png files showing cross-fit segmentations should be written out (default: FALSE)
save.model.plots	whether pdf files showing model fits should be written out (default = TRUE)
n.cores	number of cores to use

`min.size.entries`
: minimum number of genes to use when determining expected expression magnitude during model fitting

`max.pairs`
: maximum number of cross-fit comparisons that should be performed per group (default: 5000)

`min.pairs.per.cell`
: minimum number of pairs that each cell should be cross-compared with

`verbose`
: 1 for increased output

`linear.fit`
: Boolean of whether to use a linear fit in the regression (default: TRUE).

`local.theta.fit`
: Boolean of whether to fit the overdispersion parameter theta, ie. the negative binomial size parameter, based on local regression (default: set to be equal to the linear.fit parameter)

`theta.fit.range`
: Range of valid values for the overdispersion parameter theta, ie. the negative binomial size parameter (default: c(1e-2, 1e2))

Details

Note: the default implementation has been changed to use linear-scale fit with expression-dependent NB size (overdispersion) fit. This represents an interative improvement on the originally published model. Use linear.fit=F to revert back to the original fitting procedure.

Value

a model matrix, with rows corresponding to different cells, and columns representing different parameters of the determined models

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)
sg <- factor(gsub("(MEF|ESC).*", "\\1", colnames(cd)), levels = c("ESC", "MEF"))
names(sg) <- colnames(cd)

o.ifm <- scde.error.models(counts = cd, groups = sg, n.cores = 10, threshold.segmentation = TRUE)
```

---

scde.expression.difference

Test for expression differences between two sets of cells

Description

Use the individual cell error models to test for differential expression between two groups of cells.

Usage

```
scde.expression.difference(models, counts, prior, groups = NULL,
  batch = NULL, n.randomizations = 150, n.cores = 10,
  batch.models = models, return.posteriors = FALSE, verbose = 0)
```

Arguments

models	models determined by scde.error.models
counts	read count matrix
prior	gene expression prior as determined by scde.expression.prior
groups	a factor determining the two groups of cells being compared. The factor entries should correspond to the rows of the model matrix. The factor should have two levels. NAs are allowed (cells will be omitted from comparison).
batch	a factor (corresponding to rows of the model matrix) specifying batch assignment of each cell, to perform batch correction
n.randomizations	number of bootstrap randomizations to be performed
n.cores	number of cores to utilize
batch.models	(optional) separate models for the batch data (if generated using batch-specific group argument). Normally the same models are used.
return.posteriors	whether joint posterior matrices should be returned
verbose	integer verbose level (1 for verbose)

Value

default: a data frame with the following fields:

- lb, mle, ub lower bound, maximum likelihood estimate, and upper bound of the 95 ce conservative estimate of expression-fold change (equals to the min(abs(c(lb, ub))), or 0 if the CI crosses the 0 Z uncorrected Z-score of expression difference cZ expression difference Z-score corrected for multiple hypothesis testing using Holm procedure
  If batch correction has been performed (batch has been supplied), analogous data frames are returned in slots $batch.adjusted for batch-corrected results, and $batch.effect for the differences explained by batch effects alone.

return.posteriors = TRUE: A list is returned, with the default results data frame given in the $results slot. difference.posterior returns a matrix of estimated expression difference posteriors (rows - genes, columns correspond to different magnitudes of fold-change - log2 values are given in the column names) joint.posteriors a list of two joint posterior matrices (rows - genes, columns correspond to the expression levels, given by prior$x grid)

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)
sg <- factor(gsub("(MEF|ESC).*", "\\1", colnames(cd)), levels = c("ESC", "MEF"))
```

```
names(sg) <- colnames(cd)

o.ifm <- scde.error.models(counts = cd, groups = sg, n.cores = 10, threshold.segmentation = TRUE)
o.prior <- scde.expression.prior(models = o.ifm, counts = cd, length.out = 400, show.plot = FALSE)
# make sure groups corresponds to the models (o.ifm)
groups <- factor(gsub("(MEF|ESC).*", "\\1", rownames(o.ifm)), levels = c("ESC", "MEF"))
names(groups) <- row.names(o.ifm)
ediff <- scde.expression.difference(o.ifm, cd, o.prior, groups = groups, n.randomizations = 100, n.cores = n.cores,
```

`scde.expression.magnitude` *Return scaled expression magnitude estimates*

Description

Return point estimates of expression magnitudes of each gene across a set of cells, based on the regression slopes determined during the model fitting procedure.

Usage

```
scde.expression.magnitude(models, counts)
```

Arguments

`models`	models determined by `scde.error.models`
`counts`	count matrix

Value

a matrix of expression magnitudes on a log scale (rows - genes, columns - cells)

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)
data(o.ifm) # Load precomputed model. Use ?scde.error.models to see how o.ifm was generated
# get expression magnitude estimates
lfpm <- scde.expression.magnitude(o.ifm, cd)
```

scde.expression.prior *Estimate prior distribution for gene expression magnitudes*

Description

Use existing count data to determine a prior distribution of genes in the dataset

Usage

```
scde.expression.prior(models, counts, length.out = 400, show.plot = FALSE,
  pseudo.count = 1, bw = 0.1, max.quantile = 1 - 0.001,
  max.value = NULL)
```

Arguments

models	models determined by scde.error.models
counts	count matrix
length.out	number of points (resolution) of the expression magnitude grid (default: 400). Note: larger numbers will linearly increase memory/CPU demands.
show.plot	show the estimate posterior
pseudo.count	pseudo-count value to use (default 1)
bw	smoothing bandwidth to use in estimating the prior (default: 0.1)
max.quantile	determine the maximum expression magnitude based on a quantile (default : 0.999)
max.value	alternatively, specify the exact maximum expression magnitude value

Value

a structure describing expression magnitude grid ($x, on log10 scale) and prior ($y)

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)
data(o.ifm) # Load precomputed model. Use ?scde.error.models to see how o.ifm was generated
o.prior <- scde.expression.prior(models = o.ifm, counts = cd, length.out = 400, show.plot = FALSE)
```

scde.failure.probability *Calculate drop-out probabilities given a set of counts or expression magnitudes*

Description

Returns estimated drop-out probability for each cell (row of `models` matrix), given either an expression magnitude

Usage

```
scde.failure.probability(models, magnitudes = NULL, counts = NULL)
```

Arguments

`models`	models determined by `scde.error.models`
`magnitudes`	a vector (`length(counts) == nrows(models)`) or a matrix (columns correspond to cells) of expression magnitudes, given on a log scale
`counts`	a vector (`length(counts) == nrows(models)`) or a matrix (columns correspond to cells) of read counts from which the expression magnitude should be estimated

Value

a vector or a matrix of drop-out probabilities

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)
data(o.ifm) # Load precomputed model. Use ?scde.error.models to see how o.ifm was generated
o.prior <- scde.expression.prior(models = o.ifm, counts = cd, length.out = 400, show.plot = FALSE)
# calculate probability of observing a drop out at a given set of magnitudes in different cells
mags <- c(1.0, 1.5, 2.0)
p <- scde.failure.probability(o.ifm, magnitudes = mags)
# calculate probability of observing the dropout at a magnitude corresponding to the
# number of reads actually observed in each cell
self.p <- scde.failure.probability(o.ifm, counts = cd)
```

scde.fit.models.to.reference *Fit scde models relative to provided set of expression magnitudes*

Description

If group-average expression magnitudes are available (e.g. from bulk measurement), this method can be used to fit individual cell error models relative to that reference

Usage

```
scde.fit.models.to.reference(counts, reference, n.cores = 10,
  zero.count.threshold = 1, nrep = 1, save.plots = FALSE,
  plot.filename = "reference.model.fits.pdf", verbose = 0, min.fpm = 1)
```

Arguments

counts	count matrix
reference	a vector of expression magnitudes (read counts) corresponding to the rows of the count matrix
n.cores	number of cores to use
zero.count.threshold	read count to use as an initial guess for the zero threshold
nrep	number independent of mixture fit iterations to try (default = 1)
save.plots	whether to write out a pdf file showing the model fits
plot.filename	model fit pdf filename
verbose	verbose level
min.fpm	minimum reference fpm of genes that will be used to fit the models (defaults to 1). Note: fpm is calculated from the reference count vector as reference/sum(reference)*1e6

Value

matrix of scde models

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)

o.ifm <- scde.error.models(counts = cd, groups = sg, n.cores = 10, threshold.segmentation = TRUE)
o.prior <- scde.expression.prior(models = o.ifm, counts = cd, length.out = 400, show.plot = FALSE)
# calculate joint posteriors across all cells
jp <- scde.posteriors(models = o.ifm, cd, o.prior, n.cores = 10, return.individual.posterior.modes = TRUE, n.random
# use expected expression magnitude for each gene
av.mag <- as.numeric(jp$jp %*% as.numeric(colnames(jp$jp)))
# translate into counts
```

```
av.mag.counts <- as.integer(round(av.mag))
# now, fit alternative models using av.mag as a reference (normally this would correspond to bulk RNA expression mag
ref.models <- scde.fit.models.to.reference(cd, av.mag.counts, n.cores = 1)
```

---

scde.posteriors *Calculate joint expression magnitude posteriors across a set of cells*

Description

Calculates expression magnitude posteriors for the individual cells, and then uses bootstrap re-sampling to calculate a joint expression posterior for all the specified cells. Alternatively during batch-effect correction procedure, the joint posterior can be calculated for a random composition of cells of different groups (see `batch` and `composition` parameters).

Usage

```
scde.posteriors(models, counts, prior, n.randomizations = 100, batch = NULL,
  composition = NULL, return.individual.posteriors = FALSE,
  return.individual.posterior.modes = FALSE, ensemble.posterior = FALSE,
  n.cores = 20)
```

Arguments

`models`	models models determined by `scde.error.models`
`counts`	read count matrix
`prior`	gene expression prior as determined by `scde.expression.prior`
`n.randomizations`	number of bootstrap iterations to perform
`batch`	a factor describing which batch group each cell (i.e. each row of `models` matrix) belongs to
`composition`	a vector describing the batch composition of a group to be sampled
`return.individual.posteriors`	whether expression posteriors of each cell should be returned
`return.individual.posterior.modes`	whether modes of expression posteriors of each cell should be returned
`ensemble.posterior`	Boolean of whether to calculate the ensemble posterior (sum of individual posteriors) instead of a joint (product) posterior. (default: FALSE)
`n.cores`	number of cores to utilize

Value

default: a posterior probability matrix, with rows corresponding to genes, and columns to expression levels (as defined by `prior$x`)

return.individual.posterior.modes: a list is returned, with the `$jp` slot giving the joint posterior matrix, as described above. The `$modes` slot gives a matrix of individual expression posterior mode values on log scale (rows - genes, columns -cells)

return.individual.posteriors: a list is returned, with the `$post` slot giving a list of individual posterior matrices, in a form analogous to the joint posterior matrix, but reported on log scale

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)
data(o.ifm) # Load precomputed model. Use ?scde.error.models to see how o.ifm was generated
o.prior <- scde.expression.prior(models = o.ifm, counts = cd, length.out = 400, show.plot = FALSE)
# calculate joint posteriors
jp <- scde.posteriors(o.ifm, cd, o.prior, n.cores = 1)
```

---

scde.test.gene.expression.difference

Test differential expression and plot posteriors for a particular gene

---

Description

The function performs differential expression test and optionally plots posteriors for a specified gene.

Usage

```
scde.test.gene.expression.difference(gene, models, counts, prior,
  groups = NULL, batch = NULL, batch.models = models,
  n.randomizations = 1000, show.plots = TRUE, return.details = FALSE,
  verbose = FALSE, ratio.range = NULL, show.individual.posteriors = TRUE,
  n.cores = 1)
```

Arguments

`gene`	name of the gene to be tested
`models`	models
`counts`	read count matrix (must contain the row corresponding to the specified gene)
`prior`	expression magnitude prior
`groups`	a two-level factor specifying between which cells (rows of the models matrix) the comparison should be made

batch	optional multi-level factor assigning the cells (rows of the model matrix) to different batches that should be controlled for (e.g. two or more biological replicates). The expression difference estimate will then take into account the likely difference between the two groups that is explained solely by their difference in batch composition. Not all batch configuration may be corrected this way.
batch.models	optional set of models for batch comparison (typically the same as models, but can be more extensive, or recalculated within each batch)
n.randomizations	number of bootstrap/sampling iterations that should be performed
show.plots	whether the plots should be shown
return.details	whether the posterior should be returned
verbose	set to T for some status output
ratio.range	optionally specifies the range of the log2 expression ratio plot
show.individual.posteriors	whether the individual cell expression posteriors should be plotted
n.cores	number of cores to use (default = 1)

Value

by default returns MLE of log2 expression difference, 95

Examples

```
data(es.mef.small)
cd <- clean.counts(es.mef.small, min.lib.size=1000, min.reads = 1, min.detected = 1)
data(o.ifm) # Load precomputed model. Use ?scde.error.models to see how o.ifm was generated
o.prior <- scde.expression.prior(models = o.ifm, counts = cd, length.out = 400, show.plot = FALSE)
scde.test.gene.expression.difference("Tdh", models = o.ifm, counts = cd, prior = o.prior)
```

---

show.app *View PAGODA application*

Description

Installs a given pagoda app (or any other rook app) into a server, optionally making a call to show it in the browser.

Usage

```
show.app(app, name, browse = TRUE, port = NULL, ip = "127.0.0.1",
  server = NULL)
```

Arguments

app	pagoda app (output of make.pagoda.app()) or another rook app
name	URL path name for this app
browse	whether a call should be made for browser to show the app
port	optional port on which the server should be initiated
ip	IP on which the server should listen (typically localhost)
server	an (optional) Rook server instance (defaults to ___scde.server)

Value

Rook server instance

Examples

```
app <- make.pagoda.app(tamr2, tam, varinfo, go.env, pwpca, clpca, col.cols=col.cols, cell.clustering=hc, title="NP
# show app in the browser (port 1468)
show.app(app, "pollen", browse = TRUE, port=1468)
```

---

view.aspects *View heatmap*

Description

Internal function to visualize aspects of transcriptional heterogeneity as a heatmap. Used by pagoda.view.aspects.

Usage

```
view.aspects(mat, row.clustering = NA, cell.clustering = NA, zlim = c(-1,
  1) * quantile(mat, p = 0.95), row.cols = NULL, col.cols = NULL,
  cols = colorRampPalette(c("darkgreen", "white", "darkorange"), space =
  "Lab")(1024), show.row.var.colors = TRUE, top = Inf, ...)
```

Arguments

mat	Numeric matrix
row.clustering	Row dendrogram
cell.clustering	Column dendrogram
zlim	Range of the normalized gene expression levels, inputted as a list: c(lower_bound, upper_bound). Values outside this range will be Winsorized. Useful for increasing the contrast of the heatmap visualizations. Default, set to the 5th and 95th percentiles.
row.cols	Matrix of row colors.

col.cols	Matrix of column colors. Useful for visualizing cell annotations such as batch labels.
cols	Heatmap colors
show.row.var.colors	Boolean of whether to show row variance as a color track
top	Restrict output to the top n aspects of heterogeneity
...	additional arguments for heatmap plotting

Value

A heatmap

ViewPagodaApp-class *A Reference Class to represent the PAGODA application*

Description

This ROOK application class enables communication with the client-side ExtJS framework and Inchlib HTML5 canvas libraries to create the graphical user interface for PAGODA Refer to the code in make.pagoda.app for usage example

Fields

results Output of the pathway clustering and redundancy reduction

genes List of genes to display in the Detailed clustering panel

mat Matrix of posterior mode count estimates

matw Matrix of weights associated with each estimate in mat

goenv Gene set list as an environment

renv Global environment

name Name of the application page; for display as the page title

trim Trim quantity used for Winsorization for visualization

batch Any batch or other known confounders to be included in the visualization as a column color track

`winsorize.matrix` *Winsorize matrix*

Description

Sets the ncol(mat)*trim top outliers in each row to the next lowest value same for the lowest outliers

Usage

```
winsorize.matrix(mat, trim)
```

Arguments

`mat`	matrix
`trim`	fraction of outliers (on each side) that should be Winsorized, or (if the value is >= 1) the number of outliers to be trimmed on each side

Value

Winsorized matrix

Examples

```
set.seed(0)
mat <- matrix( c(rnorm(5*10,mean=0,sd=1), rnorm(5*10,mean=5,sd=1)), 10, 10)  # random matrix
mat[1,1] <- 1000  # make outlier
range(mat)  # look at range of values
win.mat <- winsorize.matrix(mat, 0.1)
range(win.mat)  # note outliers removed
```

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