

Package ‘clusterExperiment’

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Title Compare Clusterings for Single-Cell Sequencing

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Description Provides functionality for running and comparing many different clusterings of single-cell sequencing data or other large mRNA Expression data sets.

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BugReports <https://github.com/epurdom/clusterExperiment/issues>

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'AllHelperClusterFunction.R' 'AllHelperFilter.R'
'JiashinJiCode.R' 'RcppExports.R' 'addClusters.R'
'internalClusterFunctions.R' 'internalFunctions.R'
'builtInClusterFunctions.R' 'clusterContrasts.R'
'clusterLabels.R' 'clusterMany.R' 'clusterSingle.R'
'combineMany.R' 'dataCreation.R' 'getFeatures.R' 'getParams.R'
'mainClustering.R' 'makeBlankData.R' 'makeDendrogram.R'
'makeFilterStats.R' 'makeReducedDims.R' 'mergeClusters.R'
'plotBarplot.R' 'plotClusters.R' 'plotClustersWorkflow.R'
'plotContrastHeatmap.R' 'plotDendrogram.R'
'plotFeatureBoxplot.R' 'plotHeatmap.R' 'plotReduceDim.R'
'plottingHelpers.R' 'rsec.R' 'seqCluster.R'
'subsampleClustering.R' 'subsampleLoop.R' 'transformFunction.R'
'workflowClusters.R'

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 addClusterings, ClusterExperiment, matrix-method

Functions to add/remove clusters to ClusterExperiment

Description

These functions are used to add or remove clusters to a [ClusterExperiment](#) object.

Usage

```
## S4 method for signature 'ClusterExperiment,matrix'
addClusterings(x, y,
  clusterTypes = "User", clusterLabels = NULL, clusterLegend = NULL)

## S4 method for signature 'ClusterExperiment,ClusterExperiment'
addClusterings(x, y)

## S4 method for signature 'ClusterExperiment,vector'
addClusterings(x, y, makePrimary = FALSE,
  ...)

## S4 method for signature 'ClusterExperiment,character'
removeClusterings(x, whichClusters, ...)

## S4 method for signature 'ClusterExperiment,numeric'
removeClusterings(x, whichClusters)

## S4 method for signature 'ClusterExperiment'
removeUnclustered(x)

## S4 method for signature 'ClusterExperiment,numeric'
removeClusters(x, whichClusters,
  clustersToRemove, clusterLabels = NULL)

## S4 method for signature 'ClusterExperiment,character'
removeClusters(x, whichClusters, ...)
```

Arguments

x	a ClusterExperiment object.
y	additional clusters to add to x. Can be a ClusterExperiment object or a matrix/vector of clusters.
clusterTypes	a string describing the nature of the clustering. The values 'clusterSingle', 'clusterMany', 'mergeClusters', 'combineMany' are reserved for the clustering coming from the package workflow and should not be used when creating a new object with the constructor.
clusterLabels	label(s) for the clusters being added. If y a matrix, the column names of that matrix will be used by default, if clusterLabels is not given.
clusterLegend	a list giving the cluster legend for the clusters added.

<code>makePrimary</code>	whether to make the added cluster the primary cluster (only relevant if <code>y</code> is a vector)
<code>...</code>	Passed to signature <code>ClusterExperiment, matrix</code> .
<code>whichClusters</code>	optional argument that can be either numeric or character value. If numeric, gives the indices of the <code>clusterMatrix</code> to return; this can also be used to define an ordering for the clusterings. <code>whichClusters</code> can be a character value identifying the <code>clusterTypes</code> to be used, or if not matching <code>clusterTypes</code> then <code>clusterLabels</code> ; alternatively <code>whichClusters</code> can be either 'all' or 'workflow' to indicate choosing all clusters or choosing all workflowClusters . If missing, the entire matrix of all clusterings is returned.
<code>clustersToRemove</code>	numeric vector identifying the clusters to remove (whose samples will be reassigned to -1 value).

Details

`addClusterings` adds `y` to `x`, and is thus not symmetric in the two arguments. In particular, the `primaryCluster`, all of the dendrogram information, `coClustering`, and `orderSamples` are all kept from the `x` object, even if `y` is a `ClusterExperiment`.

`removeClusterings` removes the clusters given by `whichClusters`. If the `primaryCluster` is one of the clusters removed, the `primaryClusterIndex` is set to 1 and the dendrogram and `coClustering` matrix are discarded and `orderSamples` is set to `1:NCOL(x)`.

`removeUnclustered` removes all samples that are unclustered (i.e. -1 or -2 assignment) in the `primaryCluster` of `x` (so they may be unclustered in other clusters found in `clusterMatrix(x)`).

`removeClusters` creates a new cluster that unassigns samples in `cluster clustersToRemove` (in the clustering defined by `whichClusters`) and assigns them to -1 (unassigned)

Value

A [ClusterExperiment](#) object with the added clusters.

`removeClusterings` returns a `ClusterExperiment` object, unless all clusters are removed, in which case it returns a [SingleCellExperiment](#) object.

Examples

```
data(simData)

c11 <- clusterSingle(simData, subsample=FALSE,
  sequential=FALSE, mainClusterArgs=list(clusterArgs=list(k=3), clusterFunction="pam"))
c12 <- clusterSingle(simData, subsample=FALSE,
  sequential=FALSE, mainClusterArgs=list(clusterArgs=list(k=3), clusterFunction="pam"))

addClusterings(c11, c12)
```

clusterContrasts *Create contrasts for testing DE of a cluster*

Description

Uses clustering to create different types of contrasts to be tested that can then be fed into DE testing programs.

Usage

```
## S4 method for signature 'ClusterExperiment'
clusterContrasts(cluster, contrastType, ...)

## S4 method for signature 'vector'
clusterContrasts(cluster, contrastType = c("Dendro",
  "Pairs", "OneAgainstAll"), dendro = NULL, pairMat = NULL,
  outputType = c("limma", "MAST"), removeNegative = TRUE)
```

Arguments

cluster	Either a vector giving contrasts assignments or a ClusterExperiment object
contrastType	What type of contrast to create. 'Dendro' traverses the given dendrogram and does contrasts of the samples in each side, 'Pairs' does pair-wise contrasts based on the pairs given in pairMat (if pairMat=NULL, does all pairwise), and 'OneAgainstAll' compares each cluster to the average of all others.
...	arguments that are passed to from the ClusterExperiment version to the most basic numeric version.
dendro	The dendrogram to traverse if contrastType="Dendro". Note that this should be the dendrogram of the clusters, not of the individual samples, either of class "dendrogram" or "phylo4"
pairMat	matrix giving the pairs of clusters for which to do pair-wise contrasts (must match to elements of cl). If NULL, will do all pairwise of the clusters in cluster (excluding "-1" categories). Each row is a pair to be compared and must match the names of the clusters in the vector cluster.
outputType	character string. Gives format for the resulting contrast matrix. Currently the two options are the format appropriate for limma and MAST package.
removeNegative	logical, whether to remove negative valued clusters from the design matrix. Appropriate to pick TRUE (default) if design will be input into linear model on samples that excludes -1.

Details

The input vector must be numeric clusters, but the external commands that make the contrast matrix (e.g. [makeContrasts](#)) require syntactically valid R names. For this reason, the names of the levels will be "X1" instead of "1". And negative values (if removeNegative=FALSE) will be "X.1", "X.2", etc.

Value

List with components:

- `contrastMatrix` Contrast matrix, the form of which depends on `outputType`. If `outputType=="limma"`, the result of running `makeContrasts`: a matrix with number of columns equal to the number of contrasts, and rows equal to the number of levels of the factor that will be fit in a linear model.
- `contrastNamesA` vector of names for each of the contrasts. NULL if no such additional names.

Author(s)

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References

Ritchie, ME, Phipson, B, Wu, D, Hu, Y, Law, CW, Shi, W, and Smyth, GK (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research* 43, e47. <http://nar.oxfordjournals.org/content/43/7/e47>

Finak, et al. MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. *Genome Biology* (2015).

Examples

```
data(simData)
c1 <- clusterMany(simData,nReducedDims=c(5,10,50), reduceMethod="PCA",
clusterFunction="pam", ks=2:4, findBestK=c(FALSE), removeSil=TRUE,
subsample=FALSE)
#Pairs:
clusterContrasts(c1,contrastType="Pairs")
#Dendrogram
c1<-makeDendrogram(c1)
clusterContrasts(c1,contrastType="Pairs")
```

ClusterExperiment-class

Class ClusterExperiment

Description

ClusterExperiment is a class that extends SingleCellExperiment and is used to store the data and clustering information.

In addition to the slots of the SingleCellExperiment class, the ClusterExperiment object has the additional slots described in the Slots section.

There are several methods implemented for this class. The most important methods (e.g., `clusterMany`, `combineMany`, ...) have their own help page. Simple helper methods are described in the Methods section. For a comprehensive list of methods specific to this class see the Reference Manual.

The constructor `ClusterExperiment` creates an object of the class `ClusterExperiment`. However, the typical way of creating these objects is the result of a call to `clusterMany` or `clusterSingle`.

Note that when subsetting the data, the co-clustering and dendrogram information are lost.

Usage

```

ClusterExperiment(object, clusters, ...)

## S4 method for signature 'matrixOrHDF5,ANY'
ClusterExperiment(object, clusters, ...)

## S4 method for signature 'SummarizedExperiment,ANY'
ClusterExperiment(object, clusters, ...)

## S4 method for signature 'SingleCellExperiment,numeric'
ClusterExperiment(object, clusters,
  ...)

## S4 method for signature 'SingleCellExperiment,character'
ClusterExperiment(object, clusters,
  ...)

## S4 method for signature 'SingleCellExperiment,factor'
ClusterExperiment(object, clusters, ...)

## S4 method for signature 'SingleCellExperiment,matrix'
ClusterExperiment(object, clusters,
  transformation = function(x) { x }, primaryIndex = 1,
  clusterTypes = "User", clusterInfo = NULL,
  orderSamples = 1:ncol(object), dendro_samples = NULL,
  dendro_index = NA_real_, dendro_clusters = NULL, dendro_outbranch = NA,
  coClustering = NULL, merge_index = NA_real_, merge_cutoff = NA_real_,
  merge_dendrocluster_index = NA_real_, merge_nodeProp = NULL,
  merge_nodeMerge = NULL, merge_method = NA_character_,
  clusterLegend = NULL, checkTransformAndAssay = TRUE)

```

Arguments

object	a matrix or SummarizedExperiment or SingleCellExperiment containing the data that was clustered.
clusters	can be either a numeric or character vector, a factor, or a numeric matrix, containing the cluster labels.
...	The arguments transformation, clusterTypes and clusterInfo to be passed to the constructor for signature SingleCellExperiment,matrix.
transformation	function. A function to transform the data before performing steps that assume normal-like data (i.e. constant variance), such as the log.
primaryIndex	integer. Sets the 'primaryIndex' slot (see Slots).
clusterTypes	a string describing the nature of the clustering. The values 'clusterSingle', 'clusterMany', 'mergeClusters', 'combineMany' are reserved for the clustering coming from the package workflow and should not be used when creating a new object with the constructor.
clusterInfo	a list with information on the clustering (see Slots).
orderSamples	a vector of integers. Sets the 'orderSamples' slot (see Slots).
dendro_samples	dendrogram. Sets the 'dendro_samples' slot (see Slots).
dendro_index	numeric. Sets the dendro_index slot (see Slots).

dendro_clusters	dendrogram. Sets the 'dendro_clusters' slot (see Slots).
dendro_outbranch	logical. Sets the dendro_outbranch slot (see Slots).
coClustering	matrix. Sets the coClustering slot (see Slots).
merge_index	integer. Sets the merge_index slot (see Slots)
merge_cutoff	numeric. Sets the merge_cutoff slot (see Slots)
merge_dendrocluster_index	integer. Sets the merge_dendrocluster_index slot (see Slots)
merge_nodeProp	data.frame. Sets the merge_nodeProp slot (see Slots)
merge_nodeMerge	data.frame. Sets the merge_nodeMerge slot (see Slots)
merge_method	character, Sets the merge_method slot (see Slots)
clusterLegend	list, Sets the clusterLegend slot (see Slots)
checkTransformAndAssay	logical. Whether to check the content of the assay and given transformation function for whether they are valid.

Details

The ClusterExperiment constructor function gives clusterLabels based on the column names of the input matrix/SingleCellExperiment. If missing, will assign labels "cluster1", "cluster2", etc.

Note that the validity check when creating a new ClusterExperiment object with new is less extensive than when using ClusterExperiment function with checkTransformAndAssay=TRUE (the default). Users are advised to use ClusterExperiment to create new ClusterExperiment objects.

Value

A ClusterExperiment object.

Slots

transformation	function. Function to transform the data by when methods that assume normal-like data (e.g. log)
clusterMatrix	matrix. A matrix giving the integer-valued cluster ids for each sample. The rows of the matrix correspond to clusterings and columns to samples. The integer values are assigned in the order that the clusters were found, if found by setting sequential=TRUE in clusterSingle. "-1" indicates the sample was not clustered.
primaryIndex	numeric. An index that specifies the primary set of labels.
clusterInfo	list. A list with info about the clustering. If created from clusterSingle, clusterInfo will include the parameter used for the call, and the call itself. If sequential = TRUE it will also include the following components.
merge_index	index of the current merged cluster
merge_cutoff	value for the cutoff used to determine whether to merge clusters
merge_dendrocluster_index	index of the cluster merged with the current merge
merge_nodeMerge	data.frame of information about nodes merged in the current merge
merge_nodeProp	data.frame of information of proportion estimated non-null at each node of dendrogram

`merge_method` character indicating method used for merging

- `clusterInfo` if `sequential=TRUE` and clusters were successfully found, a matrix of information regarding the algorithm behavior for each cluster (the starting and stopping K for each cluster, and the number of iterations for each cluster).
- `whyStop` if `sequential=TRUE` and clusters were successfully found, a character string explaining what triggered the algorithm to stop.

`clusterTypes` character vector with the origin of each column of `clusterMatrix`.

`dendro_samples` dendrogram. A dendrogram containing the cluster relationship (leaves are samples; see [makeDendrogram](#) for details).

`dendro_clusters` dendrogram. A dendrogram containing the cluster relationship (leaves are clusters; see [makeDendrogram](#) for details).

`dendro_index` numeric. An integer giving the cluster that was used to make the dendrograms. `NA_real_` value if no dendrograms are saved.

`dendro_outbranch` logical. Whether the `dendro_samples` dendrogram put missing/non-clustered samples in an outbranch, or intermixed in the dendrogram.

`coClustering` matrix. A matrix with the cluster co-occurrence information; this can either be based on subsampling or on co-clustering across parameter sets (see `clusterMany`). The matrix is a square matrix with number of rows/columns equal to the number of samples.

`clusterLegend` a list, one per cluster in `clusterMatrix`. Each element of the list is a matrix with n rows equal to the number of different clusters in the clustering, and consisting of at least two columns with the following column names: "clusterId" and "color".

`orderSamples` a numeric vector (of integers) defining the order of samples to be used for plotting of samples. Usually set internally by other functions.

Examples

```
sce <- matrix(data=rnorm(200), ncol=10)
labels <- gl(5, 2)

cc <- ClusterExperiment(sce, as.numeric(labels), transformation =
function(x){x})
```

ClusterExperiment-methods

Helper methods for the ClusterExperiment class

Description

This is a collection of helper methods for the `ClusterExperiment` class.

Usage

```
## S4 method for signature 'ClusterExperiment,ANY,character,ANY'
x[i, j, ..., drop = TRUE]

## S4 method for signature 'ClusterExperiment,ANY,logical,ANY'
x[i, j, ..., drop = TRUE]
```

```
## S4 method for signature 'ClusterExperiment,ANY,numeric,ANY'  
x[i, j, ..., drop = TRUE]  
  
## S4 method for signature 'ClusterExperiment'  
show(object)  
  
## S4 method for signature 'ClusterExperiment'  
clusterMatrixNamed(x)  
  
## S4 method for signature 'ClusterExperiment'  
primaryClusterNamed(x)  
  
## S4 method for signature 'ClusterExperiment'  
transformation(x)  
  
## S4 replacement method for signature 'ClusterExperiment,`function`'  
transformation(object) <- value  
  
## S4 method for signature 'ClusterExperiment'  
nClusterings(x)  
  
## S4 method for signature 'ClusterExperiment'  
nClusters(x, ignoreUnassigned = TRUE)  
  
## S4 method for signature 'ClusterExperiment'  
nFeatures(x)  
  
## S4 method for signature 'ClusterExperiment'  
nSamples(x)  
  
## S4 method for signature 'ClusterExperiment,missing'  
clusterMatrix(x, whichClusters)  
  
## S4 method for signature 'ClusterExperiment,numeric'  
clusterMatrix(x, whichClusters)  
  
## S4 method for signature 'ClusterExperiment,character'  
clusterMatrix(x, whichClusters)  
  
## S4 method for signature 'ClusterExperiment'  
primaryCluster(x)  
  
## S4 method for signature 'ClusterExperiment'  
primaryClusterIndex(x)  
  
## S4 method for signature 'ClusterExperiment'  
dendroClusterIndex(x)  
  
## S4 replacement method for signature 'ClusterExperiment,numeric'  
primaryClusterIndex(object) <- value
```

```

## S4 method for signature 'ClusterExperiment'
coClustering(x)

## S4 replacement method for signature 'ClusterExperiment,matrix'
coClustering(object) <- value

## S4 method for signature 'ClusterExperiment'
clusterTypes(x)

## S4 method for signature 'ClusterExperiment'
clusteringInfo(x)

## S4 method for signature 'ClusterExperiment'
clusterLabels(x)

## S4 replacement method for signature 'ClusterExperiment,character'
clusterLabels(object) <- value

## S4 method for signature 'ClusterExperiment'
clusterLegend(x)

## S4 replacement method for signature 'ClusterExperiment,list'
clusterLegend(object) <- value

## S4 method for signature 'ClusterExperiment'
orderSamples(x)

## S4 replacement method for signature 'ClusterExperiment,numeric'
orderSamples(object) <- value

## S4 replacement method for signature 'ClusterExperiment,character'
clusterTypes(object) <- value

## S4 method for signature 'ClusterExperiment,character'
tableClusters(x, whichClusters, ...)

## S4 method for signature 'ClusterExperiment,missing'
tableClusters(x, whichClusters, ...)

## S4 method for signature 'ClusterExperiment,numeric'
tableClusters(x, whichClusters, ...)

```

Arguments

<code>x</code> , <code>object</code>	a <code>ClusterExperiment</code> object.
<code>...</code> , <code>i</code> , <code>j</code> , <code>drop</code>	Forwarded to the SingleCellExperiment method.
<code>value</code>	The value to be substituted in the corresponding slot. See the slot descriptions in ClusterExperiment for details on what objects may be passed to these functions.
<code>ignoreUnassigned</code>	logical. If true, ignore the clusters with -1 or -2 assignments in calculating the number of clusters per clustering.

`whichClusters` optional argument that can be either numeric or character value. If numeric, gives the indices of the `clusterMatrix` to return; this can also be used to define an ordering for the clusterings. `whichClusters` can be a character value identifying the `clusterTypes` to be used, or if not matching `clusterTypes` then `clusterLabels`; alternatively `whichClusters` can be either 'all' or 'workflow' to indicate choosing all clusters or choosing all [workflowClusters](#). If missing, the entire matrix of all clusterings is returned.

Details

Note that when subsetting the data, the dendrogram information and the co-clustering matrix are lost.

Note that redefining the transformation function via `transformation(x)<-` will check the validity of the transformation on the data assay. If the assay is large, this may be time consuming. Consider using a call to `ClusterExperiment`, which has the option as to whether to check the validity of the transformation.

Value

`clusterMatrixNamed` returns a matrix with cluster labels.

`primaryClusterNamed` returns the primary cluster (using cluster labels).

`transformation` prints the function used to transform the data prior to clustering.

`nClusterings` returns the number of clusterings (i.e., `ncol` of `clusterMatrix`).

`nClusters` returns the number of clusters per clustering

`nFeatures` returns the number of features (same as 'nrow').

`nSamples` returns the number of samples (same as 'ncol').

`clusterMatrix` returns the matrix with all the clusterings.

`clusterMatrix` returns the matrix with all the clusterings.

`clusterMatrix` returns the matrix with all the clusterings.

`primaryCluster` returns the primary clustering (as numeric).

`primaryClusterIndex` returns/sets the primary clustering index (i.e., which column of `clusterMatrix` corresponds to the primary clustering).

`dendroClusterIndex` returns/sets the clustering index of the clusters used to create dendrogram (i.e., which column of `clusterMatrix` corresponds to the clustering).

`coClustering` returns/sets the co-clustering matrix.

`clusterTypes` returns/sets the `clusterTypes` slot.

`clusteringInfo` returns the `clusterInfo` slot.

`clusterLabels` returns/sets the column names of the `clusterMatrix` slot.

`clusterLegend` returns/sets the `clusterLegend` slot.

`orderSamples` returns/sets the `orderSamples` slot.

ClusterFunction-methods*Helper methods for the ClusterFunction class*

Description

This is a collection of helper methods for the ClusterExperiment class.

Usage

```
## S4 method for signature 'character'
requiredArgs(object)

## S4 method for signature 'ClusterFunction'
requiredArgs(object, genericOnly = FALSE)

## S4 method for signature 'character'
requiredArgs(object)

## S4 method for signature 'character'
requiredArgs(object)

## S4 method for signature 'factor'
requiredArgs(object)

## S4 method for signature 'ClusterFunction'
algorithmType(object)

## S4 method for signature 'character'
algorithmType(object)

## S4 method for signature 'factor'
algorithmType(object)

## S4 method for signature 'ClusterFunction'
inputType(object)

## S4 method for signature 'character'
inputType(object)

## S4 method for signature 'factor'
inputType(object)
```

Arguments

<code>object</code>	input to the method, usually either a ClusterFunction class or a character describing a built-in ClusterFunction object.
<code>genericOnly</code>	logical If TRUE, return only the generic required arguments (i.e. those required by the algorithm type) and not the arguments specific to that clustering found in the slot requiredArgs. If FALSE both sets of arguments are returned.

Details

Note that when subsetting the data, the dendrogram information and the co-clustering matrix are lost.

Value

requiredArgs returns a list of the required args of a function (via a call to [requiredArgs](#))

algorithmType returns a character value giving the type of clustering function ("01" or "K")

inputType returns a character value giving the input type of the object

clusterMany	<i>Create a matrix of clustering across values of parameters</i>
-------------	--

Description

Given a range of parameters, this function will return a matrix with the clustering of the samples across the range, which can be passed to `plotClusters` for visualization.

Usage

```
## S4 method for signature 'matrixOrHDF5'
clusterMany(x, reduceMethod = "none",
  nReducedDims = NA, transFun = NULL, isCount = FALSE, ...)

## S4 method for signature 'SingleCellExperiment'
clusterMany(x, ks = NA, clusterFunction,
  reduceMethod = "none", nFilterDims = defaultNDims(x, reduceMethod, type =
  "filterStats"), nReducedDims = defaultNDims(x, reduceMethod, type =
  "reducedDims"), alphas = 0.1, findBestK = FALSE, sequential = FALSE,
  removeSil = FALSE, subsample = FALSE, silCutoff = 0,
  distFunction = NA, betas = 0.9, minSizes = 1, transFun = NULL,
  isCount = FALSE, verbose = FALSE, mainClusterArgs = NULL,
  subsampleArgs = NULL, seqArgs = NULL, ncores = 1, random.seed = NULL,
  run = TRUE, ...)

## S4 method for signature 'ClusterExperiment'
clusterMany(x, reduceMethod = "none",
  nFilterDims = defaultNDims(x, reduceMethod, type = "filterStats"),
  nReducedDims = defaultNDims(x, reduceMethod, type = "reducedDims"),
  eraseOld = FALSE, ...)

## S4 method for signature 'SummarizedExperiment'
clusterMany(x, ...)

## S4 method for signature 'data.frame'
clusterMany(x, ...)
```

Arguments

x	the data matrix on which to run the clustering. Can be object of the following classes: matrix (with genes in rows), SummarizedExperiment , SingleCellExperiment or ClusterExperiment .
reduceMethod	character A character identifying what type of dimensionality reduction to perform before clustering. Options are 1) "none", 2) one of <code>listBuiltInReducedDims()</code> or <code>listBuiltInFiltlerStats</code> OR 3) stored filtering or <code>reducedDim</code> values in the object.
nReducedDims	vector of the number of dimensions to use (when <code>reduceMethod</code> gives a dimensionality reduction method).
transFun	a transformation function to be applied to the data. If the transformation applied to the data creates an error or NA values, then the function will throw an error. If object is of class <code>ClusterExperiment</code> , the stored transformation will be used and giving this parameter will result in an error.
isCount	if <code>transFun=NULL</code> , then <code>isCount=TRUE</code> will determine the transformation as defined by <code>function(x){log2(x+1)}</code> , and <code>isCount=FALSE</code> will give a transformation function <code>function(x){x}</code> . Ignored if <code>transFun=NULL</code> . If object is of class <code>ClusterExperiment</code> , the stored transformation will be used and giving this parameter will result in an error.
...	For signature <code>list</code> , arguments to be passed on to <code>mclapply</code> (if <code>ncores>1</code>). For all the other signatures, arguments to be passed to the method for signature <code>list</code> .
ks	the range of k values (see details for the meaning of k for different choices of other parameters).
clusterFunction	function used for the clustering. Note that unlike in clusterSingle , this must be a character vector of pre-defined clustering techniques, and can not be a user-defined function. Current functions can be found by typing <code>listBuiltInFunctions()</code> into the command-line.
nFilterDims	vector of the number of the most variable features to keep (when "var", "abscv", or "mad" is identified in <code>reduceMethod</code>).
alphas	values of alpha to be tried. Only used for clusterFunctions of type '01'. Determines tightness required in creating clusters from the dissimilarity matrix. Takes on values in [0,1]. See documentation of ClusterFunction .
findBestK	logical, whether should find best K based on average silhouette width (only used when <code>clusterFunction</code> of type "K").
sequential	logical whether to use the sequential strategy (see details of seqCluster). Can be used in combination with <code>subsample=TRUE</code> or <code>FALSE</code> .
removeSil	logical as to whether remove when <code>silhouette < silCutoff</code> (only used if <code>clusterFunction</code> of type "K")
subsample	logical as to whether to subsample via subsampleClustering . If <code>TRUE</code> , clustering in <code>mainClustering</code> step is done on the co-occurrence between clusterings in the subsampled clustering results. If <code>FALSE</code> , the <code>mainClustering</code> step will be run directly on <code>x/diss</code>
silCutoff	Requirement on minimum silhouette width to be included in cluster (only for combinations where <code>removeSil=TRUE</code>).
distFunction	a vector of character strings that are the names of distance functions found in the global environment. See the help pages of clusterSingle for details about

	the required format of distance functions. Currently, this distance function must be applicable for all clusterFunction types tried. Therefore, it is not possible in clusterMany to intermix type "K" and type "01" algorithms if you also give distances to evaluate via distFunction unless all distances give 0-1 values for the distance (and hence are possible for both type "01" and "K" algorithms).
betas	values of beta to be tried in sequential steps. Only used for sequential=TRUE. Determines the similarity between two clusters required in order to deem the cluster stable. Takes on values in [0,1]. See documentation of seqCluster .
minSizes	the minimum size required for a cluster (in the mainClustering step). Clusters smaller than this are not kept and samples are left unassigned.
verbose	logical. If TRUE it will print informative messages.
mainClusterArgs	list of arguments to be passed for the mainClustering step, see help pages of mainClustering .
subsampleArgs	list of arguments to be passed to the subsampling step (if subsample=TRUE), see help pages of subsampleClustering .
seqArgs	list of arguments to be passed to seqCluster .
ncores	the number of threads
random.seed	a value to set seed before each run of clusterSingle (so that all of the runs are run on the same subsample of the data). Note, if 'random.seed' is set, argument 'ncores' should NOT be passed via subsampleArgs; instead set the argument 'ncores' of clusterMany directly (which is preferred for improving speed anyway).
run	logical. If FALSE, doesn't run clustering, but just returns matrix of parameters that will be run, for the purpose of inspection by user (with rownames equal to the names of the resulting column names of clMat object that would be returned if run=TRUE). Even if run=FALSE, however, the function will create the dimensionality reductions of the data indicated by the user input.
eraseOld	logical. Only relevant if input x is of class ClusterExperiment. If TRUE, will erase existing workflow results (clusterMany as well as mergeClusters and combineMany). If FALSE, existing workflow results will have "_i" added to the clusterTypes value, where i is one more than the largest such existing workflow clusterTypes.

Details

Some combinations of these parameters are not feasible. See the documentation of [clusterSingle](#) for important information on how these parameter choices interact.

While the function allows for multiple values of clusterFunction, the code does not reuse the same subsampling matrix and try different clusterFunctions on it. This is because if sequential=TRUE, different subsample clusterFunctions will create different sets of data to subsample so it is not possible; if sequential=FALSE, we have not implemented functionality for this reuse. Setting the random.seed value, however, should mean that the subsampled matrix is the same for each, but there is no gain in computational complexity (i.e. each subsampled co-occurrence matrix is recalculated for each set of parameters).

The argument ks is interpreted differently for different choices of the other parameters. When/if sequential=TRUE, ks defines the argument k0 of [seqCluster](#). Otherwise, ks values are the k values for **both** the mainClustering and subsampling step (i.e. assigned to the subsampleArgs and mainClusterArgs that are passed to [mainClustering](#) and [subsampleClustering](#) unless k

is set appropriately in `subsampleArgs`. The passing of these arguments via `subsampleArgs` will only have an effect if `'subsample=TRUE'`. Similarly, the passing of `mainClusterArgs[["k"]]` will only have an effect when the `clusterFunction` argument includes a clustering algorithm of type "K". When/if `"findBestK=TRUE"`, `ks` also defines the `kRange` argument of `mainClustering` unless `kRange` is specified by the user via the `mainClusterArgs`; note this means that the default option of setting `kRange` that depends on the input `k` (see `mainClustering`) is not available in `clusterMany`, only in `clusterSingle`.

If the input is a `ClusterExperiment` object, current implementation is that existing `orderSamples`, `coClustering` or the many dendrogram slots will be retained.

Value

If `run=TRUE` and the input is not a list of data matrices, will return a `ClusterExperiment` object, where the results are stored as clusterings with clusterTypes `clusterMany`. Depending on `eraseOld` argument above, this will either delete existing such objects, or change the clusterTypes of existing objects. See argument `eraseOld` above. Arbitrarily the first clustering is set as the `primaryClusteringIndex`.

If `run=TRUE` and the input is a list of data sets, a list with the following objects:

- `c1Mat` a matrix with each column corresponding to a clustering and each row to a sample.
- `clusterInfo` a list with information regarding clustering results (only relevant entries for those clusterings with `sequential=TRUE`)
- `paramMatrix` a matrix giving the parameters of each clustering, where each column is a possible parameter set by the user and passed to `clusterSingle` and each row of `paramMatrix` corresponds to a clustering in `c1Mat`
- `mainClusterArgs` a list of (possibly modified) arguments to `mainClusterArgs`
- `seqArgs=seqArgs` a list of (possibly modified) arguments to `seqArgs`
- `subsampleArgs` a list of (possibly modified) arguments to `subsampleArgs`

If `run=FALSE` a list similar to that described above, but without the clustering results.

Examples

```
data(simData)

#Example: clustering using pam with different dimensions of pca and different
#k and whether remove negative silhouette values
#check how many and what runs user choices will imply:
checkParams <- clusterMany(simData,reduceMethod="PCA",
nReducedDims=c(5,10,50), clusterFunction="pam", isCount=FALSE,
ks=2:4,findBestK=c(TRUE,FALSE),removeSil=c(TRUE,FALSE),run=FALSE)
print(head(checkParams$paramMatrix))

#Now actually run it
cl <- clusterMany(simData,reduceMethod="PCA", nReducedDims=c(5,10,50), isCount=FALSE,
clusterFunction="pam",ks=2:4,findBestK=c(TRUE,FALSE),removeSil=c(TRUE,FALSE))
print(cl)
head(colnames(clusterMatrix(cl)))

#make names shorter for plotting
clNames <- clusterLabels(cl)
clNames <- gsub("TRUE", "T", clNames)
clNames <- gsub("FALSE", "F", clNames)
```

```

c1Names <- gsub("k=NA,", "", c1Names)

par(mar=c(2, 10, 1, 1))
plotClusters(c1, axisLine=-2, clusterLabels=c1Names)

## Not run:
#following code takes around 1+ minutes to run because of the subsampling
#that is redone each time:
system.time(clusterTrack <- clusterMany(simData, ks=2:15,
  alphas=c(0.1,0.2,0.3), findBestK=c(TRUE,FALSE), sequential=c(FALSE),
  subsample=c(FALSE), removeSil=c(TRUE), clusterFunction="pam",
  mainClusterArgs=list(minSize=5, kRange=2:15), ncores=1, random.seed=48120))

## End(Not run)

```

clusterSingle

General wrapper method to cluster the data

Description

Given input data, this function will find clusters, based on a single specification of parameters.

Usage

```

## S4 method for signature 'missing,matrixOrNULL'
clusterSingle(x, diss, ...)

## S4 method for signature 'matrixOrHDF5OrNULL,missing'
clusterSingle(x, diss, ...)

## S4 method for signature 'SummarizedExperiment,missing'
clusterSingle(x, diss, ...)

## S4 method for signature 'ClusterExperiment,missing'
clusterSingle(x,
  replaceCoClustering = FALSE, ...)

## S4 method for signature 'SingleCellExperiment,missing'
clusterSingle(x,
  reduceMethod = "none", nDims = defaultNDims(x, reduceMethod), ...)

## S4 method for signature 'matrixOrHDF5OrNULL,matrixOrNULL'
clusterSingle(x, diss,
  subsample = TRUE, sequential = FALSE, mainClusterArgs = NULL,
  subsampleArgs = NULL, seqArgs = NULL, isCount = FALSE,
  transFun = NULL, reduceMethod = c("none", listBuiltInReducedDims(),
  listBuiltInFilterStats()), nDims = defaultNDims(x, reduceMethod),
  clusterLabel = "clusterSingle", checkDiss = TRUE)

```

Arguments

x	the data on which to run the clustering (features in rows), or a SummarizedExperiment , SingleCellExperiment , or ClusterExperiment object.
diss	n x n data matrix of dissimilarities between the samples on which to run the clustering.
...	arguments to be passed on to the method for signature matrix.
replaceCoClustering	logical. Applicable if x is a ClusterExperiment object. If TRUE, the co-clustering resulting from subsampling is returned in the coClustering object and replaces any existing coClustering object in the slot coClustering.
reduceMethod	character A character identifying what type of dimensionality reduction to perform before clustering. Options are 1) "none", 2) one of listBuiltInReducedDims() or listBuiltInFiltlerStats OR 3) stored filtering or reducedDim values in the object.
nDims	integer An integer identifying how many dimensions to reduce to in the reduction specified by reduceMethod. Defaults to output of defaultNDims
subsample	logical as to whether to subsample via subsampleClustering . If TRUE, clustering in mainClustering step is done on the co-occurrence between clusterings in the subsampled clustering results. If FALSE, the mainClustering step will be run directly on x/diss
sequential	logical whether to use the sequential strategy (see details of seqCluster). Can be used in combination with subsample=TRUE or FALSE.
mainClusterArgs	list of arguments to be passed for the mainClustering step, see help pages of mainClustering .
subsampleArgs	list of arguments to be passed to the subsampling step (if subsample=TRUE), see help pages of subsampleClustering .
seqArgs	list of arguments to be passed to seqCluster .
isCount	if transFun=NULL, then isCount=TRUE will determine the transformation as defined by <code>function(x){log2(x+1)}</code> , and isCount=FALSE will give a transformation function <code>function(x){x}</code> . Ignored if transFun=NULL. If object is of class ClusterExperiment , the stored transformation will be used and giving this parameter will result in an error.
transFun	a transformation function to be applied to the data. If the transformation applied to the data creates an error or NA values, then the function will throw an error. If object is of class ClusterExperiment , the stored transformation will be used and giving this parameter will result in an error.
clusterLabel	a string used to describe the clustering. By default it is equal to "clusterSingle", to indicate that this clustering is the result of a call to <code>clusterSingle</code> .
checkDiss	logical. Whether to check whether the input diss is valid.

Details

`clusterSingle` is an 'expert-oriented' function, intended to be used when a user wants to run a single clustering and/or have a great deal of control over the clustering parameters. Most users will find [clusterMany](#) more relevant. However, [clusterMany](#) makes certain assumptions about the intention of certain combinations of parameters that might not match the user's intent; similarly

`clusterMany` does not directly take a dissimilarity matrix but only a matrix of values x (though a user can define a distance function to be applied to x in `clusterMany`).

Unlike `clusterMany`, most of the relevant arguments for the actual clustering algorithms in `clusterSingle` are passed to the relevant steps via the arguments `mainClusterArgs`, `subsampleArgs`, and `seqArgs`. These arguments should be *named* lists with parameters that match the corresponding functions: `mainClustering`, `subsampleClustering`, and `seqCluster`. These functions are not meant to be called by the user, but rather accessed via calls to `clusterSingle`. But the user can look at the help files of those functions for more information regarding the parameters that they take.

Only certain combinations of parameters are possible for certain choices of `sequential` and `subsample`. These restrictions are documented below.

- `clusterFunction` for `mainClusterArgs`: The choice of `subsample=TRUE` also controls what algorithm type of clustering functions can be used in the `mainClustering` step. When `subsample=TRUE`, then resulting co-clustering matrix from subsampling is converted to a dissimilarity (specifically 1-coclustering values) and is passed to `diss` of `mainClustering`. For this reason, the `ClusterFunction` object given to `mainClustering` via the argument `mainClusterArgs` must take input of the form of a dissimilarity. When `subsample=FALSE` and `sequential=TRUE`, the `clusterFunction` passed in `clusterArgs` element of `mainClusterArgs` must define a `ClusterFunction` object with `algorithmType 'K'`. When `subsample=FALSE` and `sequential=FALSE`, then there are no restrictions on the `ClusterFunction` and that clustering is applied directly to the input data.
- `clusterFunction` for `subsampleArgs`: If the `ClusterFunction` object given to the `clusterArgs` of `subsampleArgs` is missing the algorithm will use the default for `subsampleClustering` (currently "pam"). If `sequential=TRUE`, this `ClusterFunction` object must be of type 'K'.
- Setting `k` for subsampling: If `subsample=TRUE` and `sequential=TRUE`, the current `K` of the sequential iteration determines the 'k' argument passed to `subsampleClustering` so setting 'k=' in the list given to the `subsampleArgs` will not do anything and will produce a warning to that effect (see documentation of `seqCluster`).
- Setting `k` for `mainClustering` step: If `sequential=TRUE` then the user should not set `k` in the `clusterArgs` argument of `mainClusterArgs` because it must be set by the sequential code, which has a iterative resetting of the parameters. Specifically if `subsample=FALSE`, then the sequential method iterates over choices of `k` to cluster the input data. And if `subsample=TRUE`, then the `k` in the clustering of `mainClustering` step (assuming the clustering function is of type 'K') will use the `k` used in the subsampling step to make sure that the `k` used in the `mainClustering` step is reasonable.
- Setting `findBestK` in `mainClusterArgs`: If `sequential=TRUE` and `subsample=FALSE`, the user should not set 'findBestK=TRUE' in `mainClusterArgs`. This is because in this case the sequential method changes `k`; an error message will be given if this combination of options are set. However, if `sequential=TRUE` and `subsample=TRUE`, then passing either 'findBestK=TRUE' or 'findBestK=FALSE' via `mainClusterArgs` will function as expected (assuming the `clusterFunction` argument passed to `mainClusterArgs` is of type 'K'). In particular, the sequential step will set the number of clusters `k` for clustering of each subsample. If `findBestK=FALSE`, that same `k` will be used for `mainClustering` step that clusters the resulting co-occurrence matrix after subsampling. If `findBestK=TRUE`, then `mainClustering` will search for best `k`. Note that the default 'kRange' over which `mainClustering` searches when `findBestK=TRUE` depends on the input value of `k` which is set by the sequential method if `sequential=TRUE`, see above. The user can change `kRange` to not depend on `k` and to be fixed across all of the sequential steps by setting `kRange` explicitly in the `mainClusterArgs` list.

To provide a distance matrix via the argument `distFunction`, the function must be defined to take the distance of the rows of a matrix (internally, the function will call `distFunction(t(x))`). This is

to be compatible with the input for the `dist` function. `as.matrix` will be performed on the output of `distFunction`, so if the object returned has a `as.matrix` method that will convert the output into a symmetric matrix of distances, this is fine (for example the class `dist` for objects returned by `dist` have such a method). If `distFunction=NA`, then a default distance will be calculated based on the type of clustering algorithm of `clusterFunction`. For type "K" the default is to take `dist` as the distance function. For type "01", the default is to take the $(1-\text{cor}(x))/2$.

Value

A `ClusterExperiment` object if `run=TRUE`.

If input was `diss`, then the result is a list with values

- `clustering`: The vector of clustering results
- `clusterInfo`: A list with information about the parameters run in the clustering
- `diss`: The dissimilarity matrix used in the clustering

See Also

[clusterMany](#) to compare multiple choices of parameters, and [mainClustering](#), [subsampleClustering](#), and [seqCluster](#) for the underlying functions called by `clusterSingle`.

Examples

```
data(simData)

## Not run:
#following code takes some time.
#use clusterSingle to do sequential clustering
#(same as example in seqCluster only using clusterSingle ...)
  clusterFunction="hierarchical01",clusterArgs=list(alpha=0.1))

## End(Not run)

#use clusterSingle to do just clustering k=3 with no subsampling
clustNothing <- clusterSingle(simData,
  subsample=FALSE, sequential=FALSE,
  mainClusterArgs=list(clusterFunction="pam", clusterArgs=list(k=3)))
#compare to standard pam
cluster::pam(t(simData),k=3,cluster.only=TRUE)
```

combineMany,matrix,missing-method

Find sets of samples that stay together across clusterings

Description

Find sets of samples that stay together across clusterings in order to define a new clustering vector.

Usage

```
## S4 method for signature 'matrix,missing'
combineMany(x, whichClusters, proportion,
  clusterFunction = "hierarchical01", propUnassigned = 0.5, minSize = 5,
  ...)

## S4 method for signature 'ClusterExperiment,numeric'
combineMany(x, whichClusters,
  eraseOld = FALSE, clusterLabel = "combineMany", ...)

## S4 method for signature 'ClusterExperiment,character'
combineMany(x, whichClusters, ...)

## S4 method for signature 'ClusterExperiment,missing'
combineMany(x, whichClusters, ...)
```

Arguments

x	a matrix or ClusterExperiment object.
whichClusters	a numeric or character vector that specifies which clusters to compare (missing if x is a matrix)
proportion	The proportion of times that two sets of samples should be together in order to be grouped into a cluster (if <1, passed to mainClustering via $\alpha = 1 - \text{proportion}$)
clusterFunction	the clustering to use (passed to mainClustering); currently must be of type '01'.
propUnassigned	samples with greater than this proportion of assignments equal to '-1' are assigned a '-1' cluster value as a last step (only if $\text{proportion} < 1$)
minSize	minimum size required for a set of samples to be considered in a cluster because of shared clustering, passed to mainClustering
...	arguments to be passed on to the method for signature <code>matrix,missing</code> .
eraseOld	logical. Only relevant if input x is of class <code>ClusterExperiment</code> . If TRUE, will erase existing workflow results (<code>clusterMany</code> as well as <code>mergeClusters</code> and <code>combineMany</code>). If FALSE, existing workflow results will have "_i" added to the <code>clusterTypes</code> value, where i is one more than the largest such existing workflow <code>clusterTypes</code> .
clusterLabel	a string used to describe the type of clustering. By default it is equal to "combineMany", to indicate that this clustering is the result of a call to <code>combineMany</code> . However, a more informative label can be set (see vignette).

Details

The function tries to find a consensus cluster across many different clusterings of the same samples. It does so by creating a `nSamples x nSamples` matrix of the percentage of co-occurrence of each sample and then calling `mainClustering` to cluster the co-occurrence matrix. The function assumes that '-1' labels indicate clusters that are not assigned to a cluster. Co-occurrence with the unassigned cluster is treated differently than other clusters. The percent co-occurrence is taken only with respect to those clusterings where both samples were assigned. Then samples with more than

propUnassigned values that are '-1' across all of the clusterings are assigned a '-1' regardless of their cluster assignment.

The method calls [mainClustering](#) on the proportion matrix with clusterFunction as the O1 clustering algorithm, alpha=1-proportion, minSize=minSize, and evalClusterMethod=c("average"). See help of [mainClustering](#) for more details.

Value

If x is a matrix, a list with values

- clustering vector of cluster assignments, with "-1" implying unassigned
- percentageShared a nSample x nSample matrix of the percent co-occurrence across clusters used to find the final clusters. Percentage is out of those not '-1'
- noUnassignedCorrection a vector of cluster assignments before samples were converted to '-1' because had >propUnassigned '-1' values (i.e. the direct output of the mainClustering output.)

If x is a [ClusterExperiment](#), a [ClusterExperiment](#) object, with an added clustering of clusterTypes equal to combineMany and the percentageShared matrix stored in the coClustering slot.

Examples

```
data(simData)

cl <- clusterMany(simData,nReducedDims=c(5,10,50), reduceMethod="PCA",
clusterFunction="pam", ks=2:4, findBestK=c(FALSE), removeSil=TRUE,
subsample=FALSE)

#make names shorter for plotting
clMat <- clusterMatrix(cl)
colnames(clMat) <- gsub("TRUE", "T", colnames(clMat))
colnames(clMat) <- gsub("FALSE", "F", colnames(clMat))
colnames(clMat) <- gsub("k=NA,", "", colnames(clMat))

#require 100% agreement -- very strict
clCommon100 <- combineMany(clMat, proportion=1, minSize=10)

#require 70% agreement based on clustering of overlap
clCommon70 <- combineMany(clMat, proportion=0.7, minSize=10)

oldpar <- par()
par(mar=c(1.1, 12.1, 1.1, 1.1))
plotClusters(cbind("70%Similarity"=clCommon70$clustering, clMat,
"100%Similarity"=clCommon100$clustering), axisLine=-2)

#method for ClusterExperiment object
clCommon <- combineMany(cl, whichClusters="workflow", proportion=0.7,
minSize=10)
plotClusters(clCommon)
par(oldpar)
```

getBestFeatures,matrixOrHDF5-method

Function for finding best features associated with clusters

Description

Calls limma on input data to determine features most associated with found clusters (based on an F-statistic, pairwise comparisons, or following a tree that clusters the clusters).

Usage

```
## S4 method for signature 'matrixOrHDF5'
getBestFeatures(x, cluster, contrastType = c("F",
      "Dendro", "Pairs", "OneAgainstAll"), dendro = NULL, pairMat = NULL,
      contrastAdj = c("All", "PerContrast", "AfterF"), isCount = FALSE,
      normalize.method = "none", ...)

## S4 method for signature 'ClusterExperiment'
getBestFeatures(x, contrastType = c("F",
      "Dendro", "Pairs", "OneAgainstAll"), isCount = FALSE, ...)
```

Arguments

x	data for the test. Can be a numeric matrix or a ClusterExperiment .
cluster	A numeric vector with cluster assignments. "-1" indicates the sample was not assigned to a cluster.
contrastType	What type of test to do. 'F' gives the omnibus F-statistic, 'Dendro' traverses the given dendrogram and does contrasts of the samples in each side, 'Pairs' does pair-wise contrasts based on the pairs given in pairMat (if pairMat=NULL, does all pairwise), and 'OneAgainstAll' compares each cluster to the average of all others. Passed to clusterContrasts
dendro	The dendrogram to traverse if contrastType="Dendro". Note that this should be the dendrogram of the clusters, not of the individual samples, either of class "dendrogram" or "phylo4"
pairMat	matrix giving the pairs of clusters for which to do pair-wise contrasts (must match to elements of cl). If NULL, will do all pairwise of the clusters in cluster (excluding "-1" categories). Each row is a pair to be compared and must match the names of the clusters in the vector cluster.
contrastAdj	What type of FDR correction to do for contrasts tests (i.e. if contrastType='Dendro' or 'Pairs').
isCount	logical as to whether input data is count data, in which case to perform voom correction to data. See details.
normalize.method	character value, passed to voom in limma package. Only used if countData=TRUE. Note that the default value is set to "none", which is not the default value of voom .
...	options to pass to topTable or topTableF (see limma package)

Details

getBestFeatures returns the top ranked features corresponding to a cluster assignment. It uses limma to fit the models, and limma's functions `topTable` or `topTableF` to find the best features. See the options of these functions to put better control on what gets returned (e.g. only if significant, only if log-fc is above a certain amount, etc.). In particular, set `'number='` to define how many significant features to return (where number is per contrast for the `'Pairs'` or `'Dendro'` option)

When `'contrastType'` argument implies that the best features should be found via contrasts (i.e. `'contrastType'` is `'Pairs'` or `'Dendro'`), then then `'contrastAdj'` determines the type of multiple testing correction to perform. `'PerContrast'` does FDR correction for each set of contrasts, and does not guarantee control across all the different contrasts (so probably not the preferred method). `'All'` calculates the corrected p-values based on FDR correction of all of the contrasts tested. `'AfterF'` controls the FDR based on a hierarchical scheme that only tests the contrasts in those genes where the omnibus F statistic is significant. If the user selects `'AfterF'`, the user must also supply an option `'p.value'` to have any effect, and then only those significant at that p.value level will be returned. Note that currently the correction for `'AfterF'` is not guaranteed to control the FDR; improvements will be added in the future.

Note that the default option for `topTable` is to not filter based on adjusted p-values (`p.value = 1`) and return only the top 10 most significant (`number = 10`) – these are options the user can change (these arguments are passed via the `...` in `getBestFeatures`). In particular, it only makes sense to set `requireF = TRUE` if p.value is meaningful (e.g. 0.1 or 0.05); the default value of `p.value = 1` will not result in any effect on the adjusted p-value otherwise.

`isCount` triggers whether the "voom" correction will be performed in limma. If the input data is a matrix is counts (or a `'ClusterExperiment'` object with counts as the primary data before transformation) this should be set to `TRUE` and they will be log-transformed internally by voom for the differential expression analysis in a way that accounts for the difference in the mean-variance relationships. Otherwise, `dat` should be on the correct (log) scale for differential expression analysis without a need a variance stabilization (e.g. microarray data). Currently the default is set to `FALSE`, simply because the `isCount` has not been heavily tested. If the But `TRUE` with `x` being counts really should be the default for RNA-Seq data. If the input data is a `'ClusterExperiment'` object, setting `'isCount=TRUE'` will cause the program to ignore the internally stored transformation function and instead use voom with $\log_2(x+0.5)$. Alternatively, `isCount=FALSE` for a `ClusterExperiment` object will cause the DE to be performed with limma after transforming the data with the stored transformation. Although some writing about "voom" seem to suggest that it would be appropriate for arbitrary transformations, the authors have cautioned against using it for anything other than count data on mailing lists. For this reason we are not implementing it for arbitrary transformations at this time (e.g. $\log(\text{FPKM}+\text{epsilon})$ transformations).

Value

A data.frame in the same format as `topTable`, except for the following additional or changed columns:

- `Feature` This is the column called `'ProbeID'` by `topTable`
- `IndexInOriginal` Gives the index of the feature to the original input dataset, `x`
- `Contrast` The contrast that the results corresponds to (if applicable, depends on `contrastType` argument)
- `ContrastName` The name of the contrast that the results corresponds to. For dendrogram searches, this will be the node of the tree of the dendrogram.

References

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Law, CW, Chen, Y, Shi, W, and Smyth, GK (2014). Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology* 15, R29. <http://genomebiology.com/2014/15/2/R29>

Smyth, G. K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Statistical Applications in Genetics and Molecular Biology*, Volume 3, Article 3. <http://www.statsci.org/smyth/pubs/ebayes.pdf>

Examples

```
data(simData)

#create a clustering, for 8 clusters (truth was 4)
cl <- clusterSingle(simData, subsample=FALSE,
sequential=FALSE, mainClusterArgs=list(clusterFunction="pam", clusterArgs=list(k=8)))

#basic F test, return all, even if not significant:
testF <- getBestFeatures(cl, contrastType="F", number=nrow(simData),
isCount=FALSE)

#Do all pairwise, only return significant, try different adjustments:
pairsPerC <- getBestFeatures(cl, contrastType="Pairs", contrastAdj="PerContrast",
p.value=0.05, isCount=FALSE)
pairsAfterF <- getBestFeatures(cl, contrastType="Pairs", contrastAdj="AfterF",
p.value=0.05, isCount=FALSE)
pairsAll <- getBestFeatures(cl, contrastType="Pairs", contrastAdj="All",
p.value=0.05, isCount=FALSE)

#not useful for this silly example, but could look at overlap with Venn
allGenes <- paste("Row", 1:nrow(simData), sep="")
if(require(limma)){
  vennC <- vennCounts(cbind(PerContrast= allGenes %in% pairsPerC$Feature,
  AllJoint=allGenes %in% pairsAll$Feature, FHier=allGenes %in%
  pairsAfterF$Feature))
  vennDiagram(vennC, main="FDR Overlap")
}

#Do one cluster against all others
oneAll <- getBestFeatures(cl, contrastType="OneAgainstAll", contrastAdj="All",
p.value=0.05)

#Do dendrogram testing
hcl <- makeDendrogram(cl)
allDendro <- getBestFeatures(hcl, contrastType="Dendro", contrastAdj=c("All"),
number=ncol(simData), p.value=0.05)

# do DE on counts using voom
# compare results to if used simData instead (not on count scale).
# Again, not relevant for this silly example, but basic principle useful
testFVoom <- getBestFeatures(simCount, primaryCluster(cl), contrastType="F",
number=nrow(simData), isCount=TRUE)
plot(testF$P.Value[order(testF$Index)],
testFVoom$P.Value[order(testFVoom$Index)], log="xy")
```

```
getClusterManyParams, ClusterExperiment-method
```

Get parameter values of clusterMany clusters

Description

Takes an input a ClusterExperiment object and returns the parameter values used in creating the clusters that were created by 'clusterMany'

Usage

```
## S4 method for signature 'ClusterExperiment'
getClusterManyParams(x,
  whichClusters = "clusterMany", simplify = TRUE)
```

Arguments

x	a ClusterExperiment object that contains clusterings from running <code>clusterMany</code> .
whichClusters	The indices (or clusterLabels) of those clusters whose labels will be parsed to determine the parameters; should be subset of the clusterMany results.
simplify	logical. Whether to simplify the output so as to remove features that do not change across the clusterings.

Details

The method simply parses the clusterLabels of the indicated clusterings, relying on the specific format used by clusterMany to create labels. The function will only allow the parsing to be performed on those clusterings with a 'clusterMany' clusterType. If the user has manipulated the clusterLabels manually or manually identified the clusterType of a clustering as 'clusterMany', this function may create unexpected results or errors. Similarly, it cannot be used on 'clusterMany' results from an old iteration (e.g. that have type 'clusterMany.1')

Specifically, it splits the label of each clustering by the character ",", as indicating the different parameters; this should return a value of form "ABC=123". The function then pulls out the numeric value ('123') and associates that value as the value of the parameter ('ABC')

Value

Returns a data.frame where the column names are the parameter names, and the entries are the values of the parameter for the indicated clustering. The column 'clusteringIndex' identifies the index of the clustering in the full set of clusterings of the given ClusterExperiment object.

Examples

```
data(simData)

c1 <- clusterMany(simData, nReducedDims=c(5, 10, 50), reduceMethod="PCA",
  clusterFunction="pam", ks=2:4, findBestK=c(TRUE,FALSE),
  removeSil=c(TRUE,FALSE))
getClusterManyParams(c1)
```

internalFunctionCheck *Class ClusterFunction*

Description

ClusterFunction is a class for holding functions that can be used for clustering in the clustering algorithms in this package.

The constructor ClusterFunction creates an object of the class ClusterFunction.

Usage

```
internalFunctionCheck(clusterFUN, inputType, algorithmType, outputType)
```

```
ClusterFunction(clusterFUN, ...)
```

```
## S4 method for signature ``function``
ClusterFunction(clusterFUN, inputType, outputType,
  algorithmType, inputClassifyType = NA_character_,
  requiredArgs = NA_character_, classifyFUN = NULL, checkFunctions = TRUE)
```

Arguments

clusterFUN	function passed to slot clusterFUN.
inputType	character for slot inputType
algorithmType	character for slot inputType
outputType	character for slot outputType
...	arguments passed to different methods of ClusterFunction
inputClassifyType	character for slot inputClassifyType
requiredArgs	character for slot requiredArgs
classifyFUN	function for slot classifyFUN
checkFunctions	logical for whether to check the input functions with internalFunctionsCheck

Details

internalFunctionCheck is the function that is called by the validity check of the ClusterFunction constructor (if checkFunctions=TRUE). It is available as an S3 function for the user to be able to test their functions and debug them, which is difficult to do with a S4 validity function.

Required arguments for clusterFUN:

- "x or diss" either x and/or diss depending on inputType. If x, then x is assumed to be nfeatures x nsamples (like assay(CEObj) would give)
- "checkArgs" logical argument. If checkArgs=TRUE, the clusterFUN should check if the arguments passed in ... are valid and return an error if not; otherwise, no error will be given, but the check should be done and only valid arguments in ... passed along. This is necessary for the function to work with clusterMany which passes all arguments to all functions without checking.

- "cluster.only" logical argument. If `cluster.only=TRUE`, then `clusterFUN` should return only the vector of cluster assignments (or list if `outputType="list"`). If `cluster.only=FALSE` then the `clusterFUN` should return a named list where one of the elements entitled `clustering` contains the vector described above (no list!); anything else needed by the `classifyFUN` to classify new data should be contained in the output list as well. `cluster.only` is set internally depending on whether `classifyFUN` will be used by subsampling or only for clustering the final product.
- "... Any additional arguments specific to the algorithm used by `clusterFUN` should be passed via `...` and NOT passed via arguments to `clusterFUN`
- "Other required arguments" `clusterFUN` must also accept arguments required for its `algorithmType` (see Details below).

`algorithmType`: Type "01" is for clustering functions that expect as an input a dissimilarity matrix that takes on 0-1 values (e.g. from subclustering) with 1 indicating more dissimilarity between samples. "01" algorithm types must also have `inputType` equal to "diss". It is also generally expected that "01" algorithms use the 0-1 nature of the input to set criteria as to where to find clusters. "01" functions must take as an argument `alpha` between 0 and 1 to determine the clusters, where larger values of `alpha` require less similarity between samples in the same cluster. "K" is for clustering functions that require an argument `k` (the number of clusters), but arbitrary `inputType`. On the other hand, "K" algorithms are assumed to need a predetermined 'k' and are also assumed to cluster all samples to a cluster. If not, the post-processing steps in `mainClustering` such as `findBestK` and `removeSil` may not operate correctly since they rely on silhouette distances.

Value

A `ClusterFunction` object.

Slots

`clusterFUN` a function defining the clustering function. See details for required arguments.

`inputType` a character defining what type of input `clusterFUN` takes. Must be one of either "diss", "X", or "either"

`algorithmType` a character defining what type of clustering algorithm `clusterFUN` is. Must be one of either "01" or "K". `clusterFUN` must take the corresponding required arguments (see details below).

`classifyFUN` a function that takes as input new data and the output of `clusterFUN` (when `cluster.only=FALSE` and results in cluster assignments of the new data. Note that the function should assume that the input 'x' is not the same samples that were input to the `ClusterFunction` (but can assume that it is the same number of features/columns). Used in subsampling clustering. If given value NULL then subsampling can only be "InSample", see `subsampleClustering`.

`inputClassifyType` the input type for the classification function (if not NULL); like `inputType`, must be one of "diss", "X", or "either"

`outputType` the type of output given by `clusterFUN`. Must either be "vector" or "list". If "vector" then the output should be a vector of length equal to the number of observations with integer-valued elements identifying them to different clusters; the vector assignments should be in the same order as the original input of the data. Samples that are not assigned to any cluster should be given a '-1' value. If "list", then it must be a list equal to the length of the number of clusters, and the elements of the list contain the indices of the samples in that cluster. Any indices not in any of the list elements are assumed to be -1. The main advantage of "list" is that it can preserve the order of the clusters if the `clusterFUN` desires to do so. In which case the `orderBy` argument of `mainClustering` can preserve this ordering (default is to order by size).

`requiredArgs` Any additional required arguments for `clusterFUN` (beyond those required of all `clusterFUN`, described in details).

`checkFunctions` logical. If TRUE, the validity check of the `ClusterFunction` object will check the `clusterFUN` with simple toy data using the function `internalFunctionCheck`.

Examples

```
#Use internalFunctionCheck to check possible function
goodFUN<-function(x,diss,k,checkArgs,cluster.only,...){
  cluster::pam(x=t(x),k=k,cluster.only=cluster.only)
}
#passes internal check
internalFunctionCheck(goodFUN,inputType="X",algorithmType="K",outputType="vector")
#Note it doesn't pass if inputType="either" because no catches for x=NULL
internalFunctionCheck(goodFUN, inputType="either",algorithmType="K",outputType="vector")
myCF<-ClusterFunction(clusterFUN=goodFUN, inputType="X",algorithmType="K", outputType="vector")
badFUN<-function(x,diss,k,checkArgs,cluster.only,...){cluster::pam(x=x,k=k)}
internalFunctionCheck(badFUN,inputType="X",algorithmType="K",outputType="vector")
```

listBuiltInFunctions *Built in ClusterFunction options*

Description

Documents the built-in clustering options that are available in the `clusterExperiment` package.

Usage

```
listBuiltInFunctions()

## S4 method for signature 'character'
getBuiltInFunction(object)

listBuiltInTypeK()

listBuiltInType01()
```

Arguments

`object` name of built in function.

Details

`listBuiltInFunctions` will return the character names of the built-in clustering functions available.

`listBuiltInTypeK` returns the names of the built-in functions that have type 'K'

`listBuiltInType01` returns the names of the built-in functions that have type '01'

`getBuiltInFunction` will return the `ClusterFunction` object of a character value that corresponds to a built-in function.

`algorithmType` and `inputType` will return the `algorithmType` and `inputType` of the built-in `clusterFunction` corresponding to the character value.

Built-in clustering methods: The built-in clustering methods, the names of which can be accessed by `listBuiltInFunctions()` are the following:

- "pam" Based on `pam` in `cluster` package. Arguments to that function can be passed via `clusterArgs`. Input is "either" (x or diss); algorithm type is "K"
- "clara" Based on `clara` in `cluster` package. Arguments to that function can be passed via `clusterArgs`. Note that we have changed the default arguments of that function to match the recommendations in the documentation of `clara` (numerous functions are set to less than optimal settings for back-compatibility). Specifically, the following defaults are implemented `samples=50`, `keep.data=FALSE`, `mediods.x=FALSE`, `rngR=TRUE`, `pamLike=TRUE`, `correct.d=TRUE`. Input is "X"; algorithm type is "K".
- "kmeans" Based on `kmeans` in `stats` package. Arguments to that function can be passed via `clusterArgs` except for `centers` which is reencoded here to be the argument 'k' Input is "X"; algorithm type is "K"
- "hierarchical01" `hclust` in `stats` package is used to build hierarchical clustering. Arguments to that function can be passed via `clusterArgs`. The `hierarchical01` cuts the hierarchical tree based on the parameter `alpha`. It does not use the `cutree` function, but instead transversing down the tree until getting a block of samples with whose summary of the values is greater than or equal to `1-alpha`. Arguments that can be passed to 'hierarchical01' are 'evalClusterMethod' which determines how to summarize the samples' values of `D[samples,samples]` for comparison to `1-alpha`: "maximum" (default) takes the minimum of `D[samples,samples]` and requires it to be less than or equal to `1-alpha`; "average" requires that each row mean of `D[samples,samples]` be less than or equal to `1-alpha`. Additional arguments of `hclust` can also be passed via `clusterArgs` to control the hierarchical clustering of `D`. Input is "diss"; algorithm type is "01"
- "hierarchicalK" `hclust` in `stats` package is used to build hierarchical clustering and `cutree` is used to cut the tree into `k` clusters. Input is "diss"; algorithm type is "K"
- "tight" Based on the algorithm in Tsang and Wong, specifically their method of picking clusters from a co-occurrence matrix after subsampling. The clustering encoded here is not the entire tight clustering algorithm, only that single piece that identifies clusters from the co-occurrence matrix. Arguments for the tight method are 'minSize.core' (default=2), which sets the minimum number of samples that form a core cluster. Input is "diss"; algorithm type is "01"
- "spectral" `specc` in `kernlab` package is used to perform spectral clustering. Note that spectral clustering can produce errors if the number of clusters (`K`) is not sufficiently smaller than the number of samples (`N`). $K < N$ is not always sufficient. Input is "X"; algorithm type is "K".

Value

`listBuiltInFunctions` returns a character vector of all the built-in cluster functions' names.

`getBuiltInFunction` returns the `ClusterFunction` object that corresponds to the character name of a function

`listBuiltInTypeK` returns a character vector of the names of built-in cluster functions that are of type "K"

`listBuiltInType01` returns a character vector of the names of built-in cluster functions that are of type "01"

See Also

[ClusterFunction](#), [algorithmType](#), [inputType](#)

Examples

```
listBuiltInFunctions()
algorithmType(c("kmeans","pam","hierarchical01"))
inputType(c("kmeans","pam","hierarchical01"))
listBuiltInTypeK()
listBuiltInType01()
```

mainClustering	<i>Cluster distance matrix from subsampling</i>
----------------	---

Description

Given input data, this function will try to find the clusters based on the given ClusterFunction object.

Usage

```
## S4 method for signature 'character'
mainClustering(clusterFunction, ...)

## S4 method for signature 'ClusterFunction'
mainClustering(clusterFunction, x = NULL,
  diss = NULL, distFunction = NA, clusterArgs = NULL, minSize = 1,
  orderBy = c("size", "best"), format = c("vector", "list"),
  checkArgs = TRUE, checkDiss = TRUE, returnData = FALSE, ...)

## S4 method for signature 'ClusterFunction'
getPostProcessingArgs(clusterFunction)
```

Arguments

clusterFunction	a ClusterFunction object that defines the clustering routine. See ClusterFunction for required format of user-defined clustering routines. User can also give a character value to the argument clusterFunction to indicate the use of clustering routines provided in package. Type listBuiltInFunctions at command prompt to see the built-in clustering routines. If clusterFunction is missing, the default is set to "pam".
...	arguments passed to the post-processing steps of the clustering. The available post-processing arguments for a ClusterFunction object depend on it's algorithm type and can be found by calling getPostProcessingArgs . See details below for documentation.
x	p x n data matrix on which to run the clustering (samples in columns).
diss	n x n data matrix of dissimilarities between the samples on which to run the clustering
distFunction	a distance function to be applied to D. Only relevant if input is only x (a matrix of data), and diss=NULL. See details of clusterSingle for the required format of the distance function.
clusterArgs	arguments to be passed directly to the clusterFUN slot of the ClusterFunction object

minSize	the minimum number of samples in a cluster. Clusters found below this size will be discarded and samples in the cluster will be given a cluster assignment of "-1" to indicate that they were not clustered.
orderBy	how to order the cluster (either by size or by maximum alpha value). If orderBy="size" the numbering of the clusters are reordered by the size of the cluster, instead of by the internal ordering of the clusterFUN defined in the ClusterFunction object (an internal ordering is only possible if slot outputType of the ClusterFunction is "list").
format	whether to return a list of indices in a cluster or a vector of clustering assignments. List is mainly for compatibility with sequential part.
checkArgs	logical as to whether should give warning if arguments given that don't match clustering choices given. Otherwise, inapplicable arguments will be ignored without warning.
checkDiss	logical. Whether to check whether the input diss is valid.
returnData	logical as to whether to return the diss or x matrix in the output. If FALSE only the clustering vector is returned.

Details

mainClustering is not meant to be called by the user. It is only an exported function so as to be able to clearly document the arguments for mainClustering which can be passed via the argument mainClusterArgs in functions like [clusterSingle](#) and [clusterMany](#).

Post-processing Arguments: For post-processing the clustering, currently only type 'K' algorithms have a defined post-processing. Specifically

- "findBestK" logical, whether should find best K based on average silhouette width (only used if clusterFunction of type "K").
- "kRange" vector of integers to try for k values if findBestK=TRUE. If k is given in clusterArgs, then default is k-2 to k+20, subject to those values being greater than 2; if not the default is 2:20. Note that default values depend on the input k, so running for different choices of k and findBestK=TRUE can give different answers unless kRange is set to be the same.
- "removeSil" logical as to whether remove the assignment of a sample to a cluster when the sample's silhouette value is less than silCutoff
- "silCutoff" Cutoff on the minimum silhouette width to be included in cluster (only used if removeSil=TRUE).

Value

mainClustering returns a vector of cluster assignments (if format="vector") or a list of indices for each cluster (if format="list"). Clusters less than minSize are removed.

Examples

```
data(simData)
c11<-mainClustering(x=simData,clusterFunction="pam",clusterArgs=list(k=3))
c12<-mainClustering(simData,clusterFunction="hierarchical01",clusterArgs=list(alpha=.1))
c13<-mainClustering(simData,clusterFunction="tight",clusterArgs=list(alpha=.1))
#change distance to manhattan distance
c14<-mainClustering(simData,clusterFunction="pam",clusterArgs=list(k=3),
  distFunction=function(x){dist(x,method="manhattan")})
```

```

#run hierarchical method for finding blocks, with method of evaluating
#coherence of block set to evalClusterMethod="average", and the hierarchical
#clustering using single linkage:
clustSubHier <- mainClustering(simData, clusterFunction="hierarchical01",
minSize=5, clusterArgs=list(alpha=0.1,evalClusterMethod="average", method="single"))

#do tight
clustSubTight <- mainClustering(simData, clusterFunction="tight", clusterArgs=list(alpha=0.1),
minSize=5)

#two twists to pam
clustSubPamK <- mainClustering(simData, clusterFunction="pam", silCutoff=0, minSize=5,
removeSil=TRUE, clusterArgs=list(k=3))
clustSubPamBestK <- mainClustering(simData, clusterFunction="pam", silCutoff=0,
minSize=5, removeSil=TRUE, findBestK=TRUE, kRange=2:10)

# note that passing the wrong arguments for an algorithm results in warnings
# (which can be turned off with checkArgs=FALSE)
clustSubTight_test <- mainClustering(simData, clusterFunction="tight",
clusterArgs=list(alpha=0.1), minSize=5, removeSil=TRUE)
clustSubTight_test2 <- mainClustering(simData, clusterFunction="tight",
clusterArgs=list(alpha=0.1,evalClusterMethod="average"))

```

makeDendrogram

Make hierarchy of set of clusters

Description

Makes a dendrogram of a set of clusters based on hclust on the medoids of the cluster.

Usage

```

## S4 method for signature 'ClusterExperiment'
makeDendrogram(x,
  whichCluster = "primaryCluster", reduceMethod = "mad",
  nDims = defaultNDims(x, reduceMethod), ignoreUnassignedVar = TRUE,
  unassignedSamples = c("outgroup", "cluster"), ...)

## S4 method for signature 'dist'
makeDendrogram(x, cluster, unassignedSamples = c("outgroup",
  "cluster", "remove"), ...)

## S4 method for signature 'matrixOrHDF5'
makeDendrogram(x, cluster,
  unassignedSamples = c("outgroup", "cluster", "remove"), ...)

```

Arguments

x data to define the medoids from. Matrix and [ClusterExperiment](#) supported.

whichCluster an integer index or character string that identifies which cluster should be used to make the dendrogram. Default is primaryCluster.

reduceMethod	character A character identifying what type of dimensionality reduction to perform before clustering. Can be either a value stored in either of reducedDims or filterStats slot or a built-in dimensionality reduction/filtering. The option "co-Cluster" will use the co-Clustering matrix stored in the 'coClustering' slot of the ClusterExperiment object
nDims	The number of dimensions to keep from reduceMethod. If missing calls <code>defaultNDims</code> .
ignoreUnassignedVar	logical. Whether filtering statistics should ignore the unassigned samples within the clustering. Only relevant if 'reduceMethod' matches one of built-in filtering statistics in <code>listBuiltInFilterStats()</code> . In which case the clustering identified in whichCluster is passed to <code>makeFilterStats</code> . See <code>makeFilterStats</code> for more details.
unassignedSamples	how to handle unassigned samples("-1") ; only relevant for sample clustering. See details.
...	for <code>makeDendrogram</code> , if signature <code>matrix</code> , arguments passed to <code>hclust</code> ; if signature <code>ClusterExperiment</code> passed to the method for signature <code>matrix</code> . For <code>plotDendrogram</code> , passed to <code>plot.dendrogram</code> .
cluster	A numeric vector with cluster assignments. If <code>x</code> is a <code>ClusterExperiment</code> object, <code>cluster</code> is automatically the <code>primaryCluster(x)</code> . "-1" indicates the sample was not assigned to a cluster.

Details

The function returns two dendrograms (as a list if `x` is a matrix or in the appropriate slots if `x` is `ClusterExperiment`). The cluster dendrogram is created by applying `hclust` to the medoids of each cluster. In the sample dendrogram the clusters are again clustered, but now the samples are also part of the resulting dendrogram. This is done by giving each sample the value of the medoid of its cluster.

The argument `unassignedSamples` governs what is done with unassigned samples (defined by a -1 cluster value). If `unassigned=="cluster"`, then the dendrogram is created by `hclust` of the expanded medoid data plus the original unclustered observations. If `unassignedSamples` is "outgroup", then all unassigned samples are put as an outgroup. If the `x` object is a matrix, then `unassignedSamples` can also be "remove", to indicate that samples with "-1" should be discarded. This is not a permitted option, however, when `x` is a `ClusterExperiment` object, because it would return a dendrogram with less samples than `NCOL(x)`, which is not permitted for the `@dendro_samples` slot.

If any merge information is stored in the input object, it will be erased by a call to `mergeDendrogram`.

If `nDims` is missing, it will be given a default value depending on the value of `reduceMethod`. See `defaultNDims` for details.

Value

If `x` is a matrix, a list with two dendrograms, one in which the leaves are clusters and one in which the leaves are samples. If `x` is a `ClusterExperiment` object, the dendrograms are saved in the appropriate slots.

See Also

`makeFilterStats`, `makeReducedDims`

Examples

```

data(simData)

#create a clustering, for 8 clusters (truth was 3)
cl <- clusterSingle(simData, subsample=FALSE,
  sequential=FALSE, mainClusterArgs=list(clusterFunction="pam", clusterArgs=list(k=8)))

#create dendrogram of clusters:
hcl <- makeDendrogram(cl)
plotDendrogram(hcl)
plotDendrogram(hcl, leafType="samples",plotType="colorblock")

```

mergeClusters

Merge clusters based on dendrogram

Description

Takes an input of hierarchical clusterings of clusters and returns estimates of number of proportion of non-null and merges those below a certain cutoff.

Usage

```

## S4 method for signature 'matrixOrHDF5'
mergeClusters(x, cl, dendro = NULL,
  mergeMethod = c("none", "Storey", "PC", "adjP", "locfdr", "MB", "JC"),
  plotInfo = "none", nodePropTable = NULL, calculateAll = TRUE,
  showWarnings = FALSE, cutoff = 0.05, plot = TRUE, isCount = TRUE,
  logFCcutoff = 0, ...)

## S4 method for signature 'ClusterExperiment'
mergeClusters(x, eraseOld = FALSE,
  isCount = FALSE, mergeMethod = "none", plotInfo = "all",
  clusterLabel = "mergeClusters", leafType = c("samples", "clusters"),
  plotType = c("colorblock", "name", "ids"), plot = TRUE, ...)

## S4 method for signature 'ClusterExperiment'
nodeMergeInfo(x)

## S4 method for signature 'ClusterExperiment'
mergeCutoff(x)

## S4 method for signature 'ClusterExperiment'
mergeMethod(x)

## S4 method for signature 'ClusterExperiment'
mergeClusterIndex(x)

## S4 method for signature 'ClusterExperiment'
getMergeCorrespond(x, by = c("merge",
  "original"))

```

Arguments

x	data to perform the test on. It can be a matrix or a ClusterExperiment .
cl	A numeric vector with cluster assignments to compare to. “-1” indicates the sample was not assigned to a cluster.
dendro	dendrogram providing hierarchical clustering of clusters in cl. If x is a matrix, then the default is dendro=NULL and the function will calculate the dendrogram with the given (x, cl) pair using makeDendrogram . If x is a ClusterExperiment object, the dendrogram in the slot dendro_clusters will be used. In this case, this means that makeDendrogram needs to be called before mergeClusters.
mergeMethod	method for calculating proportion of non-null that will be used to merge clusters (if 'none', no merging will be done). See details for description of methods.
plotInfo	what type of information about the merging will be shown on the dendrogram. If 'all', then all the estimates of proportion non-null will be plotted at each node of the dendrogram; if 'mergeMethod', then only the value used in the mergeClusters command is plotted at each node. If 'none', then no proportions will be added to the dendrogram, though the dendrogram will be drawn. 'plotInfo' can also be one of the valid input to mergeMethod (even if that method is not the method chosen in mergeMethod argument). plotInfo can also show the information corresponding to "adjP" with a fold-change cutoff, by giving a value to this argument in the form of "adjP_2.0", for example.
nodePropTable	Only for matrix version. Matrix of results from previous run of mergeClusters as returned by matrix version of mergeClusters. Useful if just want to change the cutoff. Not generally intended for user but used internally by package.
calculateAll	logical. Whether to calculate the estimates for all methods. This reduces computation costs for any future calls to mergeClusters since the results can be passed to future calls of mergeClusters (and for ClusterExperiment objects this is done automatically).
showWarnings	logical. Whether to show warnings given by the methods. The 'locfdr' method in particular frequently spits out warnings (which may indicate that its estimates are not reliable). Setting showWarnings=FALSE will suppress all warnings from all methods (not just "locfdr"). By default this is set to showWarnings=FALSE by default to avoid large number of warnings being produced by "locfdr", but users may want to be more careful to check the warnings for themselves.
cutoff	minimum value required for NOT merging a cluster, i.e. two clusters with the proportion of DE below cutoff will be merged. Must be a value between 0, 1, where lower values will make it harder to merge clusters.
plot	logical as to whether to plot the dendrogram with the merge results
isCount	logical as to whether input data is a count matrix. See details.
logFCcutoff	Relevant only if the mergeMethod selected is "adjP", in which case the calculation of the proportion of individual tests significant will also require that the estimated log-fold change of the features to be at least this large in absolute value. Value will be rounded to nearest tenth of an integer via round(logFCcutoff, digits=1). For any other method, this parameter is ignored.
...	for signature matrix, arguments passed to the plot.phylo function of ape that plots the dendrogram. For signature ClusterExperiment arguments passed to the method for signature matrix and then if do not match those arguments, will be passed onto plot.phylo .

eraseOld	logical. Only relevant if input <code>x</code> is of class <code>ClusterExperiment</code> . If <code>TRUE</code> , will erase existing workflow results (<code>clusterMany</code> as well as <code>mergeClusters</code> and <code>combineMany</code>). If <code>FALSE</code> , existing workflow results will have " <code>_i</code> " added to the <code>clusterTypes</code> value, where <code>i</code> is one more than the largest such existing workflow <code>clusterTypes</code> .
clusterLabel	a string used to describe the type of clustering. By default it is equal to " <code>mergeClusters</code> ", to indicate that this clustering is the result of a call to <code>mergeClusters</code> (only if <code>x</code> is a <code>ClusterExperiment</code> object)
leafType	if plotting, whether the leaves should be the clusters or the samples. Choosing 'samples' allows for visualization of how many samples are in the merged clusters (only if <code>x</code> is a <code>ClusterExperiment</code> object), which is the main difference between choosing "clusters" and "samples", particularly if <code>plotType="colorblock"</code>
plotType	if plotting, then whether leaves of dendrogram should be labeled by rectangular blocks of color (" <code>colorblock</code> ") or with the names of the leaves (" <code>name</code> ") (only if <code>x</code> is a <code>ClusterExperiment</code> object).
by	indicates whether output from <code>getMergeCorrespond</code> should be a vector/list with elements corresponding to merge cluster ids or elements corresponding to the original clustering ids. See return value for details.

Details

Estimation of Proportion non-null "Storey" refers to the method of Storey (2002). "PC" refers to the method of Pounds and Cheng (2004). "JC" refers to the method of Ji and Cai (2007), and implementation of "JC" method is copied from code available on Jiashin Ji's website, December 16, 2015 (<http://www.stat.cmu.edu/~jiashun/Research/software/NullandProp/>). "locfdr" refers to the method of Efron (2004) and is implemented in the package `locfdr`. "MB" refers to the method of Meinshausen and Bühlmann (2005) and is implemented in the package `howmany`. "adjP" refers to the proportion of genes that are found significant based on a FDR adjusted p-values (method "BH") and a cutoff of 0.05.

Count correction If `isCount=TRUE`, and the input is a matrix, $\log_2(\text{count} + 1)$ will be used for `makeDendrogram` and the original data with voom correction will be used in `getBestFeatures`. If input is `ClusterExperiment`, then setting `isCount=TRUE` also means that the $\log_2(1+\text{count})$ will be used as the transformation, like for the matrix case as well as the voom calculation, and will NOT use the transformation stored in the object. If `FALSE`, then `transformData(x)` will be given to the input and will be used for both `makeDendrogram` and `getBestFeatures`, with no voom correction.

Control of Plotting If `mergeMethod` is not equal to 'none' then the plotting will indicate where the clusters will be merged by making dotted lines of edges that are merged together (assuming `plotInfo` is not 'none'). `plotInfo` controls simultaneously what information will be plotted on the nodes as well as whether the dotted lines will be shown for the merged cluster. Notice that the choice of `plotInfo` (as long as it is not 'none') has no effect on how the dotted edges are drawn – they are always drawn based on the `mergeMethod`. If you choose `plotInfo` to not be equal to the `mergeMethod`, then you will have a confusing picture where the dotted edges will be based on the clustering created by `mergeMethod` while the information on the nodes is based on a different method. Note that you can override `plotInfo` by setting `show.node.label=FALSE` (passed to `plot.phylo`), so that no information is plotted on the nodes, but the dotted edges are still drawn. If you just want plot of the dendrogram, with no merging performed nor demonstrated on the plot, see `plotDendrogram`.

If the dendrogram was made with option `unassignedSamples="cluster"` (i.e. unassigned were clustered in with other samples), then you cannot choose the option `leafType='samples'`. This is because the current code cannot reliably link up the internal nodes of the sample dendrogram to the internal nodes of the cluster dendrogram when the unassigned samples are intermixed.

When the input is a `ClusterExperiment` object, the function attempts to update the merge information in that object. This is done by checking that the existing dendrogram stored in the object (and run on the clustering stored in the slot `dendro_index`) is the same clustering that is stored in the slot `merge_dendrocluster_index`. For this reason, new calls to `makeDendrogram` will erase the merge information saved in the object.

If `mergeClusters` is run with `mergeMethod="none"`, the function may still calculate the proportions per node if `plotInfo` is not equal to "none" or `calculateAll=TRUE`. If the input object was a `ClusterExperiment` object, the resulting information will be still saved, though no new clustering was created; if there was not an existing merge method, the slot `merge_dendrocluster_index` will be updated.

Value

If 'x' is a matrix, it returns (invisibly) a list with elements

- `clustering` a vector of length equal to `ncol(x)` giving the integer-valued cluster ids for each sample. "-1" indicates the sample was not clustered.
- `oldClToNew` A table of the old cluster labels to the new cluster labels.
- `nodeProp` A table of the proportions that are DE on each node.
- `nodeMerge` A table of indicating for each node whether merged or not and the cluster id in the new clustering that corresponds to the node
- `originalClusterDendro` The dendrogram on which the merging was based (based on the original clustering).
- `cutoff` The cutoff value for merging.

If 'x' is a `ClusterExperiment`, it returns a new `ClusterExperiment` object with an additional clustering based on the merging. This becomes the new primary clustering.

`nodeMergeInfo` returns information collected about the nodes during merging as a `data.frame` with the following entries:

- `Node Name` of the node
- `Contrast` The contrast compared at each node, in terms of the cluster ids
- `isMerged` Logical as to whether samples from that node which were merged into one cluster during merging
- `mergeClusterId` If a node corresponds to a new, merged cluster, gives the cluster id it corresponds to. Otherwise NA
- ... The remaining columns give the estimated proportion of genes differentially expressed for each method. A column of NAs means that the method in question hasn't been calculated yet.

`mergeCutoff` returns the cutoff used for the current merging.

`mergeMethod` returns the method used for the current merge.

`mergeClusterIndex` returns the index of the clustering used for the current merge.

`getMergeCorrespond` returns the correspondence between the merged cluster and its originating cluster. If `by="original"` returns a named vector, where the names of the vector are the cluster ids of the originating cluster and the values of the vector are the cluster ids of the merged cluster. If `by="merge"` the results returned are organized by the merged clusters. This will generally be a list, with the names of the list equal to the clusterIds of the merge clusters and the entries the clusterIds of the originating clusters. However, if there was no merging done (so that the clusters are identical) the output will be a vector like with `by="original"`.

References

- Ji and Cai (2007), "Estimating the Null and the Proportion of Nonnull Effects in Large-Scale Multiple Comparisons", *JASA* 102: 495-906.
- Efron (2004) "Large-scale simultaneous hypothesis testing: the choice of a null hypothesis," *JASA*, 99: 96-104.
- Meinshausen and Buhlmann (2005) "Lower bounds for the number of false null hypotheses for multiple testing of associations", *Biometrika* 92(4): 893-907.
- Storey (2002) "A direct approach to false discovery rates", *J. R. Statist. Soc. B* 64 (3): 479-498.
- Pounds and Cheng (2004). "Improving false discovery rate estimation." *Bioinformatics* 20(11): 1737-1745.

See Also

makeDendrogram, plotDendrogram, getBestFeatures

Examples

```
data(simData)

#create a clustering, for 8 clusters (truth was 3)
cl<-clusterSingle(simData, subsample=FALSE,
sequential=FALSE, mainClusterArgs=list(clusterFunction="pam", clusterArgs=list(k=8)))

#give more interesting names to clusters:
newNames<- paste("Cluster",clusterLegend(cl)[[1]][,"name"],sep="")
clusterLegend(cl)[[1]][,"name"]<-newNames
#make dendrogram
cl <- makeDendrogram(cl)

#plot showing the before and after clustering
#(Note argument 'use.edge.length' can improve
#readability)
merged <- mergeClusters(cl, plotInfo="all",
mergeMethod="adjP", use.edge.length=FALSE)

#Simpler plot with just dendrogram and single method
merged <- mergeClusters(cl, plotInfo="mergeMethod",
mergeMethod="adjP", use.edge.length=FALSE,
leafType="clusters",plotType="name")

#compare merged to original
tableClusters(merged,whichClusters=c("mergeClusters","clusterSingle"))
```

plotBarplot, ClusterExperiment, character-method
Barplot of 1 or 2 clusterings

Description

Make a barplot of sample's assignments to clusters for single clustering, or cross comparison for two clusterings.

Usage

```
## S4 method for signature 'ClusterExperiment,character'
plotBarplot(object, whichClusters, ...)

## S4 method for signature 'ClusterExperiment,missing'
plotBarplot(object, whichClusters, ...)

## S4 method for signature 'ClusterExperiment,numeric'
plotBarplot(object, whichClusters,
  labels = c("names", "ids"), ...)

## S4 method for signature 'ClusterExperiment,missing'
plotBarplot(object, whichClusters, ...)

## S4 method for signature 'vector,missing'
plotBarplot(object, whichClusters, ...)

## S4 method for signature 'matrix,missing'
plotBarplot(object, whichClusters, xNames = NULL,
  legNames = NULL, legend = ifelse(ncol(object) == 2, TRUE, FALSE),
  xlab = NULL, legend.title = NULL, unassignedColor = "white",
  missingColor = "grey", colPalette = NULL, ...)
```

Arguments

object	A matrix of with each column corresponding to a clustering and each row a sample or a ClusterExperiment object.
whichClusters	If numeric, a predefined order for the clusterings in the plot. If x is a ClusterExperiment object, whichClusters can be a character value identifying the clusterTypes to be used, or if not matching clusterTypes then clusterLabels; alternatively whichClusters can be either 'all' or 'workflow' to indicate choosing all clusters or choosing all workflowClusters .
...	for plotBarplot arguments passed either to the method of plotBarplot for matrices or ultimately to barplot .
labels	if object is a ClusterExperiment object, then labels defines whether the clusters will be identified by their names values in clusterLegend (labels="names", the default) or by their clusterIds value in clusterLegend (labels="ids").
xNames	names for the clusters on x-axis (i.e. clustering given 1st). By default uses names of the 1st column of clusters matrix. See details.
legNames	names for the clusters dividing up the 1st clusters (will appear in legend). By default uses names of the 2nd cluster of clusters matrix. If only one clustering, xNames and legNames refer to the same clustering. See details.
legend	whether to plot the legend
xlab	label for x-axis. By default or if equal NULL the column name of the 1st cluster of clusters matrix
legend.title	label for legend. By default or if equal NULL the column name of the 2st cluster of clusters matrix
unassignedColor	If "-1" in clusters, will be given this color (meant for samples not assigned to cluster).

missingColor	If “-2” in clusters, will be given this color (meant for samples that were missing from the clustering, mainly when comparing clusterings run on different sets of samples)
colPalette	a vector of colors used for the different clusters. See details.

Details

The first column of the cluster matrix will be on the x-axis and the second column (if present) will separate the groups of the first column.

All arguments of the matrix version can be passed to the ClusterExperiment version. As noted above, however, some arguments have different interpretations.

If whichClusters = "workflow", then the most recent two clusters of the workflow will be chosen where recent is based on the following order (most recent first): final, mergeClusters, combineMany, clusterMany.

xNames, legNames and colPalette should all be named vectors, with the names referring to the clusters they should match to (for ClusterExperiment objects, it is determined by the argument labels as to whether the names should match the cluster names or the clusterIds). colPalette and legNames must be same length of the number of clusters found in the second clustering, or if only a single clustering, the same length as the number of clusters in that clustering.

Value

A plot is produced, nothing is returned

Author(s)

Elizabeth Purdom

Examples

```
#clustering using pam: try using different dimensions of pca and different k
data(simData)

cl <- clusterMany(simData, nReducedDims=c(5, 10, 50), reduceMethod="PCA",
clusterFunction="pam", ks=2:4, findBestK=c(TRUE,FALSE),
removeSil=c(TRUE,FALSE))

plotBarplot(cl)
plotBarplot(cl,whichClusters=1:2)
```

plotClusters

Visualize cluster assignments across multiple clusterings

Description

Align multiple clusterings of the same set of samples and provide a color-coded plot of their shared cluster assignments

Usage

```
## S4 method for signature 'ClusterExperiment,character'
plotClusters(object,
  whichClusters = c("workflow", "all"), ...)

## S4 method for signature 'ClusterExperiment,numeric'
plotClusters(object, whichClusters,
  existingColors = c("ignore", "all", "firstOnly"), resetNames = FALSE,
  resetColors = FALSE, resetOrderSamples = FALSE, sampleData = NULL,
  clusterLabels = NULL, ...)

## S4 method for signature 'ClusterExperiment,missing'
plotClusters(object, whichClusters, ...)

## S4 method for signature 'matrix,missing'
plotClusters(object, whichClusters,
  orderSamples = NULL, sampleData = NULL, reuseColors = FALSE,
  matchToTop = FALSE, plot = TRUE, unassignedColor = "white",
  missingColor = "grey", minRequireColor = 0.3, startNewColors = FALSE,
  colPalette = massivePalette, input = c("clusters", "colors"),
  clusterLabels = colnames(object), add = FALSE, xCoord = NULL,
  ylim = NULL, tick = FALSE, ylab = "", xlab = "", axisLine = 0,
  box = FALSE, ...)
```

Arguments

object	A matrix of with each column corresponding to a clustering and each row a sample or a ClusterExperiment object. If a matrix, the function will plot the clusterings in order of this matrix, and their order influences the plot greatly.
whichClusters	If numeric, a predefined order for the clusterings in the plot. If x is a ClusterExperiment object, whichClusters can be a character value identifying the clusterTypes to be used, or if not matching clusterTypes then clusterLabels; alternatively whichClusters can be either 'all' or 'workflow' to indicate choosing all clusters or choosing all workflowClusters .
...	for plotClusters arguments passed either to the method of plotClusters for matrices, or ultimately to plot (if add=FALSE).
existingColors	how to make use of the exiting colors in the ClusterExperiment object. 'ignore' will ignore them and assign new colors. 'firstOnly' will use the existing colors of only the 1st clustering, and then align the remaining clusters and give new colors for the remaining only. 'all' will use all of the existing colors.
resetNames	logical. Whether to reset the names of the clusters in clusterLegend to be the aligned integer-valued ids from plotClusters.
resetColors	logical. Whether to reset the colors in clusterLegend in the ClusterExperiment returned to be the colors from the plotClusters.
resetOrderSamples	logical. Whether to replace the existing orderSamples slot in the ClusterExperiment object with the new order found.
sampleData	If clusters is a matrix, sampleData gives a matrix of additional cluster/sampleData on the samples to be plotted with the clusterings given in clusters. Values in sampleData will be added to the end (bottom) of the plot. NAs in the

sampleData matrix will trigger an error. If clusters is a ClusterExperiment object, the input in sampleData refers to columns of the colData slot of the ClusterExperiment object to be plotted with the clusters. In this case, sampleData can be TRUE (i.e. all columns will be plotted), or an index or a character vector that references a column or column name, respectively, of the colData slot of the ClusterExperiment object. If there are NAs in the colData columns, they will be encoded as 'unassigned' and receive the same color as 'unassigned' samples in the clustering.

clusterLabels	names to go with the columns (clusterings) in matrix colorMat. If sampleData argument is not NULL, the clusterLabels argument must include names for the sample data too. If the user gives only names for the clusterings, the code will try to anticipate that and use the column names of the sample data, but this is fragile. If set to FALSE, then no labels will be plotted.
orderSamples	A predefined order in which the samples will be plotted. Otherwise the order will be found internally by aligning the clusters (assuming input="clusters")
reuseColors	Logical. Whether each row should consist of the same set of colors. By default (FALSE) each cluster that the algorithm doesn't identify to the previous rows clusters gets a new color.
matchToTop	Logical as to whether all clusters should be aligned to the first row. By default (FALSE) each cluster is aligned to the ordered clusters of the row above it.
plot	Logical as to whether a plot should be produced.
unassignedColor	If "-1" in clusters, will be given this color (meant for samples not assigned to cluster).
missingColor	If "-2" in clusters, will be given this color (meant for samples that were missing from the clustering, mainly when comparing clusterings run on different sets of samples)
minRequireColor	In aligning colors between rows of clusters, require this percent overlap.
startNewColors	logical, indicating whether in aligning colors between rows of clusters, should the colors restart at beginning of colPalette as long as colors are not in immediately preceding row (the colors at the end of massivePalette are all of colors() and many will be indistinguishable, so this option can be useful if you have a large cluster matrix).
colPalette	a vector of colors used for the different clusters. Must be as long as the maximum number of clusters found in any single clustering/column given in clusters or will otherwise return an error.
input	indicate whether the input matrix is matrix of integer assigned clusters, or contains the colors. If input="colors", then the object clusters is a matrix of colors and there is no alignment (this option allows the user to manually adjust the colors and replot, for example).
add	whether to add to existing plot.
xCoord	values on x-axis at which to plot the rows (samples).
ylim	vector of limits of y-axis.
tick	logical, whether to draw ticks on x-axis for each sample.
ylab	character string for the label of y-axis.
xlab	character string for the label of x-axis.

axisLine	the number of lines in the axis labels on y-axis should be (passed to line = ... in the axis call).
box	logical, whether to draw box around the plot.

Details

All arguments of the matrix version can be passed to the ClusterExperiment version. As noted above, however, some arguments have different interpretations.

If whichClusters = "workflow", then the workflow clusterings will be plotted in the following order: final, mergeClusters, combineMany, clusterMany.

Value

If clusters is a [ClusterExperiment](#) Object, then plotClusters returns an updated ClusterExperiment object, where the clusterLegend and/or orderSamples slots have been updated (depending on the arguments).

If clusters is a matrix, plotClusters returns (invisibly) the orders and other things that go into making the matrix. Specifically, a list with the following elements.

- orderSamples a vector of length equal to nrows(clusters) giving the order of the samples (rows) to use to get the original clusters matrix into the order made by plotClusters.
- colors matrix of color assignments for each element of original clusters matrix. Matrix is in the same order as original clusters matrix. The matrix colors[orderSamples,] is the matrix that can be given back to plotClusters to recreate the plot (see examples).
- alignedClusterIds a matrix of integer valued cluster assignments that match the colors. This is useful if you want to have cluster identification numbers that are better aligned than that given in the original clusters. Again, the rows/samples are in same order as original input matrix.
- clusterLegend list of length equal to the number of columns of input matrix. The elements of the list are matrices, each with three columns named "Original", "Aligned", and "Color" giving, respectively, the correspondence between the original cluster ids in clusters, the aligned cluster ids in aligned, and the color.
- origClustersThe original matrix of clusters given to plotClusters

Author(s)

Elizabeth Purdom and Marla Johnson (based on the tracking plot in [ConsensusClusterPlus](#) by Matt Wilkerson and Peter Waltman).

References

Wilkerson, D. M, Hayes and Neil D (2010). "ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking." *Bioinformatics*, 26(12), pp. 1572-1573.

See Also

The [ConsensusClusterPlus](#) package.

Examples

```

#clustering using pam: try using different dimensions of pca and different k
data(simData)

cl <- clusterMany(simData, nReducedDims=c(5, 10, 50), reduceMethod="PCA",
clusterFunction="pam", ks=2:4, findBestK=c(TRUE,FALSE),
removeSil=c(TRUE,FALSE))

clusterLabels(cl)

#make names shorter for better plotting
x <- clusterLabels(cl)
x <- gsub("TRUE", "T", x)
x <- gsub("FALSE", "F", x)
x <- gsub("k=NA,", "", x)
x <- gsub("Features", "", x)
clusterLabels(cl) <- x

par(mar=c(2,10,1,1))
#this will make the choices of plotClusters
cl <- plotClusters(cl, axisLine=-1, resetOrderSamples=TRUE, resetColors=TRUE)

#see the new cluster colors
clusterLegend(cl)[1:2]

#We can also change the order of the clusterings. Notice how this
#dramatically changes the plot!
clOrder <- c(3:6, 1:2, 7:ncol(clusterMatrix(cl)))
cl <- plotClusters(cl, whichClusters=clOrder, resetColors=TRUE,
resetOrder=TRUE, axisLine=-2)

#We can manually switch the red ("E31A1C") and green ("33A02C") in the
#first cluster:

#see what the default colors are and their names
showPalette(wh=1:5)

#change "E31A1C" to "33A02C"
newColorMat <- clusterLegend(cl)[[clOrder[1]]]
newColorMat[2:3, "color"] <- c("#33A02C", "#E31A1C")
clusterLegend(cl)[[clOrder[1]]]<-newColorMat

#replot by setting 'input="colors"'
par(mfrow=c(1,2))
plotClusters(cl, whichClusters=clOrder, orderSamples=orderSamples(cl),
existingColors="all")
plotClusters(cl, whichClusters=clOrder, resetColors=TRUE, resetOrder=TRUE,
axisLine=-2)
par(mfrow=c(1,1))

#set some of clusterings arbitrarily to "-1", meaning not clustered (white),
#and "-2" (another possible designation getting gray, usually for samples not
#include in original clustering)
clMatNew <- apply(clusterMatrix(cl), 2, function(x) {
wh <- sample(1:nSamples(cl), size=10)
x[wh]<- -1

```

```

wh <- sample(1:nSamples(c1), size=10)
x[wh]<- -2
return(x)
})

#make a new object
c12 <- ClusterExperiment(assay(c1), c1MatNew,
transformation=transformation(c1))
plotClusters(c12)

```

plotClustersWorkflow, ClusterExperiment-method

A plot of clusterings specific for clusterMany and workflow visualization

Description

A realization of `plotClusters` call specific to separating out the results of `clusterMany` and other clustering results.

Usage

```

## S4 method for signature 'ClusterExperiment'
plotClustersWorkflow(object,
  whichClusters = c("mergeClusters", "combineMany"),
  whichClusterMany = NULL, nBlankLines = ceiling(nClusterings(object) *
0.05), existingColors = c("ignore", "all", "highlightOnly"),
  nSizeResult = ceiling(nClusterings(object) * 0.02), clusterLabels = TRUE,
  clusterManyLabels = TRUE, sortBy = c("highlighted", "clusterMany"),
  highlightOnTop = TRUE, ...)

```

Arguments

<code>object</code>	A <code>ClusterExperiment</code> object on which <code>clusterMany</code> has been run
<code>whichClusters</code>	which clusterings to "highlight", i.e draw separately from the <code>clusterMany</code> results. Can be numeric or character vector, indicating the indices or <code>clusterLabels/clusterTypes</code> of the clusterings of interest, respectively.
<code>whichClusterMany</code>	numeric indices of which of the <code>clusterMany</code> clusterings to plot (if <code>NULL</code> , defaults to all). Unlike <code>whichClusters</code> , these must be numeric indices. They must also refer to clusterings of <code>clusterType clusterMany</code> .
<code>nBlankLines</code>	the number of blank (i.e. white) rows to add between the <code>clusterMany</code> clusterings and the highlighted clusterings.
<code>existingColors</code>	one of "ignore","all","highlightOnly". Whether the plot should use the stored colors in the <code>ClusterExperiment</code> object given. "highlightOnly" means only the highlighted clusters will use the stored colors, not the <code>clusterMany</code> clusterings.
<code>nSizeResult</code>	the number of rows each highlighted clustering should take up. Increasing the number increases the thickness of the rectangles representing the highlighted clusterings.

<code>clusterLabels</code>	either logical, indicating whether to plot the labels for the clusterings identified to be highlighted in the <code>whichClusters</code> argument, or a character vector of labels to use.
<code>clusterManyLabels</code>	either logical, indicating whether to plot the labels for the clusterings from <code>clusterMany</code> identified in the <code>whichClusterMany</code> , or a character vector of labels to use.
<code>sortBy</code>	how to align the clusters. If "highlighted" then the highlighted clusters indicated in the argument <code>whichClusters</code> are first in the alignment done by <code>plotClusters</code> . If "clusterMany", then the <code>clusterMany</code> results are first in the alignment. (Note this does not determine where they will be plotted, but how they are ordered in the aligning step done by <code>plotClusters</code>)
<code>highlightOnTop</code>	logical. Whether the highlighted clusters should be plotted on the top of <code>clusterMany</code> results or underneath.
<code>...</code>	arguments passed to the matrix version of plotClusters

Details

This plot is solely intended to make it easier to use the [plotClusters](#) visualization when there are a large number of clusterings from a call to [clusterMany](#). This plot separates out the `clusterMany` results from a designated clustering of interest, as indicated by the `whichClusters` argument (by default clusterings from a call to [combineMany](#) or [mergeClusters](#)). In addition the highlighted clusters are made bigger so that they can be easily seen.

Value

A plot is produced, nothing is returned.

See Also

[plotClusters](#), [clusterMany](#)

Examples

```
#clustering using pam: try using different dimensions of pca and different k
data(simData)

cl <- clusterMany(simData, nReducedDims=c(5, 10, 50), reduceMethod="PCA",
  clusterFunction="pam", ks=2:4, findBestK=c(TRUE,FALSE),
  removeSil=c(TRUE,FALSE))
cl <- combineMany(cl, proportion=0.7)
plotClustersWorkflow(cl)
```

`plotContrastHeatmap, ClusterExperiment-method`

Plot heatmaps showing significant genes per contrast

Description

Plots a heatmap of the data, with the genes grouped based on the contrast for which they were significant.

Usage

```
## S4 method for signature 'ClusterExperiment'
plotContrastHeatmap(object, signifTable,
  whichCluster = NULL, contrastColors = NULL, ...)
```

Arguments

object	ClusterExperiment object on which biomarkers were found
signifTable	A data.frame in format of the result of getBestFeatures . It must minimally contain columns 'Contrast' and 'IndexInOriginal' giving the grouping and original index of the features in the assay(object)
whichCluster	if not NULL, indicates cluster used in making the significance table. Used to match to names in clusterLegend(object) (relevant for one-vs-all contrast so that color aligns).
contrastColors	vector of colors to be given to contrasts. Should match the name of the contrasts in the 'Contrast' column of signifTable or 'ContrastName', if given.. If missing, default colors given by match to the cluster names of whichCluster (see above), or otherwise given a default assignment.
...	Arguments passed to plotHeatmap

Details

If the column 'ContrastName' is given in signifTable, these names will be used to describe the contrast in the legend.

Within each contrast, the genes are sorted by log fold-change if the column "logFC" is in the signifTable data.frame

Value

A heatmap is created. The output of [plotHeatmap](#) is returned.

See Also

[plotHeatmap](#), [makeBlankData](#), [getBestFeatures](#)

Examples

```
data(simData)

cl <- clusterSingle(simData, subsample=FALSE,
  sequential=FALSE, mainClusterArgs=list(clusterFunction="pam", clusterArgs=list(k=8)))

#Do all pairwise, only return significant, try different adjustments:
pairsPerC <- getBestFeatures(cl, contrastType="Pairs", number=5,
  p.value=0.05, isCount=FALSE)
plotContrastHeatmap(cl,pairsPerC)
```

plotDendrogram, ClusterExperiment-method

Plot dendrogram of ClusterExperiment object

Description

Plots the dendrogram saved in a ClusterExperiment object

Usage

```
## S4 method for signature 'ClusterExperiment'
plotDendrogram(x, whichClusters = "dendro",
  leafType = c("samples", "clusters"), plotType = c("colorblock", "name",
  "ids"), mergeInfo = "none", main, sub, clusterLabelAngle = 45,
  removeOutbranch = TRUE, legend = c("side", "below", "none"),
  nodeColors = NULL, ...)
```

Arguments

x	a ClusterExperiment object.
whichClusters	only used if leafType="samples"). If numeric, an index for the clusterings to be plotted with dendrogram. Otherwise, whichClusters can be a character value identifying the clusterTypes to be used, or if not matching clusterTypes then clusterLabels; alternatively whichClusters can be either 'all' or 'workflow' or 'primaryCluster' to indicate choosing all clusters or choosing all workflowClusters . Default 'dendro' indicates using the clustering that created the dendrogram.
leafType	if "samples" the dendrogram has one leaf per sample, otherwise it has one per cluster.
plotType	one of 'name', 'colorblock' or 'id'. If 'Name' then dendrogram will be plotted, and name of cluster or sample (depending on type of value for leafType) will be plotted next to the leaf of the dendrogram. If 'colorblock', rectangular blocks, corresponding to the color of the cluster will be plotted, along with cluster name legend. If 'id' the internal clusterIds value will be plotted (only appropriate if leafType="clusters").
mergeInfo	What kind of information about merge to plot on dendrogram. If not equal to "none", will replicate the kind of plot that mergeClusters creates, and the input to mergeInfo corresponds to that of plotInfo in mergeClusters.
main	passed to the plot.phylo function to set main title.
sub	passed to the plot.phylo function to set subtitle.
clusterLabelAngle	angle at which label of cluster will be drawn. Only applicable if plotType="colorblock".
removeOutbranch	logical, only applicable if there are missing samples (i.e. equal to -1 or -2), leafType="samples" and the dendrogram for the samples was made by putting missing samples in an outbranch. In which case, if this parameter is TRUE, the outbranch will not be plotted, and if FALSE it will be plotted.

legend	character, only applicable if plotType="colorblock". Passed to <code>phydataplot</code> in <code>ape</code> package that is used to draw the color values of the clusters/samples next to the dendrogram. Options are 'none', 'below', or 'side'. (Note 'none' is only available for 'ape' package >= 4.1-0.6).
nodeColors	named vector of colors to be plotted on a node in the dendrogram. Names should match the name of the node (calls <code>nodeLabels</code>).
...	arguments passed to the <code>plot.phylo</code> function of <code>ape</code> that plots the dendrogram.

Details

If leafType="clusters", the plotting function will work best if the clusters in the dendrogram correspond to the primary cluster. This is because the function colors the cluster labels based on the colors of the clusterIds of the primaryCluster

Value

A dendrogram is plotted. Returns (invisibly) a list with elements

- plottedObject the phylo object that is plotted.
- originalObject the phylo object before adjusting the node/tip labels.

See Also

[mergeClusters](#), [plot.phylo](#), [nodeLabels](#), [tiplabels](#)

Examples

```
data(simData)

#create a clustering, for 8 clusters (truth was 3)
cl <- clusterSingle(simData, subsample=FALSE,
  sequential=FALSE, mainClusterArgs=list(clusterFunction="pam", clusterArgs=list(k=8)))

#create dendrogram of clusters and then
# merge clusters based on dendrogram:
cl <- makeDendrogram(cl)
cl <- mergeClusters(cl, mergeMethod="adjP", cutoff=0.1, plot=FALSE)
plotDendrogram(cl)
plotDendrogram(cl, leafType="samples", whichClusters="all", plotType="colorblock")
```

plotFeatureBoxplot *Plot boxplot of feature values by cluster*

Description

Plot a boxplot of the (transformed) values for a particular gene, separated by cluster

Usage

```
## S4 method for signature 'ClusterExperiment,character,ANY'
plotFeatureBoxplot(object,
  whichCluster, feature, ...)

## S4 method for signature 'ClusterExperiment,missing,ANY'
plotFeatureBoxplot(object,
  whichCluster, feature, ...)

## S4 method for signature 'ClusterExperiment,numeric,character'
plotFeatureBoxplot(object,
  whichCluster, feature, ...)

## S4 method for signature 'ClusterExperiment,numeric,numeric'
plotFeatureBoxplot(object,
  whichCluster, feature, plotUnassigned = FALSE, unassignedColor = NULL,
  missingColor = NULL, main = NULL, assay = NULL, ...)
```

Arguments

object	a ClusterExperiment object
whichCluster	which clusters to show on the plot
feature	identification of feature to plot, either row name or index
...	arguments passed to boxplot
plotUnassigned	whether to plot the unassigned samples as a cluster (either -1 or -2)
unassignedColor	If not NULL, should be character value giving the color for unassigned (-2) samples (overrides clusterLegend) default.
missingColor	If not NULL, should be character value giving the color for missing (-2) samples (overrides clusterLegend) default.
main	title of plot. If NULL, given default title.
assay	Identifies which assay in the object should be used for the data to be plotted.

Value

A plot is created. The output of boxplot is returned

See Also

[boxplot](#)

Examples

```
#clustering using pam: try using different dimensions of pca and different k
data(simData)

c1 <- clusterMany(simData, nReducedDims=c(5, 10, 50), reducedDim="PCA",
  clusterFunction="pam", ks=2:4, findBestK=c(TRUE,FALSE),
  removeSil=c(TRUE,FALSE))
clusterLegend(c1)[[1]][,"name"]<-letters[1:nClusters(c1,ignoreUnassigned = FALSE)[1]]
plotFeatureBoxplot(c1,feature=1)
```


Arguments

<code>data</code>	data to use to determine the heatmap. Can be a matrix, <code>ClusterExperiment</code> , <code>SingleCellExperiment</code> or <code>SummarizedExperiment</code> object. The interpretation of parameters depends on the type of the input to <code>data</code> .
<code>isCount</code>	if <code>transFun=NULL</code> , then <code>isCount=TRUE</code> will determine the transformation as defined by <code>function(x){log2(x+1)}</code> , and <code>isCount=FALSE</code> will give a transformation function <code>function(x){x}</code> . Ignored if <code>transFun=NULL</code> . If object is of class <code>ClusterExperiment</code> , the stored transformation will be used and giving this parameter will result in an error.
<code>transFun</code>	a transformation function to be applied to the data. If the transformation applied to the data creates an error or NA values, then the function will throw an error. If object is of class <code>ClusterExperiment</code> , the stored transformation will be used and giving this parameter will result in an error.
<code>...</code>	for signature <code>matrix</code> , arguments passed to <code>aheatmap</code> . For the other signatures, passed to the method for signature <code>matrix</code> . Not all arguments can be passed to <code>aheatmap</code> effectively, see details.
<code>clusterSamplesData</code>	If <code>data</code> is a matrix, <code>clusterSamplesData</code> is either a matrix that will be used by <code>hclust</code> to define the hierarchical clustering of samples (e.g. normalized data) or a pre-existing dendrogram that clusters the samples. If <code>data</code> is a <code>ClusterExperiment</code> object, <code>clusterSamplesData</code> should be either character or integers or logical which indicates how (and whether) the samples should be clustered (or gives indices of the order for the samples). See details.
<code>clusterFeaturesData</code>	If <code>data</code> is a matrix, either a matrix that will be used in <code>hclust</code> to define the hierarchical clustering of features (e.g. normalized data) or a pre-existing dendrogram that clusters the features. If <code>data</code> is a <code>ClusterExperiment</code> object, the input should be either character or integers indicating which features should be used (see details).
<code>nFeatures</code>	integer indicating how many features should be used (if <code>clusterFeaturesData</code> is <code>'var'</code> or <code>'PCA'</code>).
<code>visualizeData</code>	either a character string, indicating what form of the data should be used for visualizing the data (i.e. for making the color-scale), or a <code>data.frame</code> /matrix with same number of samples as <code>assay(data)</code> . If a new <code>data.frame</code> /matrix, any character arguments to <code>clusterFeaturesData</code> will be ignored.
<code>whichClusters</code>	character string, or vector of characters or integers, indicating what clusters should be visualized with the heatmap.
<code>sampleData</code>	If input to <code>data</code> is either a <code>ClusterExperiment</code> , or <code>SummarizedExperiment</code> object or <code>SingleCellExperiment</code> , then <code>sampleData</code> must index the <code>sampleData</code> stored as a <code>DataFrame</code> in <code>colData</code> slot of the object. Whether that data is continuous or not will be determined by the properties of <code>colData</code> (no user input is needed). If input to <code>data</code> is matrix, <code>sampleData</code> is a matrix of additional data on the samples to show above heatmap. In this case, unless indicated by <code>whSampleDataCont</code> , <code>sampleData</code> will be converted into factors, even if numeric. <code>"-1"</code> indicates the sample was not assigned to a cluster and gets color <code>'unassignedColor'</code> and <code>"-2"</code> gets the color <code>'missingColor'</code> .
<code>clusterFeatures</code>	Logical as to whether to do hierarchical clustering of features (if <code>FALSE</code> , any input to <code>clusterFeaturesData</code> is ignored).

nBlankLines	Only applicable if input is ClusterExperiment object. Indicates the number of lines to put between groups of features if clusterFeaturesData gives groups of genes (see details and makeBlankData).
colorScale	palette of colors for the color scale of the heatmap.
whSampleDataCont	Which of the sampleData columns are continuous and should not be converted to counts. NULL indicates no additional sampleData. Only used if data input is matrix.
clusterSamples	Logical as to whether to do hierarchical clustering of cells (if FALSE, any input to clusterSamplesData is ignored).
showSampleNames	Logical as to whether show sample names.
showFeatureNames	Logical as to whether show feature names.
clusterLegend	Assignment of colors to the clusters. If NULL, sampleData columns will be assigned colors internally. See details for more.
alignSampleData	Logical as to whether should align the colors of the sampleData (only if clusterLegend not given and sampleData is not NULL).
unassignedColor	color assigned to cluster values of '-1' ("unassigned").
missingColor	color assigned to cluster values of '-2' ("missing").
breaks	Either a vector of breaks (should be equal to length 52), or a number between 0 and 1, indicating that the breaks should be equally spaced (based on the range in the data) upto the 'breaks' quantile, see setBreaks
symmetricBreaks	logical as to whether the breaks created for the color scale should be symmetrical around 0
capBreaksLegend	logical as to whether the legend for the breaks should be capped. Only relevant if breaks is a value < 1, in which case if capBreaksLegend=TRUE, only the values between the quantiles requested will show in the color scale legend.
isSymmetric	logical. if TRUE indicates that the input matrix is symmetric. Useful when plotting a co-clustering matrix or other sample by sample matrices (e.g., correlation).
overRideClusterLimit	logical. Whether to override the internal limit that only allows 10 clusterings/annotations. If overridden, may result in incomprehensible errors from aheatmap. Only override this if you have a very large plotting device and want to see if aheatmap can render it.
plot	logical indicating whether to plot the heatmap. Mainly useful for package maintenance to avoid calls to aheatmap on unit tests that take a long time.
invert	logical determining whether the coClustering matrix should be inverted to be 1-coClustering for plotting. By default, if the diagonal elements are all zero, invert=TRUE, and otherwise invert=FALSE. If coClustering matrix is not a 0-1 matrix (e.g. if equal to a distance matrix output from clusterSingle , then the user should manually set this parameter to FALSE.)

Details

The `plotHeatmap` function calls `aheatmap` to draw the heatmap. The main points of `plotHeatmap` are to 1) allow for different matrix inputs, separating out the color scale visualization and the clustering of the samples/features. 2) to visualize the clusters and meta data with the heatmap. The intended use case is to allow the user to visualize the original count scale of the data (on the log-scale), but create the hierarchical clustering on another, more appropriate dataset for clustering, such as normalized data. Similarly, some of the palettes in the package were developed assuming that the visualization might be on unscaled/uncentered data, rather than the residual from the mean of the gene, and thus palettes need to take on a greater range of relevant values so as to show meaningful comparisons with genes on very different scales.

If data is a `ClusterExperiment` object, `visualizeData` indicates what kind of transformation should be done to `assay(data)` for calculating the color scale. The features will be clustered based on these data as well. A different data.frame or matrix can be given for the visualization. For example, if the `ClusterExperiment` object contains normalized data, but the user wishes that the color scale be based on the log-counts for easier interpretation, `visualizeData` could be set to be the `log2(counts + 1)`.

If data is a `ClusterExperiment` object, `clusterSamplesData` can be used to indicate the type of clustering for the samples. If equal to `'dendrogramValue'` the dendrogram stored in data will be used; if `dendrogram` is missing, a new one will be created based on the `primaryCluster` of data using `makeDendrogram`, assuming no errors are created (if errors are created, then `clusterSamplesData` will be set to `"primaryCluster"`). If `clusterSamplesData` is equal to `"hclust"`, then standard hierarchical clustering of the transformed data will be used. If `clusterSamplesData` is equal to `'orderSamplesValue'` no clustering of the samples will be done, and instead the samples will be ordered as in the `slot orderSamples` of data. If `clusterSamplesData` is equal to `'primaryCluster'`, again no clustering will be done, and instead the samples will be ordered based on grouping the samples to match the `primaryCluster` of data; however, if the `primaryCluster` of data is only one cluster or consists solely of -1/-2 values, `clusterSamplesData` will be set to `"hclust"`. If `clusterSamplesData` is not a character value, `clusterSamplesData` can be a integer valued vector giving the order of the samples.

If data is a matrix, then `sampleData` is a data.frame of annotation data to be plotted above the heatmap and `whSampleDataCont` gives the index of the column(s) of this dataset that should be considered continuous. Otherwise the annotation data for `sampleData` will be forced into a factor (which will be nonsensical for continuous data). If data is a `ClusterExperiment` object, `sampleData` should refer to an index or column name of the `colData` slot of data. In this case `sampleData` will be added to any choices of clusterings chosen by the `whichClusters` argument (if any). If both clusterings and sample data are chosen, the clusterings will be shown closest to data (i.e. on bottom).

If data is a `ClusterExperiment` object, `clusterFeaturesData` is not a dataset, but instead indicates which features should be shown in the heatmap. In this case `clusterFeatures` can be one of the following:

- `"all"` All rows/genes will be shown
- character giving dimensionality reduction Should match one of values saved in `reducedDims` slot or a builtin function in `listBuiltInReducedDims()`. `nFeatures` then gives the number of dimensions to show. The heatmap will then be of the dimension reduction vectors
- character giving filtering Should match one of values saved in `filterStats` slot or a builtin function in `listBuiltInFilterStats()`. `nFeatures` gives the number of genes to keep after filtering.
- character giving gene/row names
- vector of integers giving row indices

- a list of indices or rownames This is used to indicate that the features should be grouped according to the elements of the list, with blank (white) space between them (see [makeBlankData](#) for more details). In this case, no clustering is done of the features.

If `breaks` is a numeric value between 0 and 1, then `breaks` is assumed to indicate the upper quantile (on the log scale) at which the heatmap color scale should stop. For example, if `breaks=0.9`, then the breaks will evenly spaced up until the 0.9 upper quantile of data, and then all values after the 0.9 quantile will be absorbed by the upper-most color bin. This can help to reduce the visual impact of a few highly expressed genes (features).

Note that `plotHeatmap` calls `aheatmap` under the hood. This allows you to plot multiple heatmaps via `par(mfrow=c(2,2))`, etc. However, the dendrograms do not resize if you change the size of your plot window in an interactive session of R (this might be a problem for RStudio if you want to pop it out into a large window...). Also, plotting to a pdf adds a blank page; see help pages of [aheatmap](#) for how to turn this off.

`clusterLegend` takes the place of argument `annColors` from `aheatmap` for giving colors to the annotation on the heatmap. `clusterLegend` should be list of length equal to `ncol(sampleData)` with names equal to the `colnames` of `sampleData`. Each element of the list should be either the format requested by `aheatmap` (a vector of colors with names corresponding to the levels of the column of `sampleData`), or should be format of the `clusterLegend` slot in a `ClusterExperiment` object. Color assignments to the rows/genes should also be passed via `clusterLegend` (assuming `annRow` is an argument passed to `...`). If `clusterFeaturesData` is a *named* list describing groupings of genes then the colors for those groups can be given in `clusterLegend` under the name "Gene Group".

If you have a factor with many levels, it is important to note that `aheatmap` does not recycle colors across factors in the `sampleData`, and in fact runs out of colors and the remaining levels get the color white. Thus if you have many factors or many levels in those factors, you should set their colors via `clusterLegend`.

Many arguments can be passed on to `aheatmap`, however, some are set internally by `plotHeatmap`. In particular, setting the values of `Rowv` or `Colv` will cause errors. `color` in `aheatmap` is replaced by `colorScale` in `plotHeatmap`. The `annCol` to give annotation to the samples is replaced by the `sampleData`; moreover, the `annColors` option in `aheatmap` will also be set internally to give more vibrant colors than the default in `aheatmap` (for `ClusterExperiment` objects, these values can also be set in the `clusterLegend` slot). Other options should be passed on to `aheatmap`, though they have not been all tested.

`plotCoClustering` is a convenience function to plot the heatmap of the co-clustering matrix stored in the `coClustering` slot of a `ClusterExperiment` object.

Value

Returns (invisibly) a list with elements

- `aheatmapOut` The output from the final call of [aheatmap](#).
- `sampleData` the annotation data.frame given to the argument `annCol` in `aheatmap`.
- `clusterLegend` the annotation colors given to the argument `annColors` `aheatmap`.
- `breaks` The breaks used for `aheatmap`, after adjusting for quantile.

Author(s)

Elizabeth Purdom

See Also

[aheatmap](#), [makeBlankData](#), [showHeatmapPalettes](#)

Examples

```

data(simData)

cl <- rep(1:3,each=100)
cl2 <- cl
changeAssign <- sample(1:length(cl), 80)
cl2[changeAssign] <- sample(cl[changeAssign])
ce <- ClusterExperiment(simCount, cl2, transformation=function(x){log2(x+1)})

#simple, minimal, example. Show counts, but cluster on underlying means
plotHeatmap(ce)

#assign cluster colors
colors <- bigPalette[20:23]
names(colors) <- 1:3
plotHeatmap(data=simCount, clusterSamplesData=simData,
sampleData=data.frame(cl), clusterLegend=list(colors))

#show two different clusters
anno <- data.frame(cluster1=cl, cluster2=cl2)
out <- plotHeatmap(simData, sampleData=anno)

#return the values to see format for giving colors to the annotations
out$clusterLegend

#assign colors to the clusters based on plotClusters algorithm
plotHeatmap(simData, sampleData=anno, alignSampleData=TRUE)

#assign colors manually
annoColors <- list(cluster1=c("black", "red", "green"),
cluster2=c("blue", "purple", "yellow"))

plotHeatmap(simData, sampleData=anno, clusterLegend=annoColors)

#give a continuous valued -- need to indicate columns
anno2 <- cbind(anno, Cont=c(rnorm(100, 0), rnorm(100, 2), rnorm(100, 3)))
plotHeatmap(simData, sampleData=anno2, whSampleDataCont=3)

#compare changing breaks quantile on visual effect
## Not run:
par(mfrow=c(2,2))
plotHeatmap(simData, colorScale=seqPal1, breaks=1, main="Full length")
plotHeatmap(simData,colorScale=seqPal1, breaks=.99, main="0.99 Quantile Upper
Limit")
plotHeatmap(simData,colorScale=seqPal1, breaks=.95, main="0.95 Quantile Upper
Limit")
plotHeatmap(simData, colorScale=seqPal1, breaks=.90, main="0.90 Quantile
Upper Limit")

## End(Not run)

```

plotReducedDims *Plot 2-dimensional representation with clusters*

Description

Plot a 2-dimensional representation of the data, color-code by a clustering.

Usage

```
## S4 method for signature 'ClusterExperiment,character'
plotReducedDims(object, whichCluster,
  ...)

## S4 method for signature 'ClusterExperiment,missing'
plotReducedDims(object, whichCluster, ...)

## S4 method for signature 'ClusterExperiment,numeric'
plotReducedDims(object, whichCluster,
  reducedDim = "PCA", whichDims = c(1:2), plotUnassigned = TRUE,
  legend = TRUE, legendTitle = "", clusterLegend = NULL,
  unassignedColor = NULL, missingColor = NULL, pch = 19, xlab = NULL,
  ylab = NULL, ...)
```

Arguments

object	a ClusterExperiment object
whichCluster	which clusters to show on the plot
...	arguments passed to <code>plot.default</code>
reducedDim	What dimensionality reduction method to use. Should match either a value in <code>reducedDimNames(object)</code> or one of the built-in functions of <code>listBuiltInReducedDims()</code>
whichDims	vector of length 2 giving the indices of which dimensions to show. The first value goes on the x-axis and the second on the y-axis.
plotUnassigned	logical as to whether unassigned (either -1 or -2 cluster values) should be plotted.
legend	either logical, indicating whether to plot legend, or character giving the location of the legend (passed to <code>legend</code>)
legendTitle	character value giving title for the legend. If NULL, uses the <code>clusterLabels</code> value for clustering.
clusterLegend	matrix with three columns and colnames 'clusterIds', 'name', and 'color' that give the color and name of the clusters in whichCluster. If NULL, pulls the information from object.
unassignedColor	If not NULL, should be character value giving the color for unassigned (-2) samples (overrides <code>clusterLegend</code>) default.
missingColor	If not NULL, should be character value giving the color for missing (-2) samples (overrides <code>clusterLegend</code>) default.
pch	the point type, passed to <code>plot.default</code>
xlab	Label for x axis
ylab	Label for y axis

Details

If `plotUnassigned=TRUE`, and the color for -1 or -2 is set to "white", will be coerced to "lightgrey" regardless of user input to `missingColor` and `unassignedColor`. If `plotUnassigned=FALSE`, the samples with -1/-2 will not be plotted, nor will the category show up in the legend.

Value

A plot is created. Nothing is returned.

See Also

[plot.default](#), [makeReducedDims](#), [listBuiltInReducedDims\(\)](#)

Examples

```
#clustering using pam: try using different dimensions of pca and different k
data(simData)

cl <- clusterMany(simData, nReducedDims=c(5, 10, 50), reducedDim="PCA",
  clusterFunction="pam", ks=2:4, findBestK=c(TRUE,FALSE),
  removeSil=c(TRUE,FALSE))

plotReducedDims(cl,legend="bottomright")
```

plottingFunctions

Convert clusterLegend into useful formats

Description

Function for converting the information stored in the `clusterLegend` slot into other useful formats. Most of these functions are called internally by plotting functions, but are exported in case the user finds them useful.

Usage

```
makeBlankData(data, groupsOfFeatures, nBlankLines = 1)

## S4 method for signature 'ClusterExperiment'
convertClusterLegend(object,
  output = c("plotAndLegend", "aheatmapFormat", "matrixNames",
    "matrixColors"), whichClusters = ifelse(output == "plotAndLegend",
    "primary", "all"))

showPalette(colPalette = bigPalette, which = NULL, cex = 1)

bigPalette

massivePalette

setBreaks(data, breaks = NA, makeSymmetric = FALSE, returnBreaks = TRUE)
```

```

showHeatmapPalettes()

seqPal5

seqPal2

seqPal3

seqPal4

seqPal1

## S4 method for signature 'ClusterExperiment'
plotClusterLegend(object,
  whichCluster = "primary", clusterNames, title, ...)

```

Arguments

data	matrix with samples on columns and features on rows.
groupsOfFeatures	list, with each element of the list containing a vector of numeric indices.
nBlankLines	the number of blank lines to add in the data matrix to separate the groups of indices (will govern the amount of white space if data is then fed to heatmap.)
object	a ClusterExperiment object.
output	character value, indicating desired type of conversion.
whichClusters	which clusters to use
colPalette	a vector of character colors. By default, the palette bigPalette is used
which	numeric. Which colors to plot. Must be a numeric vector with values between 1 and length of colPalette. If missing, all colors plotted.
cex	numeric value giving the cex for the text of the plot.
breaks	either vector of breaks, or number of breaks (integer) or a number between 0 and 1 indicating a quantile, between which evenly spaced breaks should be calculated. If missing or NA, will determine evenly spaced breaks in the range of the data.
makeSymmetric	whether to make the range of the breaks symmetric around zero (only used if not all of the data is non-positive and not all of the data is non-negative)
returnBreaks	logical as to whether to return the vector of breaks. See details.
whichCluster	which cluster to plot cluster legend
clusterNames	vector of names for the clusters; vector should have names that correspond to the clusterIds in the ClusterExperiment object. If this argument is missing, will use the names in the "name" column of the clusterLegend slot of the object.
title	title for the clusterLegend plot
...	arguments passed to legend

Format

An object of class character of length 56.

Details

`makeBlankData` pulls the data corresponding to the row indices in `groupsOfFeatures` adds lines of NA values into data between these groups. When given to `heatmap`, will create white space between these groups of features.

`convertClusterLegend` pulls out information stored in the `clusterLegend` slot of the object and returns it in useful format.

`bigPalette` is a long palette of colors (length 58) used by `plotClusters` and accompanying functions. `showPalette` creates plot that gives index of each color in a vector of colors. `massivePalette` is a combination of `bigPalette` and the non-grey colors of `colors()` (length 487). `massivePalette` is mainly useful for when doing `plotClusters` of a very large number of clusterings, each with many clusters, so that the code doesn't run out of colors. However, many of the colors will be very similar to each other.

`showPalette` will plot the `colPalette` colors with their labels and index.

if `returnBreaks` is FALSE, instead of returning the vector of breaks, the function will just return the second smallest and second largest value of the breaks. This is useful for alternatively just setting values of the data matrix larger than these values to this value if breaks was a percentile. This argument is only used if `breaks < 1`, indicating truncating the breaks for large values of data.

`setBreaks` gives a set of breaks (of length 52) equally spaced between the boundaries of the data. If `breaks` is between 0 and 1, then the evenly spaced breaks are between these quantiles of the data.

`seqPal1-seqPal14` are palettes for the heatmap. `showHeatmapPalettes` will show you these palettes.

Value

`makeBlankData` returns a list with items

- "dataWBlanks" The data with the rows of NAs separating the given indices.
- "rowNamesWBlanks" A vector of characters giving the rownames for the data, including blanks for the NA rows. These are not given as rownames to the returned data because they are not necessarily unique. However, they can be given to the `labRow` argument of `aheatmap` or `plotHeatmap`.
- "groupNamesWBlanks" A vector of characters of the same length as the number of rows of the new data (i.e. with blanks) giving the group name for the data, indicating which group (i.e. which element of `groupsOfFeatures` list) the feature came from. If `groupsOfFeatures` has unique names, these names will be used, other wise "Group1", "Group2", etc. The NA rows are given NA values.

If `output="plotAndLegend"`, "`convertClusterLegend`" will return a list that provides the necessary information to color samples according to cluster and create a legend for it:

- "colorVector" A vector the same length as the number of samples, assigning a color to each cluster of the `primaryCluster` of the object.
- "legendNames" A vector the length of the number of clusters of `primaryCluster` of the object giving the name of the cluster.
- "legendColors" A vector the length of the number of clusters of `primaryCluster` of the object giving the color of the cluster.

If `output="aheatmap"` a conversion of the `clusterLegend` to be in the format requested by `aheatmap`. The column 'name' is used for the names and the column 'color' for the color of the clusters.

If `output="matrixNames"` or "`matrixColors`" a matrix the same dimension of `clusterMatrix(object)`, but with the cluster color or cluster name instead of the `clusterIds`, respectively.

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

```

data(simData)

x <- makeBlankData(simData[,1:10], groupsOfFeatures=list(c(5, 2, 3), c(20,
34, 25)))
plotHeatmap(x$dataWBlanks, clusterFeatures=FALSE)
showPalette()
showPalette(massivePalette, cex=0.6)
setBreaks(data=simData, breaks=.9)

#show the palette colors
showHeatmapPalettes()

#compare the palettes on heatmap
cl <- clusterSingle(simData, subsample=FALSE,
sequential=FALSE, mainClusterArgs=list(clusterFunction="pam", clusterArgs=list(k=8)))

## Not run:
par(mfrow=c(2,3))
plotHeatmap(cl, colorScale=seqPal1, main="seqPal1")
plotHeatmap(cl, colorScale=seqPal2, main="seqPal2")
plotHeatmap(cl, colorScale=seqPal3, main="seqPal3")
plotHeatmap(cl, colorScale=seqPal4, main="seqPal4")
plotHeatmap(cl, colorScale=seqPal5, main="seqPal5")
par(mfrow=c(1,1))

## End(Not run)

```

reduceFunctions

Filtering statistics and Dimensionality Reduction Functions

Description

Functions for calculating and manipulating either filtering statistics, stored in `rowData`, or the dimensionality reduction results, stored in `reducedDims`.

Usage

```

## S4 method for signature 'SummarizedExperiment'
makeFilterStats(object,
  filterStats = listBuiltInFilterStats(), transFun = NULL,
  isCount = FALSE)

## S4 method for signature 'matrixOrHDF5'
makeFilterStats(object, ...)

## S4 method for signature 'ClusterExperiment'
makeFilterStats(object,

```

```

whichClusterIgnoreUnassigned = NULL,
filterStats = listBuiltInFilterStats(), ...)

listBuiltInFilterStats()

## S4 method for signature 'SummarizedExperiment'
filterData(object, filterStats, cutoff,
  percentile, absolute = FALSE, keepLarge = TRUE)

## S4 method for signature 'SingleCellExperiment'
defaultNDims(object, reduceMethod, typeToShow)

## S4 method for signature 'SummarizedExperiment'
filterNames(object)

## S4 method for signature 'SingleCellExperiment'
makeReducedDims(object, reducedDims = "PCA",
  maxDims = 500, transFun = NULL, isCount = FALSE)

## S4 method for signature 'matrixOrHDF5'
makeReducedDims(object, ...)

## S4 method for signature 'SummarizedExperiment'
makeReducedDims(object, ...)

## S4 method for signature 'ClusterExperiment'
makeReducedDims(object, ...)

listBuiltInReducedDims()

```

Arguments

object	object from which user wants to calculate per-row statistics
filterStats	character vector of statistics to calculate. Must be one of the character values given by <code>listBuildInFilterStats()</code> .
transFun	a transformation function to be applied to the data. If the transformation applied to the data creates an error or NA values, then the function will throw an error. If object is of class <code>ClusterExperiment</code> , the stored transformation will be used and giving this parameter will result in an error.
isCount	if <code>transFun=NULL</code> , then <code>isCount=TRUE</code> will determine the transformation as defined by <code>function(x){log2(x+1)}</code> , and <code>isCount=FALSE</code> will give a transformation function <code>function(x){x}</code> . Ignored if <code>transFun=NULL</code> . If object is of class <code>ClusterExperiment</code> , the stored transformation will be used and giving this parameter will result in an error.
...	Values passed on the the 'SingleCellExperiment' method.
whichClusterIgnoreUnassigned	indicates clustering that should be used to filter out unassigned samples from the calculations. If <code>NULL</code> no filtering of samples will be done. See details for more information.
cutoff	numeric. A value at which to filter the rows (genes) for the test statistic

percentile	numeric. Either a number between 0,1 indicating what percentage of the rows (genes) to keep or an integer value indicated the number of rows (genes) to keep
absolute	whether to take the absolute value of the filter statistic
keepLarge	logical whether to keep rows (genes) with large values of the test statistic or small values of the test statistic.
reduceMethod	character. A method or methods for reducing the size of the data, either by filtering the rows (genes) or by a dimensionality reduction method. Must either be 1) must match the name of a built-in method, in which case if it is not already existing in the object will be passed to <code>makeFilterStats</code> or <code>link{makeReducedDims}</code> , or 2) must match a stored filtering statistic or dimensionality reduction in the object
typeToShow	character (optional). If given, should be one of "filterStats" or "reducedDims" to indicate of the values in the reduceMethod vector, only show those corresponding to "filterStats" or "reducedDims" options.
reducedDims	a vector of character values indicating the methods of dimensionality reduction to be performed. Currently only "PCA" is implemented.
maxDims	Numeric vector of integer giving the number of PC dimensions to calculate. maxDims can also take values between (0,1) to indicate keeping the number of dimensions necessary to account for that proportion of the variance. maxDims should be of same length as reducedDims, indicating the number of dimensions to keep for each method (if maxDims is of length 1, the same number of dimensions will be used for each).

Details

`whichClusterIgnoreUnassigned` is only an option when applied to a `ClusterExperiment` class and indicates that the filtering statistics should be calculated based on samples that are unassigned by the designated clustering. The name given to the filter in this case is of the form `<filterStats>_<clusterLabel>`, i.e. the clustering label of the clustering is appended to the standard name for the filtering statistic.

Note that `filterData` returns a `SingleCellExperiment` object. To get the actual data out use either `assay` or `transformData` if transformed data is desired.

The PCA method uses either `prcomp` from the `stats` package or `svds` from the `RSpectra` package to perform PCA. Both are called on `t(assay(x))` with `center=TRUE` and `scale=TRUE` (i.e. the feature are centered and scaled), so that it is performing PCA on the correlation matrix of the features.

Value

`makeFilterStats` returns a `SummarizedExperiment` object with the requested filtering statistics will be added to the `DataFrame` in the `rowData` slot and given names corresponding to the `filterStats` values. Warning: the function will overwrite existing columns in `rowData` with the same name. Columns in the `rowData` slot with different names should not be affected.

`filterData` returns a `SingleCellExperiment` object with the rows (genes) removed based on filters

`defaultNDims` returns a numeric vector giving the default dimensions the methods in `clusterExperiment` will use for reducing the size of the data. If `typeToShow` is missing, the resulting vector will be equal to the length of `reduceMethod`. Otherwise, it will be a vector with all the unique valid default values for the `typeToShow` (note that different dimensionality reduction methods can have different maximal dimensions, so the result may not be of length one in this case).

`filterNames` returns a vector of the columns of the `rowData` that are considered valid filtering statistics. Currently any numeric column in `rowData` is a valid filtering statistic.

`makeReducedDims` returns a [SingleCellExperiment](#) containing the calculated dimensionality reduction in the `reduceDims` with names corresponding to the name given in `reducedDims`.

Examples

```
data(simData)
listBuiltInFilterStats()
scf<-makeFilterStats(simData,filterStats=c("var","mad"))
scf
scfFiltered<-filterData(scf,filterStats="mad",percentile=10)
scfFiltered
assay(scfFiltered)[1:10,1:10]
data(simData)
listBuiltInReducedDims()
scf<-makeReducedDims(simData, reducedDims="PCA", maxDims=3)
scf
```

RSEC

Resampling-based Sequential Ensemble Clustering

Description

Implementation of the RSEC algorithm (Resampling-based Sequential Ensemble Clustering) for single cell sequencing data. This is a wrapper function around the existing `ClusterExperiment` workflow that results in the output of RSEC.

Usage

```
## S4 method for signature 'SummarizedExperiment'
RSEC(x, ...)

## S4 method for signature 'data.frame'
RSEC(x, ...)

## S4 method for signature 'ClusterExperiment'
RSEC(x, eraseOld = FALSE,
      rerunClusterMany = FALSE, ...)

## S4 method for signature 'matrixOrHDF5'
RSEC(x, ...)

## S4 method for signature 'SingleCellExperiment'
RSEC(x, isCount = FALSE, transFun = NULL,
      reduceMethod = "PCA", nFilterDims = defaultNDims(x, reduceMethod, type =
"filterStats"), nReducedDims = defaultNDims(x, reduceMethod, type =
"reducedDims"), k0s = 4:15, clusterFunction = "hierarchical01",
alphas = c(0.1, 0.2, 0.3), betas = 0.9, minSizes = 1,
combineProportion = 0.7, combineMinSize, dendroReduce, dendroNDims,
mergeMethod = "adjP", mergeCutoff, mergeLogFCcutoff, verbose = FALSE,
mainClusterArgs = NULL, subsampleArgs = NULL, seqArgs = NULL,
ncores = 1, random.seed = NULL, run = TRUE)
```

Arguments

x	the data matrix on which to run the clustering. Can be object of the following classes: matrix (with genes in rows), SummarizedExperiment , SingleCellExperiment or ClusterExperiment .
...	For signature list, arguments to be passed on to mclapply (if ncores>1). For all the other signatures, arguments to be passed to the method for signature list.
eraseOld	logical. Only relevant if input x is of class ClusterExperiment . If TRUE, will erase existing workflow results (clusterMany as well as mergeClusters and combineMany). If FALSE, existing workflow results will have "_i" added to the clusterTypes value, where i is one more than the largest such existing workflow clusterTypes.
rerunClusterMany	logical. If the object is a ClusterExperiment object, determines whether to rerun the clusterMany step. Useful if want to try different parameters for combining clusters after the clusterMany step, without the computational costs of the clusterMany step.
isCount	if transFun=NULL, then isCount=TRUE will determine the transformation as defined by function(x){log2(x+1)}, and isCount=FALSE will give a transformation function function(x){x}. Ignored if transFun=NULL. If object is of class ClusterExperiment , the stored transformation will be used and giving this parameter will result in an error.
transFun	a transformation function to be applied to the data. If the transformation applied to the data creates an error or NA values, then the function will throw an error. If object is of class ClusterExperiment , the stored transformation will be used and giving this parameter will result in an error.
reduceMethod	character A character identifying what type of dimensionality reduction to perform before clustering. Options are 1) "none", 2) one of listBuiltInReducedDims() or listBuiltInFiltlerStats OR 3) stored filtering or reducedDim values in the object.
nFilterDims	vector of the number of the most variable features to keep (when "var", "abscv", or "mad" is identified in reduceMethod).
nReducedDims	vector of the number of dimensions to use (when reduceMethod gives a dimensionality reduction method).
k0s	the k0 parameter for sequential clustering (see seqCluster)
clusterFunction	function used for the clustering. Note that unlike in clusterSingle , this must be a character vector of pre-defined clustering techniques, and can not be a user-defined function. Current functions can be found by typing listBuiltInFunctions() into the command-line.
alphas	values of alpha to be tried. Only used for clusterFunctions of type '01'. Determines tightness required in creating clusters from the dissimilarity matrix. Takes on values in [0,1]. See documentation of ClusterFunction .
betas	values of beta to be tried in sequential steps. Only used for sequential=TRUE. Determines the similarity between two clusters required in order to deem the cluster stable. Takes on values in [0,1]. See documentation of seqCluster .
minSizes	the minimum size required for a cluster (in the mainClustering step). Clusters smaller than this are not kept and samples are left unassigned.
combineProportion	passed to proportion in combineMany

combineMinSize	passed to minSize in combineMany
dendroReduce	passed to reduceMethod in makeDendrogram
dendroNDims	passed to nDims in makeDendrogram
mergeMethod	passed to mergeMethod in mergeClusters
mergeCutoff	passed to cutoff in mergeClusters
mergeLogFCcutoff	passed to logFCcutoff in mergeClusters
verbose	logical. If TRUE it will print informative messages.
mainClusterArgs	list of arguments to be passed for the mainClustering step, see help pages of mainClustering .
subsampleArgs	list of arguments to be passed to the subsampling step (if subsample=TRUE), see help pages of subsampleClustering .
seqArgs	list of arguments to be passed to seqCluster .
ncores	the number of threads
random.seed	a value to set seed before each run of clusterSingle (so that all of the runs are run on the same subsample of the data). Note, if 'random.seed' is set, argument 'ncores' should NOT be passed via subsampleArgs; instead set the argument 'ncores' of clusterMany directly (which is preferred for improving speed anyway).
run	logical. If FALSE, doesn't run clustering, but just returns matrix of parameters that will be run, for the purpose of inspection by user (with rownames equal to the names of the resulting column names of cIMat object that would be returned if run=TRUE). Even if run=FALSE, however, the function will create the dimensionality reductions of the data indicated by the user input.

Value

A [ClusterExperiment](#) object is returned containing all of the clusterings from the steps of RSEC

rsecFluidigm	<i>RSEC run for vignette</i>
--------------	------------------------------

Description

RSEC run for vignette

Format

ClusterExperiment object, the result of running [RSEC](#) on fluidigm data described in vignette and available in the [scrNaseq](#) package.

Author(s)

Elizabeth Purdom <epurdom@stat.berkeley.edu>

See Also

[fluidigm](#)

Examples

```

#code used to create rsecFluidigm:
## Not run:
library(scRNAseq)
data("fluidigm")
se <- fluidigm[,colData(fluidigm)[,"Coverage_Type"]=="High"]
wh_zero <- which(rowSums(assay(se))==0)
pass_filter <- apply(assay(se), 1, function(x) length(x[x >= 10]) >= 10)
se <- se[pass_filter,]
fq <- round(limma::normalizeQuantiles(assay(se)))
assays(se) <- list(normalized_counts=fq)
wh<-which(colnames(colData(se)) %in% c("Cluster1","Cluster2"))
colnames(colData(se))[wh]<-c("Published1","Published2")
library(ClusterExperiment)
ncores<-1
system.time(rsecFluidigm<-RSEC(se, isCount = TRUE,reduceMethod="PCA",nReducedDims=10,
ncores=ncores,random.seed=176201, clusterFunction="hierarchical01",
  combineMinSize=3))
packageVersion("clusterExperiment")
devtools::use_data(rsecFluidigm,overwrite=FALSE)

## End(Not run)

```

search_pairs

Search pairs of samples that co-cluster across subsamples

Description

Assume that our input is a matrix, with N columns and B rows (the number of subsamples), storing integers – the cluster labels.

Usage

```
search_pairs(clusterings)
```

Arguments

clusterings a matrix with the cluster labels

Value

A matrix with the co-clusters, but only the lower triangle is populated.

seqCluster	<i>Program for sequentially clustering, removing cluster, and starting again.</i>
------------	---

Description

Given a data matrix, this function will call clustering routines, and sequentially remove best clusters, and iterate to find clusters.

Usage

```
seqCluster(x = NULL, diss = NULL, k0, subsample = TRUE, beta,
  top.can = 5, remain.n = 30, k.min = 3, k.max = k0 + 10,
  verbose = TRUE, subsampleArgs = NULL, mainClusterArgs = NULL,
  checkDiss = TRUE)
```

Arguments

x	p x n data matrix on which to run the clustering (samples in columns).
diss	n x n data matrix of dissimilarities between the samples on which to run the clustering
k0	the value of K at the first iteration of sequential algorithm, see details below or vignette.
subsample	logical as to whether to subsample via subsampleClustering to get the distance matrix at each iteration; otherwise the distance matrix is set by arguments to mainClustering .
beta	value between 0 and 1 to decide how stable cluster membership has to be before 'finding' and removing the cluster.
top.can	only the top.can clusters from mainClustering (ranked by 'orderBy' argument given to mainClustering) will be compared pairwise for stability. Making this very big will effectively remove this parameter and all pairwise comparisons of all clusters found will be considered. This might result in smaller clusters being found. The current default is fairly large, so probably will have little effect.
remain.n	when only this number of samples are left (i.e. not yet clustered) then algorithm will stop.
k.min	each iteration of sequential detection of clustering will decrease the beginning K of subsampling, but not lower than k.min.
k.max	algorithm will stop if K in iteration is increased beyond this point.
verbose	whether the algorithm should print out information as to its progress.
subsampleArgs	list of arguments to be passed to subsampleClustering .
mainClusterArgs	list of arguments to be passed to mainClustering .
checkDiss	logical. Whether to check whether the input diss is valid.

Details

seqCluster is not meant to be called by the user. It is only an exported function so as to be able to clearly document the arguments for seqCluster which can be passed via the argument seqArgs in functions like `clusterSingle` and `clusterMany`.

This code is adapted from the sequential portion of the code of the tightClust package of Tseng and Wong. At each iteration of the algorithm it finds a set of samples that constitute a homogeneous cluster and remove them, and iterate again to find the next set of samples that form a cluster.

In each iteration, to determine the next set of homogeneous set of samples, the algorithm will iteratively cluster the current set of samples for a series of increasing values of the parameter K , starting at a value `kinit` and increasing by 1 at each iteration, until a sufficiently homogeneous set of clusters is found. For the first set of homogeneous samples, `kinit` is set to the argument `$k0`, and for iteration, `kinit` is increased internally.

Depending on the value of `subsample` how the value of K is used differs. If `subsample=TRUE`, K is the k sent to the cluster function `clusterFunction` sent to `subsampleClustering` via `subsampleArgs`; then `mainClustering` is run on the result of the co-occurrence matrix from `subsampleClustering` with the `ClusterFunction` object defined in the argument `clusterFunction` set via `mainClusterArgs`.

The number of clusters actually resulting from this run of `mainClustering` may not be equal to the K sent to the clustering done in `subsampleClustering`. If `subsample=FALSE`, `mainClustering` is called directly on the data to determine the clusters and K set by seqCluster for this iteration determines the parameter of the clustering done by `mainClustering`. Specifically, the argument `clusterFunction` defines the clustering of the `mainClustering` step and k is sent to that `ClusterFunction` object. This means that if `subsample=FALSE`, the `clusterFunction` must be of `algorithmType "K"`.

In either setting of `subsample`, the resulting clusters from `mainClustering` for a particular K will be compared to clusters found in the previous iteration of $K-1$. For computational (and other?) convenience, only the first `top.can` clusters of each iteration will be compared to the first `top.can` clusters of previous iteration for similarity (where `top.can` currently refers to ordering by size, so first `top.can` largest clusters).

If there is no cluster of the first `top.can` in the current iteration K that has overlap similarity $>$ `beta` to any in the previous iteration, then the algorithm will move to the next iteration, increasing to $K+1$.

If, however, of these clusters there is a cluster in the current iteration K that has overlap similarity $>$ `beta` to a cluster in the previous iteration $K-1$, then the cluster with the largest such similarity will be identified as a homogenous set of samples and the samples in it will be removed and designated as such. The algorithm will then start again to determine the next set of homogenous samples, but without these samples. Furthermore, in this case (i.e. a cluster was found and removed), the value of `kinit` will be reset to `kinit-1`; i.e. the range of increasing K that will be iterated over to find a set of homogenous samples will start off one value less than was the case for the previous set of homogeneous samples. If `kinit-1 < k.min`, then `kinit` will be set to `k.min`.

If there are less than `remain.n` samples left after finding a cluster and removing its samples, the algorithm will stop, as subsampling is deemed to no longer be appropriate. If the K has to be increased to beyond `k.max` without finding any pair of clusters with overlap $>$ `beta`, then the algorithm will stop. Any samples not found as part of a homogenous set of clusters at that point will be classified as unclustered (given a value of -1)

Certain combinations of inputs to `mainClusterArgs` and `subsampleArgs` are not allowed. See `clusterSingle` for these explanations.

Value

A list with values

- `clustering` a vector of length equal to `nrows(x)` giving the integer-valued cluster ids for each sample. The integer values are assigned in the order that the clusters were found. "-1" indicates the sample was not clustered.
- `clusterInfo` if clusters were successfully found, a matrix of information regarding the algorithm behavior for each cluster (the starting and stopping K for each cluster, and the number of iterations for each cluster).
- `whyStop` a character string explaining what triggered the algorithm to stop.

References

Tseng and Wong (2005), "Tight Clustering: A Resampling-Based Approach for Identifying Stable and Tight Patterns in Data", *Biometrics*, 61:10-16.

See Also

`tight.clust`, `clusterSingle`, `mainClustering`, `subsampleClustering`

Examples

```
## Not run:
data(simData)

set.seed(12908)
clustSeqHier <- seqCluster(simData, k0=5, subsample=TRUE,
  beta=0.8, subsampleArgs=list(resamp.n=100,
  samp.p=0.7, clusterFunction="kmeans", clusterArgs=list(nstart=10)),
  mainClusterArgs=list(minSize=5, clusterFunction="hierarchical01", clusterArgs=list(alpha=0.1)))

## End(Not run)
```

simData

Simulated data for running examples

Description

Simulated data for running examples

Format

Three objects are loaded, two data frame(s) of simulated data each with 300 samples/columns and 153 variables/rows, and a vector of length 300 with the true cluster assignments.

Details

`simData` is simulated normal data of 300 observations with 51 relevant variables and the rest of the variables being noise, with observations being in one of 3 groups. `simCount` is simulated count data of the same dimensions. `trueCluster` gives the true cluster identifications of the samples. The true clusters are each of size 100 and are in order in the columns of the data.frames.

Author(s)

Elizabeth Purdom <epurdom@stat.berkeley.edu>

Examples

```

#code used to create data:
## Not run:
nvar<-51 #multiple of 3
n<-100
x<-cbind(matrix(rnorm(n*nvar,mean=5),nrow=nvar),
             matrix(rnorm(n*nvar,mean=-5),nrow=nvar),
             matrix(rnorm(n*nvar,mean=0),nrow=nvar))
#make some of them flipped effects (better for testing if both sig under/over
#expressed variables)
geneGroup<-sample(rep(1:3,each=floor(nvar/3)))
gpIndex<-list(1:n,(n+1):(n*2),(2*n+1):(n*3))
x[geneGroup==1,]<-x[geneGroup==1,unlist(gpIndex[c(3,1,2)])]
x[geneGroup==2,]<-x[geneGroup==2,unlist(gpIndex[c(2,3,1)])]

#add in differences in variable means
smp<-sample(1:nrow(x),10)
x[smp,]<-x[smp,]+10

#make different signal y
y<-cbind(matrix(rnorm(n*nvar,mean=1),nrow=nvar),
             matrix(rnorm(n*nvar,mean=-1),nrow=nvar),
             matrix(rnorm(n*nvar,mean=0),nrow=nvar))
y<-y[,sample(1:ncol(y))]+ matrix(rnorm(3*n*nvar,sd=3),nrow=nvar)

#add together the two signals
simData<-x+y

#add pure noise variables
simData<-rbind(simData,matrix(rnorm(3*n*nvar,mean=10),nrow=nvar),
               matrix(rnorm(3*n*nvar,mean=5),nrow=nvar))
#make count data
countMean<-exp(simData/2)
simCount<-matrix(rpois(n=length(as.vector(countMean)), lambda
=as.vector(countMean)+.1),nrow=nrow(countMean),ncol=ncol(countMean))
#labels for the truth
trueCluster<-rep(c(1:3),each=n)
save(list=c("simCount","simData","trueCluster"),file="data/simData.rda")

## End(Not run)

```

subsampleClustering *Cluster subsamples of the data*

Description

Given input data, this function will subsample the samples, cluster the subsamples, and return a $n \times n$ matrix with the probability of co-occurrence.

Usage

```

## S4 method for signature 'character'
subsampleClustering(clusterFunction, ...)

```

```
## S4 method for signature 'ClusterFunction'
subsampleClustering(clusterFunction, x = NULL,
  diss = NULL, distFunction = NA, clusterArgs = NULL,
  classifyMethod = c("All", "InSample", "OutOfSample"), resamp.num = 100,
  samp.p = 0.7, ncores = 1, checkArgs = TRUE, checkDiss = TRUE,
  largeDataset = FALSE, ...)
```

Arguments

clusterFunction	a ClusterFunction object that defines the clustering routine. See ClusterFunction for required format of user-defined clustering routines. User can also give a character value to the argument clusterFunction to indicate the use of clustering routines provided in package. Type listBuiltInFunctions at command prompt to see the built-in clustering routines. If clusterFunction is missing, the default is set to "pam".
...	arguments passed to mclapply (if ncores>1).
x	the data on which to run the clustering (samples in columns).
diss	a dissimilarity matrix on which to run the clustering.
distFunction	a distance function to be applied to D. Only relevant if input is only x (a matrix of data), and diss=NULL. See details of clusterSingle for the required format of the distance function.
clusterArgs	a list of parameter arguments to be passed to the function defined in the clusterFunction slot of the ClusterFunction object. For any given ClusterFunction object, use function requiredArgs to get a list of required arguments for the object.
classifyMethod	method for determining which samples should be used in calculating the co-occurrence matrix. "All"= all samples, "OutOfSample"= those not subsampled, and "InSample"=those in the subsample. See details for explanation.
resamp.num	the number of subsamples to draw.
samp.p	the proportion of samples to sample for each subsample.
ncores	integer giving the number of cores. If ncores>1, mclapply will be called.
checkArgs	logical as to whether should give warning if arguments given that don't match clustering choices given. Otherwise, inapplicable arguments will be ignored without warning.
checkDiss	logical. Whether to check whether the input diss is valid.
largeDataset	logical indicating whether a more memory-efficient version should be used because the dataset is large. This is a beta option, and is in the process of being tested before it becomes the default.

Details

subsampleClustering is not usually called directly by the user. It is only an exported function so as to be able to clearly document the arguments for subsampleClustering which can be passed via the argument subsampleArgs in functions like [clusterSingle](#) and [clusterMany](#).

requiredArgs: The choice of "All" or "OutOfSample" for requiredArgs require the classification of arbitrary samples not originally in the clustering to clusters; this is done via the classifyFUN provided in the [ClusterFunction](#) object. If the [ClusterFunction](#) object does not have such a function to define how to classify into a cluster samples not in the subsample that created the clustering then classifyMethod must be "InSample". Note that if "All" is chosen, all samples

will be classified into clusters via the classifyFUN, not just those that are out-of-sample; this could result in different assignments to clusters for the in-sample samples than their original assignment by the clustering depending on the classification function. If you do not choose 'All', it is possible to get NAs in resulting S matrix (particularly if when not enough subsamples are taken) which can cause errors if you then pass the resulting D=1-S matrix to [mainClustering](#). For this reason the default is "All".

Our subsampling algorithm is implemented in C++ and is fast and simple but may be memory inefficient if `samp.p` is low. `largeDataset = TRUE` should be more efficient in such situations, possibly at the cost of speed. Note that this feature is experimental and should be used only by the developers.

Value

A $n \times n$ matrix of co-occurrences, i.e. a symmetric matrix with $[i,j]$ entries equal to the percentage of subsamples where the i th and j th sample were clustered into the same cluster. The percentage is only out of those subsamples where the i th and j th samples were both assigned to a clustering. If `classifyMethod=="All"`, this is all subsamples for all i,j pairs. But if `classifyMethod=="InSample"` or `classifyMethod=="OutOfSample"`, then the percentage is only taken on those subsamples where the i th and j th sample were both in or out of sample, respectively, relative to the subsample.

Examples

```
## Not run:
#takes a bit of time, not run on checks:
data(simData)
coOccur <- subsampleClustering(clusterFunction="kmeans", x=simData,
clusterArgs=list(k=3,nstart=10), resamp.n=100, samp.p=0.7)

#visualize the resulting co-occurrence matrix
plotHeatmap(coOccur)

## End(Not run)
```

transformData

Transform the original data in a ClusterExperiment object

Description

Provides the transformed data

Usage

```
## S4 method for signature 'matrixOrHDF5'
transformData(object, transFun = NULL,
  isCount = FALSE)

## S4 method for signature 'ClusterExperiment'
transformData(object, ...)

## S4 method for signature 'SingleCellExperiment'
transformData(object, ...)
```

```
## S4 method for signature 'SummarizedExperiment'
transformData(object, ...)
```

Arguments

object	a matrix, SummarizedExperiment, SingleCellExperiment or ClusterExperiment object.
transFun	a transformation function to be applied to the data. If the transformation applied to the data creates an error or NA values, then the function will throw an error. If object is of class ClusterExperiment, the stored transformation will be used and giving this parameter will result in an error.
isCount	if transFun=NULL, then isCount=TRUE will determine the transformation as defined by <code>function(x){log2(x+1)}</code> , and isCount=FALSE will give a transformation function <code>function(x){x}</code> . Ignored if transFun=NULL. If object is of class ClusterExperiment, the stored transformation will be used and giving this parameter will result in an error.
...	Values passed on the the 'matrix' method.

Details

The data matrix defined by `assay(x)` is transformed based on the transformation function either defined in `x` (in the case of a ClusterExperiment object) or by user given values for other classes.

Value

A DataFrame defined by `assay(x)` suitably transformed

Examples

```
mat <- matrix(data=rnorm(200), ncol=10)
mat[1,1] <- -1 #force a negative value
labels <- gl(5, 2)
cc <- ClusterExperiment(mat, as.numeric(labels), transformation =
function(x){x^2}) #define transformation as x^2
z<-transformData(cc)
```

Description

The main workflow of the package is made of [clusterMany](#), [combineMany](#), and [mergeClusters](#). The clusterings from these functions (and not those obtained in a different way) can be obtained with the functions documented here.

Usage

```
## S4 method for signature 'ClusterExperiment'
workflowClusters(x, iteration = 0)

## S4 method for signature 'ClusterExperiment'
workflowClusterDetails(x)

## S4 method for signature 'ClusterExperiment'
workflowClusterTable(x)

## S4 method for signature 'ClusterExperiment'
setCurrent(x, whichCluster, eraseOld = FALSE)

## S4 method for signature 'ClusterExperiment'
setToFinal(x, whichCluster, clusterLabel)
```

Arguments

x	a ClusterExperiment object.
iteration	numeric. Which iteration of the workflow should be used.
whichCluster	which cluster to set to current in the workflow
eraseOld	logical. Only relevant if input x is of class ClusterExperiment. If TRUE, will erase existing workflow results (clusterMany as well as mergeClusters and combineMany). If FALSE, existing workflow results will have "_i" added to the clusterTypes value, where i is one more than the largest such existing workflow clusterTypes.
clusterLabel	optional string value to give to cluster set to be "final"

Value

workflowClusters returns a matrix consisting of the appropriate columns of the clusterMatrix slot.

workflowClusterDetails returns a data.frame with some details on the clusterings, such as the type (e.g., 'clusterMany', 'combineMany') and iteration.

workflowClusterTable returns a table of how many of the clusterings belong to each of the following possible values: 'final', 'mergeClusters', 'combineMany' and 'clusterMany'.

setCurrent returns a ClusterExperiment object where the indicated cluster of whichCluster has been set to the most current iteration in the workflow. Pre-existing clusters are appropriately updated.

setToFinal returns a ClusterExperiment object where the indicated cluster of whichCluster has clusterType set to "final". The primaryClusterIndex is also set to this cluster, and the clusterLabel, if given.

Examples

```
data(simData)

cl <- clusterMany(simData, nReducedDims=c(5,10,50), reduceMethod="PCA",
clusterFunction="pam", ks=2:4, findBestK=c(FALSE), removeSil=TRUE,
subsample=FALSE)
```

```
clCommon <- combineMany(cl, whichClusters="workflow", proportion=0.7,  
minSize=10)  
  
clCommon <- makeDendrogram(clCommon)  
  
clMerged <- mergeClusters(clCommon,mergeMethod="adjP")  
  
head(workflowClusters(clMerged))  
workflowClusterDetails(clMerged)  
workflowClusterTable(clMerged)
```

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