Package ‘flowMerge’
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Type Package
Title Cluster Merging for Flow Cytometry Data
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Description Merging of mixture components for model-based automated gating of flow cytometry data using the flowClust framework.
Note: users should have a working copy of flowClust 2.0 installed.
License Artistic-2.0
LazyLoad yes
Depends graph,feature,flowClust,Rgraphviz,foreach,snow
Enhances doMC, multicore
Imports rrcov,flowCore, graphics, methods, stats, utils
biocViews Clustering, FlowCytometry
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R topics documented:

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Merging of mixture components for automated gating of flow cytometry data.

Merges mixture components from the flowClust framework based on the entropy of clustering and provides a simple representation of complicated, non-convex cell populations.

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High density, non-convex cell populations in flow cytometry data often require multiple mixture components for a good model fit. The components are often overlapping, resulting in a complicated representation of individual cell populations. flowMerge merges overlapping mixture components (based on the max BIC flowClust model fit) in an iterative manner based on an entropy criterion, allowing these cell populations to be represented by individual mixture components while retaining the good model fitting properties of the BIC solution. Estimates of the number of clusters from a flowMerge model more accurately represent the "true" number of cell populations in the data. Running flowMerge is relatively straightforward. A flowClust object is converted to a flowObj object, which groups the model and the data (a flowFrame) into a single object. This is done by a call to flowObj(model, data) with a call to merge, which takes a flowObj object. The algorithm may be run in parallel on a multi-core machine or a networked cluster of machines. It uses the functionality in the snow package to achieve this. Parallelized calls to flowClust are available via the pFlowClust and pFlowMerge functions.

flowMerge has functionality to automatically select the "correct" number of clusters by fitting a piecewise linear model to the entropy of clustering vs number of clusters, and locating the position...
checkForRemoteErrors

of the changepoint. The piecewise linear model fitting is invoked by a call to fitPiecewiseLinreg, which returns the location of the changepoint.

Author(s)

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References

Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data; Advances in Bioinformatics (To Appear)

See Also

flowClust, flowObj, pFlowMerge, pFlowClust, fitPiecewiseLinreg, merge, getData, link{plot}

Examples

# data(rituximab)
# data(RituximabFlowClustFit)
# o <- flowObj(flowClust.res[[which.max(flowMerge:::BIC(flowClust.res))]], rituximab);
# m <- merge(o);
# i <- fitPiecewiseLinreg(m);
# m <- m[[i]];
# plot(m, pch=20, level=0.9);

checkForRemoteErrors  
Check output of snow clusters for errors

Description

Overrides the snow checkForRemoteErrors function. Try errors are returned when cluster nodes produce errors, rather than completely aborting the computation. Not meant to be called by the user.

Usage

checkForRemoteErrors(val)

Arguments

val The result returned from an individual cluster node.

Details

This function is meant to be called internally, but must be exported so that it can hide the native checkForRemoteErrors function in the snow package.

Value

The result from the snow cluster node, or an object of type try-error if there was an error.
fitPiecewiseLinreg

Author(s)

Greg Finak <<greg.finak@ircm.qc.ca>>

References

Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data; Advances in Bioinformatics (To Appear)

See Also

checkForRemoteErrors

fitPiecewiseLinreg  Fit Piecewise Linear Regression for a list of flowMerge Objects

Description

Fits a two–component piecewise linear regression to the entropy vs number of clusters for a list of merged cluster solutions.

Usage

fitPiecewiseLinreg(x, plot=FALSE, normalized=TRUE, ...)

Arguments

x  A "list" of flowMerge objects for 1 through K clusters derived from a single max BIC flowObj or flowClust object.

plot  A logical indicating whether to plot the fit or not. Default is FALSE.

normalized  A logical indicating whether the merging should be done using the normalized or unnormalized entropy

...  Additional arguments not currently used.

Details

An S4 method that takes a list of flowMerge objects output by the merge method, extracts the entropy and fits a piecewise linear regression to the entropy vs number of clusters in order to find the position of the changepoint. The location of the changepoint corresponds to the optimal merged cluster solution. The piecewise linear regression now is fitted to the entropy vs cumulative sum of merged observations at each number of clusters. This normalizes the change in entropy for the number of data points as described in Baudry et al.

Value

An integer value corresponding to the position of the changepoint.

Author(s)

Greg Finak <<greg.finak@ircm.qc.ca>>
References

Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data; Advances in Bioinformatics (To Appear)

See Also

merge

Examples

#data(rituximab)
#data(RituximabFlowClustFit)
o<-flowObj(flowClust.res[[which.max(BIC(flowClust.res))]],rituximab);
#o<-merge(o)
i<-fitPiecewiseLinreg(m);

flagOutliers

Update the flagOutliers slot in a flowMerge object

Description

Update the flagOutliers slot in a flowMerge object. This method is internal and called automatically from within the merging code.

Usage

flagOutliers(object,...)

Arguments

object An object of type flowMerge
... Additional arguments, currently unused.
flagOutliers-methods  *Methods to update the flagOutliers slot in a flowMerge object.*

**Description**

Methods that update the flagOutliers slot in a flowMerge object so that they reflect the outliers in the new merged clustering. This is an internal function, not meant for user consumption. It is called from within the merge method.

**Methods**

```
object = "flowMerge"  Update the flagOutliers slot for an object of type flowMerge
```

---

**Description**

The Rituximab data set accessible via `data(rituximab)` in the flowClust package fitted to a flowClust model containing from one to ten components. The results are in the object `flowClust.res`.

**Usage**

```
data(RituximabFlowClustFit)
```

**Format**

The format is: `flowClust.res` is a `flowClustList`, where each element of the list is a `flowClust` model of the rituximab data, for K=1 through K=10 components, respectively. The structure of `flowClustList` and `flowClust` can be found in the corresponding documentation of the flowClust package. The format of the rituximab data is found in the documentation for that data set.

**Details**

The models have been precomputed for use in `flowMerge` examples to save computation time. `flowClust` was called on the rituximab data to generate these models with the following command:

```
flowClust.res<-flowClust(rituximab,K=1:10,B=1000,B.init=100,tol=1e-5,tol.init=1e-2,nu=4,randomStart=50,trans=1,nu.est=1)
```

**Source**


**Examples**

```
#data(RituximabFlowClustFit)
#summary(flowClust.res);
```
flowMerge-class

Class "flowMerge"

Description

A class to represent flowMerge objects

Objects from the Class

The object unites the flowMerge model output and the data being modeled and contains additional slots for various characteristics of a merged cluster solution, including the entropy of clustering.

Slots

merged: The number of observations merged at the current step in the algorithm.
mtree: A tree-structured graph representing the order of merged components in the model. Inspired by SPADE. (Bendall et al.)
entropy: The entropy of clustering of the current solution.
DATA: An environment whose first element contains the flowFrame with the data modeled by this flowMerge object
expName: See the flowClust package for details
varNames: See the flowClust package for details
K: The number of clusters in the merged solution. See the flowClust package for details
w: The proportions for each component in the merged solution. See the flowClust package for details
mu: The means of the components in the merged solution. See the flowClust package for details
sigma: The covariances of the components in the merged solution. See the flowClust package for details
lambda: See the flowClust package for details
nu: See the flowClust package for details
z: See the flowClust package for details
u: The uncertainties for each data point.
label: See the flowClust package for details
uncertainty: See the flowClust package for details
ruleOutliers: See the flowClust package for details
flagOutliers: See the flowClust package for details
rm.min: See the flowClust package for details
rm.max: See the flowClust package for details
logLike: See the flowClust package for details
BIC: See the flowClust package for details
ICL: See the flowClust package for details

Extends

Class "flowObj", directly. Class "flowClust", by class "flowObj", distance 2.
Methods

**getDat**a signature(obj = "flowMerge"): Retrieves the flowFrame in the DATA environment slot.

**plot** signature(x = "flowMerge", y = "missing"): Plots the clusters in this object.

**summary** signature(x="flowMerge"): Prints a summary of the object.

**show** signature(x="flowMerge"): Prints information about the object.

Author(s)

Greg Finak <<greg.finak@ircm.qc.ca>>

References

Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data (Submitted)

See Also

flowObj-class

---

**flowObj**

Create a flowObj object from a flowClust and flowFrame object

Description

Convenience method that creates a flowObj object from a flowClust and flowFrame object, so as to group the model and data together. Useful for high-throughput analysis where one may want to access the data to compute other statistics.

Usage

flowObj(flowC = NULL, flowF = NULL)

Arguments

flowC A flowClust object representing the model fit

flowF A flowFrame object on which the flowClust model is based.

Details

Calls the new("flowObj",...) constructor.

Value

An object of class flowObj-class

Author(s)

Greg Finak <<greg.finak@ircm.qc.ca>>, Raphael Gottardo <<raphael.gottardo@ircm.qc.ca>>
flowObj-class

References
Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data; Advances in Bioinformatics (To Appear)

See Also
flowObj-class

Examples
#data(rituximab)
#data(RituximabFlowClustFit)
#o<-flowObj(flowClust.res[[which.max(flowMerge:::BIC(flowClust.res))]],rituximab);
#m<-merge(o);

flowObj-class  
Class "flowObj"

Description
A class inheriting from flowClust that groups the model and data in a single object.

Objects from the Class
Objects can be created by calls of the form new("flowObj", ...). Has a convenience method
flowObj(flowClustObj, flowFrameObj) for creating instances of the class.

Slots
DATA: An "environment" that holds a pointer to the flowFrame data in position [[1]].
expName: As described in the flowClust documentation
varNames: As described in the flowClust documentation
K: As described in the flowClust documentation
w: As described in the flowClust documentation
mu: As described in the flowClust documentation
sigma: As described in the flowClust documentation
lambda: As described in the flowClust documentation
nu: As described in the flowClust documentation
z: As described in the flowClust documentation
u: As described in the flowClust documentation
label: As described in the flowClust documentation
uncertainty: As described in the flowClust documentation
ruleOutliers: As described in the flowClust documentation
flagOutliers: As described in the flowClust documentation
rm.min: As described in the flowClust documentation
rm.max: As described in the flowClust documentation
logLike: As described in the flowClust documentation
BIC: As described in the flowClust documentation
ICL: As described in the flowClust documentation

Extends
Class "flowClust", directly.

Methods
getData signature(obj = "flowObj"): Retrieves the contents of the DATA environment
merge signature(x = "flowObj", y = "missing"): the flowMerge algorithm is called via this function on objects of type flowObj.
plot signature(x = "flowObj", y = "missing"): A simplified plotting method. Does not require specification of the data since it is contained in the flowObj object. Takes most of the same parameters as plot.flowClust, except the data parameter

Author(s)
Greg Finak <<greg.finak@ircm.qc.ca>>, Raphael Gottardo <<raphael.gottardo@ircm.qc.ca>>

References
Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data; Advances in Bioinformatics (To Appear)

See Also
flowMerge-class, flowObj

---

initPFlowMerge

Initialize a SNOW cluster for use with flowMerge

Description
Initializes a snow cluster for use with flowMerge, ensures that the flowMerge library is loaded in all environments. Not meant to be called by the user

Usage
initPFlowMerge(cl)

Arguments
cl A snow cluster

Details
A valid snow cluster.
Author(s)

Greg Finak <greg.finak@ircm.qc.ca>

References

Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data; Advances in Bioinformatics (To Appear)

See Also

pFlowMerge

---

**map**

*Map matrix of probabilities to class assignments.*

**Description**

Traverse the rows of a matrix of probabilities of size \( n \times k \), where the \( n \) rows are samples, and the \( k \) columns are the probability of assignment of the sample to each of \( k \) classes. The most probable class assignment is selected for each row and a vector of classes is returned.

**Usage**

```r
map(z, ...)
```

**Arguments**

- `z`: A matrix of probabilities.
- `...`: Additional arguments, not currently used.

**Value**

A vector of class assignments of length \( n \).

**Author(s)**

Greg Finak <greg.finak@ircm.qc.ca>, Raphael Gottardo <raphael.gottardo@ircm.qc.ca>

**Examples**

```r
z <- t(apply(t(replicate(100, rgamma(5, 0.1, 1))), 1, function(x) x/sum(x)));
map(z);
```
merge  Merge clusters in flow cytometry data

Description
Merge the clusters in a flowClust solution using the cluster merging algorithm and entropy criterion.

Usage
merge(x,y,...)

Arguments
x A flowObj object created from a flowClust object and a flowFrame using the flowObj constructor.
y missing
... Additional arguments. i.e. metric="entropy"|"mahalanobis"

Details
Run the cluster merging algorithm on the max BIC solution from a call to flowClust. The optional argument, metric specifies the measure used for clustering. Either "mahalanobis" or "entropy". Defaults to "entropy".

Value
A list of unnamed flowMerge objects. The first element of the list corresponds to the 1–cluster merged solution. The second element corresponds to the 2–cluster merged solution, and so on.

Author(s)
Greg Finak <<greg.finak@ircm.qc.cq>>

References
Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data (Submitted)

See Also
flowClust,flowObj

Examples
#data(rituximab)
data(RituximabFlowClustFit)
o<-flowObj(flowClust.res[[which.max(BIC(flowClust.res))]],rituximab)
m<-merge(o);
merge-methods

Merge mixture components

Description

Merge mixture components in a `flowObj` derived from a `flowClust` result and a `flowFrame` using the cluster merging algorithm.

Value

An unnamed list of `flowMerge` objects with the kth element corresponding to the k-cluster merged solution.

Methods

- `x = "ANY", y = "ANY"` The generic method. Should not be called.
- `x = "flowObj", y = "missing"` The merge method for a `flowObj`.

References

Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data (To Appear)

Examples

```r
#data(rituximab)
#data(RituximabFlowClustFit)
o<-flowObj(flowClust.res[[which.max(flowMerge:::BIC(flowClust.res))]],rituximab);
m<merge(o);
```

mergeClusters

Cluster merging not meant to be called by the user

Description

Internal cluster merging function.

Usage

```r
mergeClusters(object, metric)
```

Arguments

- `object` not meant to be called by the user
- `metric` not meant to be called by the user

Details

Not meant to be called by the user
mergeClusters2

Value
Not meant to be called by the user

Author(s)
Greg Finak <<greg.finak@ircm.qc.ca>>

References
Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data (Submitted)

See Also
merge

mergeClusters2  Cluster merging not meant to be called by the user

Description
Internal function not meant to be called by the user.

Usage
mergeClusters2(object, a, b)

Arguments
  object  Internal function not meant to be called by the user.
  a       Internal function not meant to be called by the user.
  b       Internal function not meant to be called by the user.

Details
Internal function not meant to be called by the user.

Value
Internal function not meant to be called by the user.

Author(s)
Greg Finak <<greg.finak@ircm.qc.ca>>

References
Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data (Submitted)

See Also
merge
Description
Extracts the normalized entropy from a list of flowMerge objects.

Usage
NENT(x)

Arguments
x
A list of flowMerge objects

Details
The normalized entropy is extracted from a flowMerge object by computing \( \frac{E}{K \times n} \) where \( E \) is the entropy, and \( K \) and \( n \) are the number of clusters and data points, respectively.

Value
Returns a vector of normalized entropy values for the flowMerge objects.

Warning
This function doesn’t do enough error checking and will try to extract the entropy from a list of anything.

Author(s)
Greg Finak <<greg.finak@ircm.qc.ca>>

References
Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data; Advances in Bioinformatics (To Appear)

See Also
ENT, merge, flowMerge-class

Examples
#data(RituximabFlowClustFit)
#data(rituximab)
#o<-flowObj(flowClust.res[[which.max(flowMerge::BIC(flowClust.res))]],rituximab);
#m<-merge(o);
#flowMerge:::ENT(m);
#flowMerge:::NENT(m);
pFlowClust

Parallelized FlowClust

Description

A parallelized call to flowClust via the snow package and framework. Not called by the user.

Usage

pFlowClust(flowData, cl, K = 1:15, B.init = 100, tol.init = 0.01, tol = 1e-05, B = 1000, randomStart = 50, nu = 4, nu.est = 1, trans = 1, varNames = NA)

Arguments

- flowData: The data object, must be a flowFrame, flowSet or list of flowFrames
- cl: The snow cluster object
- K: The number of clusters to try for each flowFrame. Can be a vector. This is what is parallelized across processors.
- B.init: See flowClust documentation
- tol.init: See flowClust documentation
- tol: See flowClust documentation
- B: See flowClust documentation
- randomStart: See flowClust documentation
- nu: See flowClust documentation
- nu.est: See flowClust documentation
- trans: See flowClust documentation
- varNames: See flowClust documentation

Details

Calls flowClust via the clusterMap method of the snow package. Parallelizes the computation of multiple components for a single flowFrame in a loop over multiple flowFrames. If the snow cluster is NULL, will make the call via mapply.

Value

Returns a list of lists of flowClust objects. The outer list corresponds to the flowFrames passed into the method. The inner list corresponds to the K cluster solutions passed into the method, for each flowFrame (ie If the input is a list of two flowFrames, and K=1:10, then the result is a list of length 2. Each element of the list is itself a list of length 10. The kth element of the inner list is the flowClust k cluster solution.)

Author(s)

Greg Finak <greg.finak@ircm.qc.ca>

References

Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data (Submitted)
pFlowMerge

See Also

flowClust, snow

pFlowMerge  Parallel call to flowMerge

Description

Calls the flowMerge methods to compute the merged solution from a flowClust object or set of objects in a parallelized manner using the snow framework.

Usage

pFlowMerge(flowData, cl, K = 1:15, B.init = 100, tol.init = 0.01, tol = 1e-05, B = 500, randomStart = 10, nu = 4, nu.est = 0, trans = 1, varNames = NA)

Arguments

flowData  The data to be fit. A list of flowFrames, a flowSet or a flowFrame
cl  The snow cluster object. Can be NULL to call the non-parallel version of flowClust
K  See flowClust documentation
B.init  See flowClust documentation
tol.init  See flowClust documentation
tol  See flowClust documentation
B  See flowClust documentation
randomStart  See flowClust documentation
nu  See flowClust documentation
nu.est  See flowClust documentation
trans  See flowClust documentation
varNames  See flowClust documentation

Details

Makes a parallelized call to flowClust. Parses the results to extract the max BIC solution, merges clusters, finds the optimal k-cluster solution using the entropy and returns it. If cl is NULL, a non-parallel call is made to the flowClust function.

Value

A list of flowMerge objects. One per flowFrame passed into the method.

Warning

This function does not do any special memory management. A large data set will likely cause it to run out of memory and start swapping incessantly. If you have lots of data, it’s best to feed it piecewise to pFlowClust.
Author(s)
Greg Finak <<greg.finak@ircm.qc.ca>>

References

Finak G, Bashasharti A, Brinkmann R, Gottardo R. Merging Mixture Model Components for Improved Cell Population Identification in High Throughput Flow Cytometry Data (Submitted)

See Also

pFlowClust, flowClust, merge, snow, fitPiecewiseLinreg

Examples

data(rituximab)
#Parallelized call below:
## Not run: cl<-makeSOCKcluster(rep("finakg@localhost",7))
## Not run: result<-pFlowMerge(rituximab,cl,varNames=c("FSC.H","SSC.H"))
## Not run: plot(result)
#cl<-NULL;
#result<-pFlowMerge(rituximab,cl=NULL,varNames=c("FSC.H","SSC.H"),K=1:8);
#plot(result);

plot-methods 

Methods for plotting flowMerge and flowObj classes

Description

Plots all possible two-dimensional projections of the parameters in a flowMerge or flowObj object and does not require specification of the flowFrame since a pointer to the data is stored in the object. Informative axis names are used, rather than the usual FL1/FL2/FS/SS channel names. This function can take most of the usual additional arguments provided to plot for the flowClust package, although some, like the axis names and the data are fixed. In order for flowMerge objects to display outliers correctly with plot (following merging), the updateU method must be called on them first.

Methods

x = "flowMerge", y = "missing" x is a flowMerge object.

x = "flowObj", y = "missing" x is a flowObj object.

See Also

flowClust

Examples

#data(rituximab)
#data(RituximabFlowClustFit)
#o<-flowObj(flowClust.res[[which.max(flowMerge:::BIC(flowClust.res))]],rituximab);
#m<-merge(o);
#i<-fitPiecewiseLinreg(m);
#for(i[[2]]; #plot(m,pch=20,level=0.9);
ptree

Generate a Function to Plot The Merging Tree

Description

This function generates and returns a new function which can be used to plot the merging tree for a flowMerge model, with nodes highlighted based on the expression of different parameters for each cell population.

Usage

ptree(x,y)

Arguments

x
A character string of the name of the variable holding the list of merged models returned from flowMerge

y
The index of the best fitting merged model in that list

Details

ptree will generate a function that will plot the merging tree from a flowMerge model. Nodes will be colored by the intensity of staining of that population in a given dimension. Calling f<-ptree("model.name",fitPiecewiseLinreg(model.name)) will assign the function to f. Calling f(3) will plot the merging tree with nodes highlighted according to parameter 3, presuming that there are that many parameters in the model.

Value

Returns a function

Side Effects

A plot will be drawn on the current device.

Author(s)

Greg Finak <gfinak@fhcrc.org>

See Also

merge.
**show-methods**

*Describe a `flowObj` or `flowMerge` object*

**Description**

Accessors to describe a `flowObj` or `flowMerge` object.

**Methods**

- `object = "flowMerge"` Describe a `flowMerge` object.
- `object = "flowObj"` Describe a `flowObj` object.

**split-methods**

*Split data in a `flowMerge` object by cluster*

**Description**

Split method defined for `flowMerge` objects. Pulls out the population based on cluster number.

**Methods**

- `itemx = "flowMerge", f = "missing"` Split a `flowMerge` object into its component clusters.

**summary-methods**

*Summary methods for `flowMerge`*

**Description**

Summary method for `flowMerge` objects.

**Methods**

- `object = "flowMerge"` Summarize a `flowMerge` object.
- `object = "flowObj"` Summarize a `flowObj` object
updateU

Description

Updates the uncertainties in a flowMerge object after merging clusters. This function is now internal and no longer exported. It is called automatically within the cluster merging method.

Usage

updateU(object)

Arguments

object

An object of type flowMerge

Details

Updates the u slot of the flowMerge object following merging. The update is computation intensive, and so, is not automatically performed on each flowMerge object. Should only be done on objects used in further analysis.

Value

A flowMerge object with the u slot updated to reflect the new parameter values.

Author(s)

Greg Finak <<greg.finak@ircm.qc.ca>>

See Also

flowMerge-class, merge

Examples

#data(rituximab)
#data(RituximabFlowClustFit)
#o<-flowObj(flowClust.res[[which.max(flowMerge::BIC(flowClust.res))]],rituximab);
#m<-merge(o);
#i<-fitPiecewiseLinreg(m);
#m<-m[[i]];
#plot(m,pch=20,level=0.9);
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