# Package ‘clusterExperiment’

March 22, 2017

**Title**  Compare clusterings for single-cell sequencing

**Version**  1.0.0

**Description**  This package provides functions for running and comparing many different clusterings of single-cell sequencing data.

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**BugReports**  https://github.com/epurdom/clusterExperiment/issues

**License**  Artistic-2.0

**Depends**  R (>= 3.3), methods, SummarizedExperiment

**Imports**  NMF, RColorBrewer, ape, phylobase, cluster, stats, limma, dendextend, howmany, locfdr, matrixStats, graphics, parallel

**Suggests**  BiocStyle, knitr, diagram, testthat, scRNAseq

**VignetteBuilder**  knitr

**LazyData**  true

**RoxygenNote**  5.0.1

**biocViews**  Clustering, RNASeq, Sequencing, Software

**NeedsCompilation**  no

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Description

These functions are used to add or remove clusters to a `ClusterExperiment` object.

Usage

```r
## S4 method for signature 'ClusterExperiment,matrix'
addClusters(x, y, clusterTypes = "User")

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

## S4 method for signature 'ClusterExperiment,numeric'
addClusters(x, y, ...)

## S4 method for signature 'ClusterExperiment,character'
removeClusters(x, whichRemove, exactMatch = TRUE)

## S4 method for signature 'ClusterExperiment,numeric'
removeClusters(x, whichRemove)

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

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.

- **...**
  - Passed to signature `ClusterExperiment,matrix`.

- **whichRemove**
  - which clusters to remove. Can be numeric or character. If numeric, must give indices of `clusterMatrix(x)` to remove. If character, should match a `clusterTypes` of x.
clusterContrasts, ClusterExperiment-method

Arguments

exactMatch logical. Whether whichRemove must exactly match a value of clusterTypes(x). Only relevant if whichRemove is character.

Details

addClusters 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.

removeClusters removes the clusters given by whichRemove. If all clusters are implied, then returns a SummarizedExperiment object. If the primaryCluster is one of the clusters removed, the primaryClusterIndex is set to 1 and the dendrogram and cooccurrence matrix are discarded and orderSamples is set to 1:NCOL(x).

removeUnclassified 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)).

Value

A ClusterExperiment object with the added clusters.

Examples

data(simData)

c1 <- clusterSingle(simData, clusterFunction="pam", subsample=FALSE, sequential=FALSE, clusterDArgs=list(k=3))

c2 <- clusterSingle(simData, clusterFunction="pam", subsample=FALSE, sequential=FALSE, clusterDArgs=list(k=5))

addClusters(c1, c2)

clusterContrasts, ClusterExperiment-method

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 = "limma", 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.
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 only option is the format appropriate for limma package, but we anticipate adding more.
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.
• contrastNames A vector of names for each of the contrasts. NULL if no such additional names.

Author(s)

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Examples

data(simData)
cl <- clusterMany(simData,nPCADims=c(5,10,50), dimReduce="PCA", clusterFunction="pam", ks=2:4, findBestK=c(FALSE), removeSil=TRUE, subsample=FALSE)
#Pairs:
clusterContrasts(cl,contrastType="Pairs")
#Dendrogram
clusterD

cl<-makeDendrogram(cl)
clusterContrasts(cl,contrastType="Pairs")

---

### Description

Given a n x n matrix of distances, these functions will try to find the clusters based on the given clustering function. cluster01 and clusterK are internal functions and clusterD is a wrapper around these two functions for easier user interface. cluster01 and clusterK are not expected to be called directly by the user, except for ease in debugging user-defined clustering functions.

### Usage

```r
clusterD(x = NULL, diss = NULL, clusterFunction = c("hierarchical01", "tight", "pam", "hierarchicalK"), typeAlg = c("01", "K"), distFunction = NA, minSize = 1, orderBy = c("size", "best"), format = c("vector", "list"), clusterArgs = NULL, checkArgs = TRUE, returnD = FALSE, ...)  
cluster01(diss, clusterFunction = c("hierarchical01", "tight"), alpha = 0.1, clusterArgs = NULL, checkArgs)  
clusterK(diss, clusterFunction = c("pam", "hierarchicalK"), findBestK = FALSE, k, kRange, removeSil = FALSE, silCutoff = 0, clusterArgs = NULL, checkArgs)
```

### 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.
- **clusterFunction**: a function that clusters a nxn matrix of dissimilarities/distances. Can also be given character values to indicate use of internal wrapper functions for default methods. See Details for the format of what the function must take as arguments and what format the function must return.
- **typeAlg**: character value of either '01' or 'K' determining whether the function given in clusterFunction should be called by clusterK or cluster01. Only used if clusterFunction is a user-defined function. Otherwise, for methods provided by the package (i.e. by user setting clusterFunction to a character value) clusterD will determine the appropriate input for 'typeAlg' and will ignore user input.
- **distFunction**: a distance function to be applied to D. Only relevant if input D is a matrix of data, rather than a distance. See details.
- **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).
clusterD

- **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.

- **clusterArgs** arguments to be passed directly to the clusterFunction, beyond the required input.

- **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.

- **returnD** logical as to whether to return the D matrix in output.

- **...** arguments given to clusterD to be passed to cluster01 or clusterK (depending on the value of typeAlg). Examples include 'k' for clusterK or 'alpha' for cluster01. These should not be the arguments needed by clusterFunction (which should be passed via the argument 'clusterArgs') but the actual arguments of cluster01 or clusterK.

- **alpha** a cutoff value of how much similarity needed for drawing blocks (lower values more strict).

- **findBestK** logical, whether should find best K based on average silhouette width (only used if clusterFunction of type "K").

- **k** single value to be used to determine how many clusters to find, if findBestK=FALSE (only used if clusterFunction of type "K").

- **kRange** vector of integers. If findBestK=TRUE, this gives the range of k’s to look over. Default is k-2 to k+20, subject to those values being greater than 2. 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 when silhouette < silCutoff (only used if clusterFunction of type "K")

- **silCutoff** Requirement on minimum silhouette width to be included in cluster (only if removeSil=TRUE).

**Details**

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-cor(x))/2.

Types of algorithms: cluster01 is for clustering functions that expect as an input D that takes on 0-1 values (e.g. from subclustering). clusterK is for clustering functions that require an input k, the number of clusters, but arbitrary distance/dissimilarity matrix. cluster01 and clusterK are given as separate functions in order to allow the user to provide different clustering functions that expect different types of input and for us to provide different shared processing of the results that is different for these different types of clustering methods (for example, removing low silhouette values is appropriate for clusterK clustering functions rather than cluster01 functions). It is also generally expected that cluster01 algorithms use the 0-1 nature of the input to set criteria as to where to find clusters and therefore do not need a pre-determined 'k'. On the other hand, clusterK functions are assumed to need a predetermined 'k' and are also assumed to cluster all samples to a cluster, and therefore clusterK gives options to exclude poorly clustered samples via silhouette distances.
clusterD

cluster01 required format for input and output for clusterFunction: clusterFunction should be a function that takes (as a minimum) an argument "D" and "alpha". 0-1 clustering algorithms are expected to use the fact that the D input is 0-1 range to find the clusters, rather than a user defined number of clusters; "alpha" is the parameter that tunes the finding of such clusters. For example, a candidate block of samples might be considered a cluster if all values of D are greater than or equal to 1-alpha. The output is a list with each element corresponding to a cluster and the elements of the list corresponding to the indices of the samples that are in the cluster. The list is expected to be in order of 'best clusters' (as defined by the clusterFunction), with first being the best and last being worst.

cluster01 methods: "tight" method refers to the method of finding clusters from a subsampling matrix given internally in the tight algorithm code of Tsang and Wong. Arguments for the tight method are 'minSize.core' (default=2), which sets the miniumum number of samples that form a core cluster. "hierarchical01" refers to running the hclust algorithm on D and 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 'hierarchical' 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. Arguments of hclust can also be passed via clusterArgs to control the hierarchical clustering of D.

clusterK required format for input and output for clusterFunction: clusterFunction should be a function that takes as a minimum an argument 'D' and 'k'. The output must be a clustering, specified by integer values. The function silhouette will be used on the clustering to calculate silhouette scores for each observation.

clusterK methods: "pam" performs pam clustering on the input \( D \) matrix using pam in the cluster package. Arguments to pam can be passed via 'clusterArgs', except for the arguments 'x' and 'k' which are given by D and k directly. "hierarchicalK" performs hierarchical clustering on the input via the hclust and then applies cutree with the specified k to obtain clusters. Arguments to hclust can be passed via clusterArgs.

Value

clusterD 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. If orderBy="size" the clusters are reordered by the size of the cluster, instead of by the internal ordering of the clusterFunction.

cluster01 and clusterK return a list of indices of the clusters found, which each element of the list corresponding to a cluster and the elements of that list a vector of indices giving the indices of the samples assigned to that cluster. Indices not included in any list are assumed to have not been clustered. The list is assumed to be ordered in terms of the 'best' cluster (as defined by the clusterFunction for cluster01 or by average silhouette for clusterK), for example in terms of most internal similarity of the elements, or average silhouette width.

Examples

data(simData)
c11<-clusterD(simData,clusterFunction="pam",k=3)
c12<-clusterD(simData,clusterFunction="hierarchical01")
c13<-clusterD(simData,clusterFunction="tight")
#change distance to manhattan distance
c14<-clusterD(simData,clusterFunction="pam",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 <- clusterD(simData, clusterFunction="hierarchical01", alpha=0.1,
  minSize=5, clusterArgs=list(evalClusterMethod="average", method="single"))

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

# two twists to pam
clustSubPamK <- clusterD(simData, clusterFunction="pam", silCutoff=0, minSize=5,
  removeSil=TRUE, k=3)
clustSubPamBestK <- clusterD(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 <- clusterD(simData, clusterFunction="tight", alpha=0.1,
  minSize=5, removeSil=TRUE)
clustSubTight_test2 <- clusterD(simData, clusterFunction="tight", alpha=0.1,
  clusterArgs=list(evalClusterMethod="average"))

ClusterExperiment-class

Class ClusterExperiment

Description

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

In addition to the slots of the SummarizedExperiment 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(se, clusters, ...)

## S4 method for signature 'matrix,ANY'
clusterExperiment(se, clusters, ...)

## S4 method for signature 'SummarizedExperiment,numeric'
clusterExperiment(se, clusters, ...)

## S4 method for signature 'SummarizedExperiment,character'
clusterExperiment(se, clusters, ...)

## S4 method for signature 'SummarizedExperiment,factor'
clusterExperiment(se, clusters, ...)

## S4 method for signature 'SummarizedExperiment,matrix'
clusterExperiment(se, clusters,
  transformation, primaryIndex = 1, clusterTypes = "User",
  clusterInfo = NULL, orderSamples = 1:ncol(se), dendro_samples = NULL,
  dendro_index = NA_real_, dendro_clusters = NULL, coClustering = NULL)

Arguments

se
  a matrix or SummarizedExperiment containing the data to be 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 SummarizedExperiment,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).

coclustering
  matrix. Sets the ‘coclustering’ slot (see Slots).

Details

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

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.

- 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.

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

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

c <- clusterExperiment(se, 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,ANY,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 method for signature 'ClusterExperiment'
 nClusters(x)

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

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

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

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

## S4 method for signature 'ClusterExperiment'
 primaryClusterIndex(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'
 clusterInfo(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

### Arguments

- **x, object**  
  a ClusterExperiment object.
- **..., i, j, drop**  
  Forwarded to the SummarizedExperiment method.
- **value**  
  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.

### Details

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

### 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.
- **nClusters** returns the number of clusterings (i.e., ncol of clusterMatrix).
- **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.
- **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).
- **coClustering** returns/sets the co-clustering matrix.
- **clusterTypes** returns/sets the clusterTypes slot.
- **clusterInfo** returns the clusterInfo slot.
- **clusterLabels** returns/sets the column names of the clusterMatrix slot.
- **clusterLegend** returns/sets the clusterLegend slot.
Create a matrix of clustering across values of parameters

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

```r
## S4 method for signature 'matrix'
clusterMany(x, dimReduce = "none", nVarDims = NA,
          nPCADims = NA, transFun = NULL, isCount = FALSE, ...)

## S4 method for signature 'list'
clusterMany(x, ks = NA, clusterFunction, alphas = 0.1,
            findBestK = FALSE, sequential = FALSE, removeSil = FALSE,
            subsample = FALSE, silCutoff = 0, distFunction = NA, betas = 0.9,
            minSizes = 1, verbose = FALSE, clusterDArgs = NULL,
            subsampleArgs = NULL, seqArgs = NULL, ncores = 1, random.seed = NULL,
            run = TRUE, ...)

## S4 method for signature 'ClusterExperiment'
clusterMany(x, dimReduce = "none",
            nVarDims = NA, nPCADims = NA, eraseOld = FALSE, ...)

## S4 method for signature 'SummarizedExperiment'
clusterMany(x, dimReduce = "none",
            nVarDims = NA, nPCADims = NA, transFun = NULL, isCount = FALSE, ...)
```

Arguments

- **x**: the data on which to run the clustering. Can be: matrix (with genes in rows), a list of datasets over which the clusterings should be run, a SummarizedExperiment object, or a ClusterExperiment object.
- **dimReduce**: character A character identifying what type of dimensionality reduction to perform before clustering. Options are "none", "PCA", "var", "cv", and "mad". See `transform` for more details.
- **nVarDims**: vector of the number of the most variable features to keep (when "var", "cv", or "mad" is identified in `dimReduce`). If NA is included, then the full dataset will also be included.
- **nPCADims**: vector of the number of PCs to use (when 'PCA' is identified in `dimReduce`). If NA is included, then the full dataset will also be included.
- **transFun**: function A function to use to transform the input data matrix before clustering.
- **isCount**: logical. Whether the data are in counts, in which case the default `transFun` argument is set as log2(x+1). This is simply a convenience to the user, and can be overridden by giving an explicit function to `transFun`. 
... 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.

ks the range of k values (see details for meaning for different choices).

clusterFunction function used for the clustering. Note that unlike in \texttt{clusterSingle}, this must be a character vector of pre-defined clustering techniques provided by \texttt{clusterSingle}, and can not be a user-defined function. Current functions are "tight", "hierarchical01", "hierarchicalK", and "pam"

alphas values of alpha to be tried. Only used for clusterFunctions of type '01' (either 'tight' or 'hierarchical01'). Determines tightness required in creating clusters from the dissimilarity matrix. Takes on values in \([0,1]\). See \texttt{clusterD}.

findBestK logical, whether should find best K based on average silhouette width (only used if clusterFunction of type "K").

sequential logical whether to use the sequential strategy (see details of seqCluster)

removeSil logical as to whether remove when silhouette < silCutoff (only used if clusterFunction of type "K")

subsample logical as to whether to subsample via \texttt{subsampleClustering} to get the distance matrix at each iteration; otherwise the distance function will be determined by argument distFunction passed in clusterDArgs.

silCutoff Requirement on minimum silhouette width to be included in cluster (only if removeSil=TRUE).

distFunction a vector of character strings that are the names of distance functions found in the global environment. See the help pages of \texttt{clusterD} 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 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 \texttt{seqCluster}.

minSizes the minimum size required for a cluster (in \texttt{clusterD}). Clusters smaller than this are not kept and samples are left unassigned.

verbose logical. If TRUE it will print informative messages.

clusterDArgs list of additional arguments to be passed to \texttt{clusterD}.

subsampleArgs list of arguments to be passed to \texttt{subsampleClustering}.

seqArgs list of additional arguments to be passed to \texttt{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

While the function allows for multiple values of clusterFunction, the code does not reuse the same subsampling matrix and try different clusterFunctions on it. If sequential=TRUE, different subsampleClusterFunctions 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-occurence 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 set in both subsampleArgs[["k"]], and clusterDArgs[["k"]], that are passed to clusterD and subsampleClustering. This passing of these arguments via subsampleArgs[["k"]], will only have an effect if 'subsample=TRUE'. Similarly, the passing of clusterDArgs[["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 clusterD unless kRange is specified by the user via the clusterDArgs; note this means that the default option of setting kRange that depends on the input k (see clusterD) is not available in clusterMany.

If the input is a ClusterExperiment object, currently existing orderSamples, coClustering or dendrogram slots will be retained.

Value

If run=TRUE and the input is either a matrix, a SummarizedExperiment object, or a ClusterExperiment object, 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=FALSE and the input is a list of data sets, a list with the following objects:

- clMat 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 clMat
- clusterDArgs a list of (possibly modified) arguments to clusterDArgs
- 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, nPCADims=c(5,10,50), dimReduce="PCA",
clusterFunction="pam",
ks=2:4, findBestK=c(TRUE, FALSE), removeSil=c(TRUE, FALSE), run=FALSE)
print(head(checkParams$paramMatrix))

#Now actually run it
c1 <- clusterMany(simData, nPCADims=c(5,10,50), dimReduce="PCA",
clusterFunction="pam", ks=2:4, findBestK=c(TRUE, FALSE), removeSil=c(TRUE, FALSE))
print(c1)
head(colnames(clusterMatrix(c1)))

#make names shorter for plotting
c1Mat <- clusterMatrix(c1)
colnames(c1Mat) <- gsub("TRUE", "T", colnames(c1Mat))
colnames(c1Mat) <- gsub("FALSE", "F", colnames(c1Mat))
colnames(c1Mat) <- gsub("k=NA,", "", colnames(c1Mat))
par(mar=c(2, 10, 1, 1))
plotClusters(c1Mat, axisLine=-2)

## 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",
clusterDArgs=list(minSize=5, kRange=2:15), ncores=1, random.seed=48120))

## End(Not run)

clusterSingle

General wrapper method to cluster the data

Description

Given a data matrix, SummarizedExperiment, or ClusterExperiment object, this function will
find clusters, based on a single specification of parameters.

Usage

## S4 method for signature 'matrixOrMissing, matrixOrMissing'
cclusterSingle(x, diss,
subsample = TRUE, sequential = FALSE, clusterFunction = c("tight", "hierarchical01", "pam", "hierarchicalK"), clusterDArgs = NULL,
subsampleArgs = NULL, seqArgs = NULL, isCount = FALSE,
transFun = NULL, dimReduce = c("none", "PCA", "var", "cv", "mad"),
ndims = NA, clusterLabel = "clusterSingle")

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

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

Arguments

x  the data on which to run the clustering (features in rows).
diss n x n data matrix of dissimilarities between the samples on which to run the clustering (only if 
    subsample=FALSE)
subsample logical as to whether to subsample via subsampleClustering to get the distance matrix at each iteration; otherwise the distance function will be determined by argument distFunction passed in clusterDArgs.
sequential logical whether to use the sequential strategy (see details of seqCluster).
clusterFunction passed to clusterD option 'clusterFunction' to indicate method of clustering, see clusterD.
clusterDArgs list of additional arguments to be passed to clusterD.
subsampleArgs list of arguments to be passed to subsampleClustering.
seqArgs list of additional arguments to be passed to seqCluster.
isCount logical. Whether the data are in counts, in which case the default transFun argument is set as log2(x+1). This is simply a convenience to the user, and can be overridden by giving an explicit function to transFun.
transFun function A function to use to transform the input data matrix before clustering.
dimReduce character A character identifying what type of dimensionality reduction to perform before clustering. Options are "none","PCA", "var","cv", and "mad". See transform for more details.
ndims integer An integer identifying how many dimensions to reduce to in the reduction specified by dimReduce
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 clusterSingle.
... arguments to be passed on to the method for signature matrix.

Details

If sequential=TRUE, the sequential clustering controls the 'k' argument of the underlying clustering so setting 'k=' in the list given to clusterDArgs or subsampleArgs will not do anything and will produce a warning to that effect.

Value

A ClusterExperiment object.

See Also

clusterMany to compare multiple choices of parameters.
Examples

data(simData)

## Not run:
# following code takes some time.
# use clusterSingle to do sequential clustering
# (same as example in seqCluster only using clusterSingle ...)
set.seed(44261)
clustSeqHier_v2 <- clusterSingle(simData, clusterFunction="hierarchical01",
sequential=TRUE, subsample=TRUE, subsampleArgs=list(resamp.n=100, samp.p=0.7,
clusterFunction="kmeans", clusterArgs=list(nstart=10)),
seqArgs=list(beta=0.8, k0=5), clusterDArgs=list(minSize=5))

## End(Not run)

# use clusterSingle to do just clustering k=3 with no subsampling
clustNothing <- clusterSingle(simData, clusterFunction="pam",
subsample=FALSE, sequential=FALSE, clusterDArgs=list(k=3))

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 = 1,
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 clusterD via alpha = 1 - proportion)
clusterFunction

the clustering to use (passed to clusterD); 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 proportion < 1)

minSize

minimum size required for a set of samples to be considered in a cluster because of shared clustering, passed to clusterD

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

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 combineMany. However, a more informative label can be set (see vignette).

... arguments to be passed on to the method for signature matrix,missing.

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 clusterD 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 clusterD on the proportion matrix with clusterFunction as the 01 clustering algorithm, alpha=1-proportion, minSize=minSize, and evalClusterMethod=c("average"). See help of clusterD 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 clusterD 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,nPCADims=c(5,10,50), dimReduce="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)

---

**convertClusterLegend**  
*Convert clusterLegend into useful formats*

**Description**

Function for converting the information stored in the clusterLegend slot into other useful formats.

**Usage**

```r
## S4 method for signature 'ClusterExperiment'
convertClusterLegend(object, 
  output = c("plotAndLegend", "aheatmapFormat", "matrixNames", 
             "matrixColors"))
```

**Arguments**

- `object`  
  a ClusterExperiment object.
- `output`  
  character value, indicating desired type of conversion.

**Details**

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

**Value**

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.
getBestFeatures, matrix-method

- "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.

getBestFeatures, matrix-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 'matrix'
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.
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').
getBestFeatures, matrix-method

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 log2(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(FPKM+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`
getBestFeatures, .matrix-method

- **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.

**Examples**

data(simData)

# create a clustering, for 8 clusters (truth was 4)
c1 <- clusterSingle(simData, clusterFunction="pam", subsample=FALSE, sequential=FALSE, clusterDArgs=list(k=8))

# basic F test, return all, even if not significant:
testF <- getBestFeatures(c1, contrastType="F", number=nrow(simData), isCount=FALSE)

# Do all pairwise, only return significant, try different adjustments:
pairsPerC <- getBestFeatures(c1, contrastType="Pairs", contrastAdj="PerContrast", p.value=0.05, isCount=FALSE)
pairsAfterF <- getBestFeatures(c1, contrastType="Pairs", contrastAdj="AfterF", p.value=0.05, isCount=FALSE)
pairsAll <- getBestFeatures(c1, 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(c1, 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")
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", dimReduce = c("none", "PCA", "var", "cv", "mad"), ndims = NA, ignoreUnassignedVar = FALSE, unassignedSamples = c("outgroup", "cluster"), ...)

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

### S4 method for signature 'ClusterExperiment'
plotDendrogram(x, leaves = c("clusters", "samples"), clusterNames = TRUE, main, sub, ...)

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.

dimReduce character A character identifying what type of dimensionality reduction to perform before clustering. Options are "none", "PCA", "var", "cv", and "mad". See transform for more details.

ndims integer An integer identifying how many dimensions to reduce to in the reduction specified by dimReduce

ignoreUnassignedVar logical indicating whether dimensionality reduction via top feature variability (i.e. 'var', 'cv', 'mad') should ignore unassigned samples in the primary clustering for calculation of the top features.

unassignedSamples how to handle unassigned samples("-1") ; only relevant for sample clustering. See details.

... for makeDendrogram, if signature matrix, arguments passed to hclust; if signature ClusterExperiment passed to the method for signature matrix. For plotDendrogram, passed to plot.dendrogram.

cluster A numeric vector with cluster assignments. If x is a ClusterExperiment object, cluster is automatically the primaryCluster(x). "-1" indicates the sample was not assigned to a cluster.

leaves if "samples" the dendrogram has one leaf per sample, otherwise it has one per cluster.
mergeClusters, matrix-method

clusterNames logical. If leaves="clusters", then clusters will be identified with their 'name' value in legend; otherwise the 'clusterIds' value will be used.

main passed to the plot function.

sub passed to the plot function.

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 leaves="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

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.

Examples

data(simData)

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

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

mergeClusters, matrix-method

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

```r
## S4 method for signature 'matrix'
mergeClusters(x, cl, dendro = NULL,
  mergeMethod = c("none", "adjP", "locfdr", "MB", "JC"),
  plotType = c("none", "all", "mergeMethod", "adjP", "locfdr", "MB", "JC"),
  cutoff = 0.1, doPlot = TRUE, isCount = TRUE, ...)

## S4 method for signature 'ClusterExperiment'
mergeClusters(x, eraseOld = FALSE,
  isCount = FALSE, mergeMethod = "none", plotType = "all",
  clusterLabel = "mergeClusters", ...)
```

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; The default for matrix (NULL) is to recalculate it with the given (x, cl) pair. If x is a `ClusterExperiment` object, the dendrogram in the slot dendro_clusters will be used. 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.
- `plotType`: what type of plotting of dendrogram. If 'all', then all the estimates of proportion non-null will be plotted; if 'mergeMethod', then only the value used in the merging is plotted for each node.
- `cutoff`: minimimum 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.
- `doPlot`: logical as to whether to plot the dendrogram (overrides plotType value). Mainly used for internal coding purposes.
- `isCount`: logical as to whether input data is a count matrix. See details.
- `...`: for signature matrix, arguments passed to the `plot.phylo` function of ade4 that plots the dendrogram. For signature `ClusterExperiment` arguments passed to the method for signature matrix.
- `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`: a string used to describe the type of clustering. By default it is equal to "mergeClusters", to indicate that this clustering is the result of a call to mergeClusters.

Details

If `isCount=TRUE`, and the input is a matrix, log2(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 log2(1+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 transform(x) will be given to the input and will be used for both 
makeDendrogram and getBestFeatures, with no voom correction.

"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 Buhlmann (2005) and is implemented in the package howmany. "adjP" refers to the proportion of genes that are found significant based on a FDR ad-
justed p-values (method "BH") and a cutoff of 0.05.

If mergeMethod is not equal to 'none' then the plotting will indicate where the clusters will be 
merged (assuming plotType is not 'none').

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.

• propDE A table of the proportions that are DE on each node.

• originalClusterDendro The dendrogram on which the merging was based (based on the 
original clustering).

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.

Examples

data(simData)

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

#make dendrogram
cl <- makeDendrogram(cl)

#merge clusters with plotting. Note argument 'use.edge.length' can improve 
#readability
merged <- mergeClusters(cl, plot=TRUE, plotType="all", 
mergeMethod="adjP", use.edge.length=FALSE)

#compare merged to original
table(primaryCluster(cl), primaryCluster(merged))

---

plotClusters,ClusterExperiment,character-method

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(clusters, 
  whichClusters = c("workflow", "all"), ...)

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

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

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

Arguments

clusters 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; 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 give new colors for the remaining (not implemented yet). '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. If clusters is a ClusterExperiment object, sampleData must be either an index or a character vector that references a column or column name, respectively, of the colData slot of the ClusterExperiment object.
**plotClusters**, ClusterExperiment, character-method

**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 proceeding row (some of the colors at the end of bigPalette are a bit wonky, and so if you have a large clusters matrix, this can be useful).

**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).

**clNames**  names to go with the columns (clusterings) in matrix `colorMat`.

**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.

- `index` a vector of length equal to `ncols(clusters)` giving the order of the columns 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[index, ]` 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 matrix is 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.

Author(s)

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

See Also

The ConsensusClusterPlus package.

Examples

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

cl <- clusterMany(simData, nPCADims=c(5, 10, 50), dimReduce="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]
The code snippet demonstrates how to manipulate clusterings in a SummarizedExperiment object using the `plotClusters` function. It shows how to change the order of clusterings, switch colors, and manipulate cluster assignments. The `plotHeatmap` function is used for visualizing the clustering results.

The `plotHeatmap` function, available in the `SummarizedExperiment` package, is used to create heatmaps for visualizing clustering results and more. It allows for showing clustering results with a heat map, using color scales from one matrix and hierarchical clustering of samples/features from another. This function is built on the `aheatmap` function of the `NMF` package.
Usage

```r
## S4 method for signature 'SummarizedExperiment'
plotHeatmap(data, isCount = FALSE,
             transFun = NULL, ...) # S4 method for signature 'ClusterExperiment'
plotHeatmap(data,
             clusterSamplesData = c("hclust", "dendrogramValue", "orderSamplesValue",
                                    "primaryCluster"),
             clusterFeaturesData = c("var", "all", "PCA"),
                                    nFeatures = NULL, visualizeData = c("transformed", "centeredAndScaled",
                                               "original"),
                                           whichClusters = c("primary", "workflow", "all", "none"),
                                           sampleData = NULL, clusterFeatures = TRUE, colorScale, ...)

## S4 method for signature 'matrix'
plotHeatmap(data, sampleData = NULL,
             clusterSamplesData = NULL, clusterFeaturesData = NULL,
             whSampleDataCont = NULL, clusterSamples = TRUE, showSampleNames = FALSE,
             clusterFeatures = TRUE, showFeatureNames = FALSE, colorScale = seqPal5,
             clusterLegend = NULL, alignSampleData = FALSE,
             unassignedColor = "white", missingColor = "grey", breaks = NA,
             isSymmetric = FALSE, overRideClusterLimit = FALSE, ...) # S4 method for signature 'ClusterExperiment'
plotCoClustering(data,
                 invert = ifelse(!is.null(data@coClustering) && all(diag(data@coClustering)
                                        == 0), TRUE, FALSE), ...)```

Arguments

data: data to use to determine the heatmap. Can be a matrix, `ClusterExperiment` or `SummarizedExperiment` object. The interpretation of parameters depends on the type of the input.

isCount: logical. Whether the data are in counts, in which case the default `transFun` argument is set as `log2(x+1)`. This is simply a convenience to the user, and can be overridden by giving an explicit function to `transFun`.

transFun: function A function to use to transform the input data matrix before clustering.

... for signature `matrix`, arguments passed to `aheatmap`. For the other signatures, passed to the method for signature `matrix`. Not all arguments can be passed to `aheatmap` effectively, see details.

clusterSamplesData: If data is a matrix, either a matrix that will be used to in `hclust` to define the hierarchical clustering of samples (e.g. normalized data) or a pre-existing dendrogram that clusters the samples. If data is a `ClusterExperiment` object, the input should be either character or integers. Indicates how (and whether) the samples should be clustered (or gives indices of the order for the samples). See details.

clusterFeaturesData: If data is a matrix, either a matrix that will be used in `hclust` to define the hierarchical clustering of features (e.g. normalized data) or a pre-existing dendrogram that clusters the features. If data is a `ClusterExperiment` object, the input should be either character or integers indicating which features should be used (see details).
plotHeatmap, SummarizedExperiment-method

nFeatures integer indicating how many features should be used (if clusterFeaturesData is 'var' or 'PCA').

visualizeData 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 data.frame/matrix with same dimensions of assay(data).

whichClusters character string, or vector of characters or integers, indicating what clusters should be visualized with the heatmap.

sampleData If input is either a ClusterExperiment or SummarizedExperiment object, then sampleData must index the sampleData stored as a DataFrame in colData slot of the object. Whether that data is continuous or not will be determined by the properties of colData (no user input is needed). If input is matrix, sampleData is a matrix of additional data on the samples to show above heatmap. Unless indicated by whSampleDataCont, sampleData will be converted into factors, even if numeric. ‘-1’ indicates the sample was not assigned to a cluster and gets color ‘unassignedColor’ and ‘-2’ gets the color ‘missingColor’.

classFeatures Logical as to whether to do hierarchical clustering of features (if FALSE, any input to clusterFeaturesData is ignored).

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.

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. 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 a 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 ClusterExperiment.

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

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.

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 missing, a new one will be created based on the primaryCluster of data. If equal to "hclust", then standard hierarchical clustering of the transformed data will be used. If 'orderSamplesValue' no clustering of the samples will be done, and instead the samples will be ordered as in the slot orderSamples of data. If 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. If not one of these values, clusterSamplesData can be a character vector matching the clusterLabels (colnames of clusterMatrix).

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 consider continuous. Otherwise the annotation data for sampleData will be forced into a factor (which will be nonsensical for continous data). If data is a ClusterExperiment object, sampleData should refer to a 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. "var" selects the nFeatures most variable genes (based on transformation(assay(data))); "PCA" results in a heatmap of the top nFeatures PCAs of the transformation(assay(data)). clusterFeaturesData can also be a vector of characters or integers, indicating the rownames or indices respectively of assay(data) that should be shown. For all of these options, the features are clustered based on the visualizeData
Finally, in the `ClusterExperiment` version of `plotHeatmap`, `clusterFeaturesData` can be a list of indices or rownames, indicating 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...).

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

**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,
plottingFunctions

Various functions useful for plotting

Description
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)

showBigPalette(wh = NULL)

setBreaks(breaks, data)

bigPalette
showHeatmapPalettes()

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.)
- **wh**  
  numeric. Which colors to plot. Must be a numeric vector with values between 1 and 62.
- **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.

Format

An object of class character of length 60.

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.
- **bigPalette** is a long palette of colors (length 62) used by **plotClusters** and accompanying functions. **showBigPalette** creates plot that gives index of each color in bigPalette.
- **showBigPalette** will plot the **bigPalette** functions with their labels and index.
- **setBreaks** gives a set of breaks (of length 52) equally spaced between the boundaries of the data.
- **seqPal1-seqPal4** 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 unique. However, they can be given to the **labRow** argument of **aheatmap** or **plotHeatmap**.
Examples

data(simData)

x <- makeBlankData(simData[,1:10], groupsOfFeatures=list(c(5, 2, 3), c(20, 34, 25)))
showBigPalette()
setBreaks(.9,simData)

#show the palette colors
showHeatmapPalettes()

#compare the palettes on heatmap
cl <- clusterSingle(simData, clusterFunction="pam", subsample=FALSE, sequential=FALSE, clusterDArgs=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)

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 'matrix'
RSEC(x, isCount = FALSE, transFun = NULL, dimReduce = "PCA", nVarDims = NA, nPCADims = c(50), k0s = 4:15, clusterFunction = c("tight", "hierarchical01"), alphas = c(0.1, 0.2, 0.3), betas = 0.9, minSizes = 1, combineProportion = 0.7, combineMinSize = 5, dendroReduce = "mad", dendroNDims = 1000, mergeMethod = "adjp", mergeCutoff = 0.05, verbose = FALSE, clusterDArgs = NULL, subsampleArgs = NULL, seqArgs = NULL, ncores = 1, random.seed = NULL, run = TRUE)

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

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

x  the data on which to run the clustering. Can be: matrix (with genes in rows), a list of datasets over which the clusterings should be run, a SummarizedExperiment object, or a ClusterExperiment object.

isCount  logical. Whether the data are in counts, in which case the default transFun argument is set as \( \log_2(x+1) \). This is simply a convenience to the user, and can be overridden by giving an explicit function to transFun.

transFun  function A function to use to transform the input data matrix before clustering.

dimReduce  character A character identifying what type of dimensionality reduction to perform before clustering. Options are "none", "PCA", "var", "cv", and "mad". See transform for more details.

nVarDims  vector of the number of the most variable features to keep (when "var", "cv", or "mad" is identified in dimReduce). If NA is included, then the full dataset will also be included.

nPCADims  vector of the number of PCs to use (when 'PCA' is identified in dimReduce). If NA is included, then the full dataset will also be included.

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 provided by clusterSingle, and can not be a user-defined function. Current functions are "tight", "hierarchical01", "hierarchicalK", and "pam"

alphas  values of alpha to be tried. Only used for clusterFunctions of type '01' (either 'tight' or 'hierarchical01'). Determines tightness required in creating clusters from the dissimilarity matrix. Takes on values in \([0,1]\). See clusterD.

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 seqCluster.

minSizes  the minimum size required for a cluster (in clusterD). 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 dimReduce in makeDendrogram

dendroNDims  passed to ndims in makeDendrogram

mergeMethod  passed to mergeMethod in mergeClusters

mergeCutoff  passed to cutoff in mergeClusters

verbose  logical. If TRUE it will print informative messages.

clusterDArgs  list of additional arguments to be passed to clusterD.

subsampleArgs  list of arguments to be passed to subsampleClustering.

seqArgs  list of additional arguments to be passed to seqCluster.

ncore  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 'ncore' should NOT be passed via subsampleArgs; instead set the argument 'ncore' 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.

### seqCluster

**Program for sequentially clustering, removing cluster, and starting again.**

**Description**

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

**Usage**

```r
seqCluster(x = NULL, diss = NULL, k0, clusterFunction = c("tight", "hierarchical01", "pam", "hierarchicalK"), subsample = TRUE, beta = 0.7, top.can = 15, remain.n = 30, k.min = 3, k.max = k0 + 10, verbose = TRUE, subsampleArgs = NULL, clusterDArgs = NULL)
```

**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.

- **clusterFunction**
  passed to clusterDMat option 'clusterFunction' to indicate method of clustering, see `clusterD`.

- **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 `clusterD`.

- **beta**
  value between 0 and 1 to decide how stable clustership membership has to be before 'finding' and removing the cluster.

- **top.can**
  only the top.can clusters from `clusterD` (ranked by 'orderBy' argument given to `clusterD`) 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. Current default is fairly large, so probably will have little effect.
seqCluster

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.

clusterDArgs list of arguments to be passed to clusterD (which can include arguments to be passed to clusterK).

Details

This code is adapted from the code of the tightClust package of Tseng and Wong

Each iteration of the algorithm will cluster the current set of samples. Depending on the method, the number of clusters resulting from clusterD may not be equal to the K used in the clustering of the (subsampled) data. The resulting clusters will then be compared to clusters found in the previous iteration that set the subsampling clustering to 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 a cluster in the current iteration that has overlap similarity > beta to a cluster in the previous iteration, then the cluster with the largest such similarity will be identified as a 'final' cluster and the samples in it will be removed for future iterations. The algorithm will then continue to the next iteration, but without these samples. Furthermore, in this case K for the next iteration will NOT be set to K+1, but will be reset to kinit-1, where kinit was the first K used after the previous 'final' cluster was removed. If kinit-1<k.min, then K will be set to k.min.

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

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 'final' cluster after the algorithm stops, will be classified as unclustered (given a value of -1)

'subsample' controls what is the D (distance) matrix used for clustering at each iteration. If subsample=TRUE, D is given via subsampleClustering function with k=K (with additional arguments passed via subsampleArgs). If subsample=FALSE, D is dist(x), for the samples currently considered in the iteration and clusterFunction must be of the 'K' type (e.g. "pam", see clusterD) or an error will be produced. The nsample x nsample matrix D is then clustered via clusterD to find clusters. The option 'clusterFunction' is passed to the argument 'clusterFunction' of clusterD to control what method is used to cluster D.

If clusterFunction is of type 'K' (e.g. "pam", see clusterD) the 'k' argument of clusterK called by clusterD is set to the current iteration of K by the sequential iteration, so setting 'k=' in the list given to clusterDArgs will not do anything and will produce a warning to that effect.

Similarly, the current K of the iteration also 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.

If subsample=FALSE and 'findBestK=FALSE' is passed to clusterDArgs, then each iteration will run the clustering given by clusterFunction on dist(x) iterating over k. However, if subsample=FALSE,
you should not set `findBestK=TRUE` (otherwise clustering dist(x) will be essentially the same for iterating over different k and there is no method implemented to change the choice of how to remove a cluster other than similarity as you change k); an error message will be given if this combination of options are set.

However, if `clusterFunction="pam"` (or is of type `"K"`) and `subsample=TRUE` passing either `findBestK=TRUE` or `findBestK=FALSE` will function as expected. In particular, the iteration over K will set the number of clusters for clustering of each subsample. If `findBestK=FALSE`, that same K will be used for clustering of DMat. If `findBestK=TRUE`, then `clusterD` will search for best k; note that the default `kRange` over which `clusterD` searches when `findBestK=TRUE` depends on the input value of `k` (you can change this to a fixed set of values by setting `kRange` explicitly in the `clusterDArgs` list).

### 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

### Examples

```r
## Not run:
data(simData)
set.seed(12908)
clustSeqHier <- seqCluster(t(simData), k0=5, subsample=TRUE,
clusterFunction="hierarchical01", beta=0.8, subsampleArgs=list(resamp.n=100,
samp.p=0.7, clusterFunction="kmeans", clusterArgs=list(nstart=10)),
clusterDArgs=list(minSize=5))
## End(Not run)
```
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.

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Examples

#code used to create data:
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):(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")

subsampleClustering  
Cluster subsamples of the data

Description
Given a data matrix, this function will subsample the rows (samples), cluster the subsamples, and return a n x n matrix with the probability of co-occurrence.

Usage
subsampleClustering(x, k, clusterFunction = "pam", clusterArgs = NULL, classifyMethod = c("All", "InSample", "OutOfSample"), classifyFunction = NULL, resamp.num = 100, samp.p = 0.7, ncores = 1, ...)

Arguments
x the data on which to run the clustering (samples in columns).
k number of clusters to find for each clustering of a subsample (passed to clusterFunction).
clusterFunction a function that clusters a p x n matrix of data. Can also be given character values 'pam' or 'kmeans' to indicate use of internal wrapper functions. Must accept arguments 'x' and 'k' (whether uses them or not). See Details for format of what must return.
clusterArgs a list of parameter arguments to be passed to clusterFunction.
classifyMethod method for determining which samples should be used in the co-occurrence matrix. "All"= all samples, "OutOfSample"= those not subsampled, and "InSample"=those in the subsample. "All" and "OutOfSample" require that you provide classifyFunction to define how to classify those samples not in the subsample into a cluster. If "All" is chosen, all samples will be classified into clusters via the classifyFunctions, not just those that are out-of-sample. Note if not choose 'All' possible to get NAs in resulting D matrix (particularly if not enough subsamples taken).
classifyFunction a function which, given the output of clusterFunction and new data points, will classify the new data points into a cluster.
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.
... arguments passed to mclapply (if ncores>1).
Details

The `clusterFunction` must be a function that takes as an argument `x` which is a p x n matrix of data and integer `k`. It minimally must return a list with element named `clustering` giving the vector of cluster ids. To be incorporated with the larger hierarchy, it should be list with elements of a partition object, just as is returned by `pam`. Generally, the user will need to write a wrapper function to do this. In the case of pam or kmeans, the user can identify `clusterFunction` as "pam" or "kmeans", and the package functions will use internally written wrappers for the `clusterFunction` and `classifyFunction` arguments. Additional arguments should be supplied via `clusterArgs`.

The `classifyFunction` should take as an object a data matrix `x` with samples on the columns, and the output of the `clusterFunction`. 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).

Value

A n x n matrix of co-occurances.

Examples

data(simData)
subD <- subsampleClustering(t(simData), k=3, clusterFunction="kmeans", clusterArgs=list(nstart=10), resamp.n=100, samp.p=0.7)
heatmap(subD)

transform

Transform the original data in a ClusterExperiment object

Description

Provides the transformed data (as defined by the object), as well as dimensionality reduction.

Usage

```r
## S4 method for signature 'ClusterExperiment'
transform(x, nPCADims = NA, nVarDims = NA, 
dimReduce = "none", ignoreUnassignedVar = FALSE)
```

Arguments

- `x` a ClusterExperiment object.
- `nPCADims` Numeric vector giving the number of PC dimensions to use in PCA dimensionality reduction. If NA no PCA dimensionality reduction is done.
- `nVarDims` Numeric vector giving the number of features (e.g. genes) to keep, based on MAD variability.
- `dimReduce` Character vector specifying the dimensionality reduction to perform, any combination of 'none', 'PCA', 'var', 'cv', and 'mad'. See details.
- `ignoreUnassignedVar` logical indicating whether dimensionality reduction via top feature variability (i.e. 'var','cv','mad') should ignore unassigned samples in the primary clustering for calculation of the top features.
Details

The data matrix defined by $\text{assay}(x)$ is transformed based on the transformation function defined in $x$. If $\text{dimReduce} = \text{"none"}$ the transformed matrix is returned. Otherwise, the user can request dimensionality reduction of the transformed data via $\text{dimReduce}$. 'PCA' refers to PCA of the transformed data with the top $n_{PCADims}$ kept. 'var', 'cv', and 'mad' refers to keeping the top most variable features, as defined by taking the variance, the mad, or the coefficient of variation (respectively) across all samples. $n_{VarDims}$ defines how many such features to keep for any of 'var', 'cv', or 'mad'; note that the number of features must be the same for all of these options (they cannot be set separately).

The PCA uses prcomp on $t(\text{assay}(x))$ with $\text{center=True}$ and $\text{scale=True}$ (i.e. the feature are centered and scaled), so that it is performing PCA on the correlation matrix of the features.

ignoreUnassignedVar has no impact for PCA reduction, which will always use all samples. At all times, regardless of the value of ignoreUnassignedVar, a matrix with the same number of columns of $\text{assay}(x)$ (i.e. the same number of samples) will be returned.

dimReduce, $n_{PCADims}$, $n_{VarDims}$ can all be a vector of values, in which case a list will be returned with the appropriate datasets as elements of the list.

Value

If dimReduce, $n_{PCADims}$, $n_{VarDims}$ are all of length 1, a matrix will be returned of the same dimensions as $\text{assay}(x)$. If these arguments are vectors, then a list of data matrices will be return, each corresponding to the multiple choices implied by these parameters.

Examples

```r
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

#transform and take top 3 dimensions
x <- transform(cc, dimReduce="PCA", nPCADims=3)

#transform and take return untransformed, top 5 features, and top 10 features
y <- transform(cc, dimReduce="var", nVarDims=c(NA, 5, 10))

z<-transform(cc) #just return tranformed data
```

Description

The main workflow of the package is made of $\text{clusterMany}$, $\text{combineMany}$, and $\text{mergeClusters}$. The clusterings from these functions (and not those obtained in a different way) can be obtained with the functions documented here.
Usage

 workflowClusters(x, iteration = 0)
 workflowClusterDetails(x)
 workflowClusterTable(x)
 setToCurrent(x, whichCluster, eraseOld = FALSE)
 setToFinal(x, whichCluster, clusterLabel)

Arguments

x an 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'.
setToCurrent 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, nPCADims=c(5,10,50), dimReduce="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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