Package: flowClust (via r-universe)

November 1, 2026

Type Package

Title Clustering for Flow Cytometry

Version 3.50.0

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Depends R(>= 2.5.0)

Imports BiocGenerics, methods, Biobase, graph, flowCore, parallel

Suggests testthat, flowWorkspace, flowWorkspaceData, knitr, rmarkdown, openCyto, flowStats(>= 4.7.1)

Description Robust model-based clustering using a t-mixture model with Box-Cox transformation. Note: users should have GSL installed. Windows users: 'consult the README file available in the inst directory of the source distribution for necessary configuration instructions'.

Collate 'SetClasses.R' 'SetMethods.R' 'flowClust.R' 'coerce.R' 'flowClust-package.R' 'hist.R' 'miscellaneous.R' 'plot.R' 'split.R'

License MIT

biocViews ImmunoOncology, Clustering, Visualization, FlowCytometry

SystemRequirements GNU make

Encoding UTF-8

RoxygenNote 7.1.1

VignetteBuilder knitr

Config/pak/sysreqs make libssl-dev

Repository Bioconductor 3.23

Date/Publication 2026-04-28 12:32:49 UTC

RemoteUrl https://github.com/bioc/flowClust

RemoteRef RELEASE_3_23

RemoteSha 3cae24ac8cd526b08e3ae101382cc91719cfd826

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`flowClust-package` *Clustering for Flow Cytometry*

Description

Robust model-based clustering using a t mixture model with Box-Cox transformation.

Details

Package:	flowClust
Type:	Package
Version:	2.0.0
Depends:	R(>= 2.5.0), methods, mnormt, mclust, ellipse, Biobase, flowCore
Collate:	SetClasses.R SetMethods.R plot.R flowClust.R SimulateMixture.R
biocViews:	Clustering, Statistics, Visualization
License:	Artistic-2.0
Built:	R 2.6.1; universal-apple-darwin8.10.1; 2008-03-26 20:54:42; unix

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list(list("plot,flowDens-method")) Contour or Image Plot of Clustering Results

list(list("plot,flowFrame,tmixFilterResult-method")) Scatterplot / 1-D Density Plot of Filtering (Clustering) Results

list(list("rbox")) Reverse Box-Cox Transformation

list(list("ruleOutliers,flowClust-method")) Showing or Modifying the Rule used to Identify Outliers

list(list("show,flowClust-method")) Show Method for `flowClust` / `tmixFilterResult` Object

list(list("show,tmixFilter-method")) Show Method for `tmixFilter` Object

list(list("SimulateMixture")) Random Generation from a t Mixture Model with Box-Cox Transformation

list(list("split,flowClust-method")) Splitting Data Based on Clustering Results

list(list("Subset,flowClust-method")) Subsetting Data Based on Clustering Results

list(list("summary,flowClust-method")) Summary Method for `flowClust` Object

list(list("tmixFilter")) Creating Filters and Filtering Flow Cytometry Data

Note

Further information is available in the vignette.

Author(s)

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Maintainer: Raphael Gottardo <raph@stat.ubc.ca>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

BIC *Extract the BIC for a flowClust fit.*

Description

Extract the bayesian information criterion for a flowClust fit.

Usage

```
## S3 method for class 'flowClustList'
BIC(object, ...)

## S3 method for class 'flowClust'
BIC(object, ...)
```

Arguments

object	flowClustList or flowClust fitted object
...	other arguments. Currently not used.

Value

vector of BIC or ICL values

box *Box-Cox Transformation*

Description

This function performs Box-Cox transformation on the inputted data matrix.

Usage

```
box(data, lambda)
```

Arguments

data	A numeric vector, matrix or data frame of observations. Negative data values are permitted.
lambda	The transformation to be applied to the data. If negative data values are present, lambda has to be positive.

Details

To allow for negative data values, a slightly modified version of the original Box-Cox (1964) is used here. This modified version originated from Bickel and Doksum (1981), taking the following form:

$$f(y) = \frac{\mathrm{sgn}(y)|y|^{\lambda} - 1}{\lambda}$$

When negative data values are involved, the transformation parameter, λ, has to be positive in order to avoid discontinuity across zero.

Value

A numeric vector, matrix or data frame of the same dimension as `data` is returned.

References

Bickel, P. J. and Doksum, K. A. (1981) An Analysis of Transformations Revisited. *J. Amer. Statist. Assoc.* **76**(374), 296-311.

Box, G. E. P. and Cox, D. R. (1964) An Analysis of Transformations. *J. R. Statist. Soc. B* **26**, 211-252.

See Also

`rbox`

Examples

```
data(rituximab)
library(flowCore)
data <- exprs(rituximab)
summary(data)
# Transform data using Box-Cox with lambda=0.3
dataTrans <- box(data, 0.3)
# Reverse transform data; this should return back to the original rituximab data
summary(rbox(dataTrans, 0.3))
```

`criterion` *Various Functions for Retrieving Information from Clustering Results*

Description

Various functions are available to retrieve the information criteria (`criterion`), the posterior probabilities of clustering memberships z (`posterior`), the "weights" u (`importance`), the uncertainty (`uncertainty`), and the estimates of the cluster proportions, means and variances (`getEstimates`) resulted from the clustering (filtering) operation.

Usage

```
criterion(object, ...)

## S4 method for signature 'flowClust'
criterion(object, type = "BIC")

## S4 method for signature 'flowClustList'
criterion(object, type = "BIC", max = FALSE, show.K = FALSE)

criterion(object) <- value

## S4 replacement method for signature 'flowClustList,character'
criterion(object) <- value

posterior(object, assign = FALSE)

importance(object, assign = FALSE)

uncertainty(object)

getEstimates(object, data)
```

Arguments

object	Object returned from flowClust or filter. For the replacement method of criterion, the object must be of class flowClustList or tmixFilterResultList.
...	Further arguments. Currently this is type, a character string. May take "BIC", "ICL" or "logLike", to specify the criterion desired.
type, value	A character string stating the criterion used to choose the best model. May take either "BIC" or "ICL".
max	whether criterion should return the max value
show.K	whether criterion should return K
assign	A logical value. If TRUE, only the quantity (z for posterior or u for importance) associated with the cluster to which an observation is assigned will be returned. Default is FALSE, meaning that the quantities associated with all the clusters will be returned.
data	A numeric vector, matrix, data frame of observations, or object of class flowFrame; an optional argument. This is the object on which flowClust or filter was performed.

Details

These functions are written to retrieve various slots contained in the object returned from the clustering operation. criterion is to retrieve object@BIC, object@ICL or object@logLike. It replacement method modifies object@index and object@criterion to select the best model according to the desired criterion. posterior and importance provide a means to conveniently retrieve information stored in object@z and object@u respectively. uncertainty is to retrieve

`object@uncertainty`. `getEstimates` is to retrieve information stored in `object@mu` (transformed back to the original scale) and `object@w`; when the data object is provided, an approximate variance estimate (on the original scale, obtained by performing one M-step of the EM algorithm without taking the Box-Cox transformation) will also be computed.

Value

Denote by K the number of clusters, N the number of observations, and P the number of variables. For `posterior` and `importance`, a matrix of size $N \times K$ is returned if `assign=FALSE` (default). Otherwise, a vector of size N is outputted. `uncertainty` always outputs a vector of size N. `getEstimates` returns a list with named elements, `proportions`, `locations` and, if the data object is provided, `dispersion`. `proportions` is a vector of size P and contains the estimates of the K cluster proportions. `locations` is a matrix of size $K \times P$ and contains the estimates of the K mean vectors transformed back to the original scale (i.e., `rbox(object@mu,object@lambda)`). `dispersion` is an array of dimensions $K \times P \times P$, containing the approximate estimates of the K covariance matrices on the original scale.

Note

When `object@nu=Inf`, the Mahalanobis distances instead of the "weights" are stored in `object@u`. Hence, `importance` will retrieve information corresponding to the Mahalanobis distances. the `assign` argument is set to `TRUE`, only the quantities corresponding to assigned observations will be returned. Quantities corresponding to unassigned observations (outliers and filtered observations) will be reported as `NA`. Hence, A change in the rule to call outliers will incur a change in the number of `NA` values returned.

Author(s)

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References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

`flowClust`, `filter`, `Map`

`dmvt` *Density of the Multivariate t Distribution with Box-Cox Tranformation*

Description

This function computes the densities at the inputted points of the multivariate t distribution with Box-Cox transformation.

Usage

```
dmvt(x, mu, sigma, nu, lambda, log = FALSE)
```

Arguments

x	A matrix or data frame of size $N \times P$, where N is the number of observations and P is the dimension. Each row corresponds to one observation.
mu	A numeric vector of length P specifying the mean.
sigma	A matrix of size $P \times P$ specifying the covariance matrix.
nu	The degrees of freedom used for the t distribution. If nu=Inf, Gaussian distribution will be used.
lambda	The Box-Cox transformation parameter. If missing, the conventional t distribution without transformation will be used.
log	A logical value. If TRUE then the logarithm of the densities is returned.

Value

A list with the following components:

value	A vector of length N containing the density values.
md	A vector of length N containing the Mahalanobis distances.

Author(s)

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dmvtmix *Density of the Multivariate t Mixture Distribution with Box-Cox Tranformation*

Description

This function computes the densities at the inputted points of the multivariate t mixture distribution with Box-Cox transformation.

Usage

```
dmvtmix(x, w, mu, sigma, nu, lambda, object, subset, include, log = FALSE)
```

Arguments

x	A matrix or data frame of size $N \times P$, where N is the number of observations and P is the dimension. Each row corresponds to one observation.
w	A numeric vector of length K containing the cluster proportions.
mu	A matrix of size $K \times P$ containing the K mean vectors.
sigma	An array of size $K \times P \times P$ containing the K covariance matrices.
nu	A numeric vector of length K containing the degrees of freedom used for the t distribution. If only one value is specified for nu, then it is used for all K clusters. If nu=Inf, Gaussian distribution will be used.
lambda	The Box-Cox transformation parameter. If missing, the conventional t distribution without transformation will be used.
object	An optional argument. If provided, it's an object returned from flowClust, and the previous arguments will be assigned values from the corresponding slots of object.
subset	An optional argument. If provided, it's a numeric vector indicating which variables are selected for computing the densities. If object is provided and object@varNames is not NULL, then a character vector containing the names of the variables is allowed.
include	An optional argument. If provided, it's a numeric vector specifying which clusters are included for computing the densities.
log	A logical value. If TRUE then the logarithm of the densities is returned.

Value

A vector of length N containing the density values.

Author(s)

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flowClust-class *Robust Model-based Clustering for Flow Cytometry*

Description

This function performs automated clustering for identifying cell populations in flow cytometry data. The approach is based on the tmixture model with the Box-Cox transformation, which provides a unified framework to handle outlier identification and data transformation simultaneously.

Usage

```
flowClust(
  x,
  expName = "Flow Experiment",
  varNames = NULL,
  K,
  nu = 4,
  lambda = 1,
  trans = 1,
  min.count = 10,
  max.count = 10,
  min = NULL,
  max = NULL,
  randomStart = 0,
  prior = NULL,
  usePrior = "no",
  criterion = "BIC",
  ...
)
```

Arguments

x	A numeric vector, matrix, data frame of observations, or object of class `flowFrame`. Rows correspond to observations and columns correspond to variables.
expName	A character string giving the name of the experiment.
varNames	A character vector specifying the variables (columns) to be included in clustering. When it is left unspecified, all the variables will be used.
K	An integer vector indicating the numbers of clusters.
nu	The degrees of freedom used for the t distribution. Default is 4. If `nu=Inf`, Gaussian distribution will be used.
lambda	The initial transformation to be applied to the data.
trans	A numeric indicating whether the Box-Cox transformation parameter is estimated from the data. May take 0 (no estimation), 1 (estimation, default) or 2 (cluster-specific estimation).
min.count	An integer specifying the threshold count for filtering data points from below. The default is 10, meaning that if 10 or more data points are smaller than or equal to `min`, they will be excluded from the analysis. If `min` is `NULL`, then the minimum of data as per each variable will be used. To suppress filtering, set it as -1.
max.count	An integer specifying the threshold count for filtering data points from above. Interpretation is similar to that of `min.count`.
min	The lower boundary set for data filtering. Note that it is a vector of length equal to the number of variables (columns), implying that a different value can be set as per each variable.
max	The upper boundary set for data filtering. Interpretation is similar to that of `min`.

`randomStart` A numeric value indicating how many times a random parition of the data is generated for initialization. The default is 0, meaning that a deterministic partition based on kmeans clustering is used. A value of 10 means random partitions of the data will be generated, each of which is followed by a short EM run. The partition leading to the highest likelihood value will be adopted to be the initial partition for the eventual long EM run.

`prior` The specification of the prior. Used if usePrior="yes"

`usePrior` Argument specifying whether or not the prior will be used. Can be "yes","no","vague". A vague prior will be automatically specified if usePrior="vague"

`criterion` A character string stating the criterion used to choose the best model. May take either `"BIC"` or `"ICL"`. This argument is only relevant when `length(K)>1`. Default is "BIC".

`...` other arguments: B: The maximum number of EM iterations.Default is 500.

tol: The tolerance used to assess the convergence of the EM. default is 1e-5.

nu.est: A numeric indicating whether `nu` is to be estimated or not. May take 0 (no estimation, default), 1 (estimation) or 2 (cluster-specific estimation). Default is 0.

level: A numeric value between 0 and 1 specifying the threshold quantile level used to call a point an outlier. The default is 0.9, meaning that any point outside the 90% quantile region will be called an outlier.

u.cutoff: Another criterion used to identify outliers. If this is `NULL`, which is default, then `level` will be used. Otherwise, this specifies the threshold (e.g., 0.5) for u, a quantity used to measure the degree of "outlyingness" based on the Mahalanobis distance. Please refer to Lo et al. (2008) for more details.

z.cutoff: A numeric value between 0 and 1 underlying a criterion which may be used together with `level`/`u.cutoff` to identify outliers. A point with the probability of assignment z (i.e., the posterior probability that a data point belongs to the cluster assigned) smaller than `z.cutoff` will be called an outlier. The default is 0, meaning that assignment will be made no matter how small the associated probability is, and outliers will be identified solely based on the rule set by `level` or `cutoff`.

B.init: The maximum number of EM iterations following each random partition in random initialization. Default is the same as B.

tol.init: The tolerance used as the stopping criterion for the short EM runs in random initialization. Default is 1e-2.

seed: An integer giving the seed number used when `randomStart>0`.Default is 1.

control: An argument reserved for internal use.

Details

Estimation of the unknown parameters (including the Box-Cox parameter) is done via an Expectation-Maximization (EM) algorithm. At each EM iteration, Brent's algorithm is used to find the optimal value of the Box-Cox transformation parameter. Conditional on the transformation parameter, all other estimates can be obtained in closed form. Please refer to Lo et al. (2008) for more details.

The **flowClust** package makes extensive use of the GSL as well as BLAS. If an optimized BLAS library is provided when compiling the package, the **flowClust** package will be able to run multi-threaded processes.

Various operations have been defined for the object returned from `flowClust`.

In addition, to facilitate the integration with the **flowCore** package for processing flow cytometry data, the `flowClust` operation can be done through a method pair (`tmixFilter` and `filter`) such that various methods defined in **flowCore** can be applied on the object created from the filtering operation.

Value

If `K` is of length 1, the function returns an object of class `flowClust` containing the following slots, where K is the number of clusters, N is the number of observations and P is the number of variables:

`expName`	Content of the `expName` argument.
`varNames`	Content of the `varNames` argument if provided; generated if available otherwise.
`K`	An integer showing the number of clusters.
`w`	A vector of length K, containing the estimates of the K cluster proportions.
`mu`	A matrix of size $K \times P$, containing the estimates of the K mean vectors.
`sigma`	An array of dimension $K \times P \times P$, containing the estimates of the K covariance matrices.
`lambda`	The Box-Cox transformation parameter estimate.
`nu`	The degrees of freedom for the t distribution.
`z`	A matrix of size $N \times K$, containing the posterior probabilities of cluster memberships. The probabilities in each row sum up to one.
`u`	A matrix of size $N \times K$, containing the "weights" (the contribution for computing cluster mean and covariance matrix) of each data point in each cluster. Since this quantity decreases monotonically with the Mahalanobis distance, it can also be interpreted as the level of "outlyingness" of a data point. Note that, when `nu=Inf`, this slot is used to store the Mahalanobis distances instead.
`label`	A vector of size N, showing the cluster membership according to the initial partition (i.e., hierarchical clustering if `randomStart=0` or random partitioning if `randomStart>0`). Filtered observations will be labelled as `NA`. Unassigned observations (which may occur since only 1500 observations at maximum are taken for hierarchical clustering) will be labelled as 0.
`uncertainty`	A vector of size N, containing the uncertainty about the cluster assignment. Uncertainty is defined as 1 minus the posterior probability that a data point belongs to the cluster to which it is assigned.
`ruleOutliers`	A numeric vector of size 3, storing the rule used to call outliers. The first element is 0 if the criterion is set by the `level` argument, or 1 if it is set by `u.cutoff`. The second element copies the content of either the `level` or `u.cutoff` argument. The third element copies the content of the `z.cutoff` argument. For instance, if points are called outliers when they lie outside the 90% quantile region or have assignment probabilities less than 0.5, then `ruleOutliers` is `c(0, 0.9,`

	`0.5`). If points are called outliers only if their "weights" in the assigned clusters are less than 0.5 regardless of the assignment probabilities, then `ruleOutliers` becomes `c(1, 0.5, 0)`.
`flagOutliers`	A logical vector of size N, showing whether each data point is called an outlier or not based on the rule defined by `level`/`u.cutoff` and `z.cutoff`.
`rm.min`	Number of points filtered from below.
`rm.max`	Number of points filtered from above.
`logLike`	The log-likelihood of the fitted mixture model.
`BIC`	The Bayesian Information Criterion for the fitted mixture model.
`ICL`	The Integrated Completed Likelihood for the fitted mixture model.

If `K` has a length >1, the function returns an object of class `flowClustList`. Its data part is a list with the same length as `K`, each element of which is a `flowClust` object corresponding to a specific number of clusters. In addition, the resultant `flowClustList` object contains the following slots:

`index` An integer giving the index of the list element corresponding to the best model as selected by `criterion`.
`criterion` The criterion used to choose the best model – either `"BIC"` or `"ICL"`.

Note that when a `flowClustList` object is used in place of a `flowClust` object, in most cases the list element corresponding to the best model will be extracted and passed to the method/function call.

Author(s)

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References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

`summary`, `plot`, `density`, `hist`, `Subset`, `split`, `ruleOutliers`, `Map`, `SimulateMixture`

Examples

```
library(flowCore)
data(rituximab)

### cluster the data using FSC.H and SSC.H
res1 <- flowClust(rituximab, varNames=c("FSC.H", "SSC.H"), K=1)

### remove outliers before proceeding to the second stage
# %in% operator returns a logical vector indicating whether each
# of the observations lies within the cluster boundary or not
rituximab2 <- rituximab[rituximab %in% res1,]
```

```
# a shorthand for the above line
rituximab2 <- rituximab[res1,]
# this can also be done using the Subset method
rituximab2 <- Subset(rituximab, res1)

### cluster the data using FL1.H and FL3.H (with 3 clusters)
res2 <- flowClust(rituximab2, varNames=c("FL1.H", "FL3.H"), K=3)
show(res2)
summary(res2)

# to demonstrate the use of the split method
split(rituximab2, res2)
split(rituximab2, res2, population=list(sc1=c(1,2), sc2=3))

# to show the cluster assignment of observations
table(Map(res2))

# to show the cluster centres (i.e., the mean parameter estimates
# transformed back to the original scale)
getEstimates(res2)$locations

### demonstrate the use of various plotting methods
# a scatterplot
plot(res2, data=rituximab2, level=0.8)
plot(res2, data=rituximab2, level=0.8, include=c(1,2), grayscale=TRUE,
    pch.outliers=2)
# a contour / image plot
res2.den <- density(res2, data=rituximab2)
plot(res2.den)
plot(res2.den, scale="sqrt", drawlabels=FALSE)
plot(res2.den, type="image", nlevels=100)
plot(density(res2, include=c(1,2), from=c(0,0), to=c(400,600)))
# a histogram (1-D density) plot
hist(res2, data=rituximab2, subset="FL1.H")

### to demonstrate the use of the ruleOutliers method
summary(res2)
# change the rule to call outliers
ruleOutliers(res2) <- list(level=0.95)
# augmented cluster boundaries lead to fewer outliers
summary(res2)

# the following line illustrates how to select a subset of data
# to perform cluster analysis through the min and max arguments;
# also note the use of level to specify a rule to call outliers
# other than the default
flowClust(rituximab2, varNames=c("FL1.H", "FL3.H"), K=3, B=100,
    min=c(0,0), max=c(400,800), level=0.95, z.cutoff=0.5)
```

---

flowClust.den *generate the curve that reflects the tmixture fitting outcome*

Description

generate the curve that reflects the tmixture fitting outcome

Usage

```
flowClust.den(x, obj, subset, include)
```

Arguments

x	the numeric vector represents the x coordinates in plot
obj	the flowClust object
subset	An integer indicating which variable is selected for the plot. Alternatively, a character string containing the name of the variable is allowed if x@varNames is not NULL.
include	A numeric vector specifying which clusters are shown on the plot. By default, all clusters are included.

---

flowDens-class *Grid of Density Values for the Fitted t Mixture Model with Box-Cox Transformation*

Description

This method constructs the flowDens object which is used to generate a contour or image plot.

Usage

```
## S4 method for signature 'flowClust'
density(
  x,
  data = NULL,
  subset = c(1, 2),
  include = 1:(x@K),
  npoints = c(100, 100),
  from = NULL,
  to = NULL
)

## S4 method for signature 'flowClustList'
density(
  x,
  data = NULL,
  subset = c(1, 2),
  include = 1:(x@K),
  npoints = c(100, 100),
  from = NULL,
  to = NULL
)
```

Arguments

x	Object returned from flowClust or from running filter on a flowFrame object.
data	A matrix, data frame of observations, or object of class flowFrame. This is the object on which flowClust or filter was performed. If this argument is not specified, the grid square upon which densities will be computed must be provided (through arguments from and to).
subset	A numeric vector of length two indicating which two variables are selected for the scatterplot. Alternatively, a character vector containing the names of the two variables is allowed if x@varNames is not NULL.
include	A numeric vector specifying which clusters are included to compute the density values. By default, all clusters are included.
npoints	A numeric vector of size two specifying the number of grid points in x (horizontal) and y (vertical) directions respectively.
from	A numeric vector of size two specifying the coordinates of the lower left point of the grid square. Note that, if this (and to) is not specified, data must be provided such that the range in the two variables (dimensions) selected will be used to define the grid square.
to	A numeric vector of size two specifying the co-ordinates of the upper right point of the grid square.

Details

The flowDens object returned is to be passed to the plot method for generating a contour or image plot.

Value

An object of class flowDens containing the following slots is constructed:

dx	A numeric vector of length npoints[1]; the x-coordinates of the grid points.
dy	A numeric vector of length npoints[2]; the y-coordinates of the grid points.
value	A matrix of size npoints[1] $\times$ npoints[2]; the density values at the grid points.

Author(s)

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See Also

plot, flowClust

`hist.flowClust` *1-D Density Plot (Histogram) of Clustering Results*

Description

This method generates a one-dimensional density plot for the specified dimension (variable) based on the robust model-based clustering results. A histogram of the actual data or cluster assignment is optional for display.

Usage

```
## S3 method for class 'flowClust'
hist(
  x,
  data = NULL,
  subset = 1,
  include = 1:(x@K),
  histogram = TRUE,
  labels = TRUE,
  ylab = "Density",
  main = NULL,
  col = NULL,
  pch = 20,
  cex = 0.6,
  ...
)

## S3 method for class 'flowClustList'
hist(x, ...)
```

Arguments

`x`	Object returned from `flowClust` or from running `filter` on a `flowFrame` object.
`data`	A numeric vector, matrix, data frame of observations, or object of class `flowFrame`. This is the object on which `flowClust` or `filter` was performed.
`subset`	An integer indicating which variable is selected for the plot. Alternatively, a character string containing the name of the variable is allowed if `x@varNames` is not `NULL`.
`include`	A numeric vector specifying which clusters are shown on the plot. By default, all clusters are included.
`histogram`	A logical value indicating whether a histogram of the actual data is made in addition to the density plot or not.
`labels`	A logical value indicating whether information about cluster assignment is shown or not.

ylab	Labels for the x- and y-axes respectively.
main	Title of the plot.
col	Colors of the plotting characters displaying the cluster assignment (if labels is TRUE). If NULL (default), it will be determined automatically.
pch	Plotting character used to show the cluster assignment.
cex	Size of the plotting character showing the cluster assignment.
...	other arguments xlim The range of x-values for the plot. If NULL, the data range will be used. ylim The range of y-values for the plot. If NULL, an optimal range will be determined automatically. breaks Content to be passed to the breaks argument of the generic hist function, if histogram is TRUE. Default is 50, meaning that 50 vertical bars with equal binwidths will be drawn. ... Further arguments passed to curve (and also hist if histogram is TRUE).

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

flowClust, plot, density

Map,flowClust-method *Cluster Assignment Based on Clustering Results*

Description

This method performs cluster assignment according to the posterior probabilities of clustering memberships resulted from the clustering (filtering) operations. Outliers identified will be left unassigned by default.

Usage

```
Map(f, ...)

## S4 method for signature 'flowClust'
Map(f, rm.outliers = TRUE, ...)

## S4 method for signature 'flowClustList'
Map(f, rm.outliers = TRUE, ...)
```

Arguments

f	Object returned from flowClust or filter.
...	Further arguments to be passed to or from other methods.
rm.outliers	A logical value indicating whether outliers will be left unassigned or not.

Value

A numeric vector of size N (the number of observations) indicating to which cluster each observation is assigned. Unassigned observations will be labelled as NA.

Note

Even if rm.outliers is set to FALSE, NA may still appear in the resultant vector due to the filtered observations; see the descriptions about the min.count, max.count, min and max arguments of flowClust.

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

flowClust, filter, posterior

---

plot,flowClust-method *Scatterplot of Clustering Results*

Description

This method generates scatterplot revealing the cluster assignment, cluster boundaries according to the specified percentile as well as supplemental information like outliers or filtered observations.

Usage

```
plot(x, y, ...)

## S4 method for signature 'flowClust,missing'
plot(
  x,
  data,
  subset = c(1, 2),
  ellipse = T,
```

```
  show.outliers = T,
  show.rm = F,
  include = 1:(x@K),
  main = NULL,
  grayscale = F,
  col = (if (grayscale) gray(1/4) else 2:(length(include) + 1)),
  pch = ".",
  cex = 0.6,
  col.outliers = gray(3/4),
  pch.outliers = ".",
  cex.outliers = cex,
  col.rm = 1,
  pch.rm = 1,
  cex.rm = 0.6,
  ecol = 1,
  elty = 1,
  level = NULL,
  u.cutoff = NULL,
  z.cutoff = NULL,
  npoints = 100,
  add = F,
  ...
)

## S4 method for signature 'flowClustList,missing'
plot(
  x,
  data,
  subset = c(1, 2),
  ellipse = T,
  show.outliers = T,
  show.rm = F,
  include = 1:(x@K),
  main = NULL,
  grayscale = F,
  col = (if (grayscale) gray(1/4) else 2:(length(include) + 1)),
  pch = ".",
  cex = 0.6,
  col.outliers = gray(3/4),
  pch.outliers = ".",
  cex.outliers = cex,
  col.rm = 1,
  pch.rm = 1,
  cex.rm = 0.6,
  ecol = 1,
  elty = 1,
  level = NULL,
  u.cutoff = NULL,
```

```
  z.cutoff = NULL,
  npoints = 501,
  add = F,
  ...
)
```

Arguments

x	Object returned from flowClust.
y	missing
...	Further graphical parameters passed to the generic function plot.
data	A matrix, data frame of observations, or object of class flowFrame. This is the object on which flowClust was performed.
subset	A numeric vector of length two indicating which two variables are selected for the scatterplot. Alternatively, a character vector containing the names of the two variables is allowed if x@varNames is not NULL.
ellipse	A logical value indicating whether the cluster boundary is to be drawn or not. If TRUE, the boundary will be drawn according to the level specified by level or cutoff.
show.outliers	A logical value indicating whether outliers will be explicitly shown or not.
show.rm	A logical value indicating whether filtered observations will be shown or not.
include	A numeric vector specifying which clusters will be shown on the plot. By default, all clusters are included.
main	Title of the plot.
grayscale	A logical value specifying if a grayscale plot is desired. This argument takes effect only if the default values of relevant graphical arguments are taken.
col	Color(s) of the plotting characters. May specify a different color for each cluster.
pch	Plotting character(s) of the plotting characters. May specify a different character for each cluster.
cex	Size of the plotting characters. May specify a different size for each cluster.
col.outliers	Color of the plotting characters denoting outliers.
pch.outliers	Plotting character(s) used to denote outliers. May specify a different character for each cluster.
cex.outliers	Size of the plotting characters used to denote outliers. May specify a different size for each cluster.
col.rm	Color of the plotting characters denoting filtered observations.
pch.rm	Plotting character used to denote filtered observations.
cex.rm	Size of the plotting character used to denote filtered observations.
ecol	Color(s) of the lines representing the cluster boundaries. May specify a different color for each cluster.
elty	Line type(s) drawing the cluster boundaries. May specify a different line type for each cluster.

`level`, `u.cutoff`, `z.cutoff`
: These three optional arguments specify the rule used to identify outliers. By default, all of them are left unspecified, meaning that the rule stated in `x@ruleOutliers` will be taken. Otherwise, these arguments will be passed to `ruleOutliers`.

`npoints`
: The number of points used to draw each cluster boundary.

`add`
: A logical value. If `TRUE`, add to the current plot.

Note

The cluster boundaries need not be elliptical since Box-Cox transformation has been performed.

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

`flowClust`

---

plot,flowDens-method *Contour or Image Plot of Clustering Results*

Description

This method makes use of the `flowDens` object returned by `density` to generate a contour or image plot.

Usage

```
## S4 method for signature 'flowDens,missing'
plot(
  x,
  type = c("contour", "image"),
  nlevels = 30,
  scale = c("raw", "log", "sqrt"),
 color = c("rainbow", "heat.colors", "terrain.colors", "topo.colors", "cm.colors",
    "gray"),
  xlab = colnames(x@dx),
  ylab = colnames(x@dy),
  ...
)
```

Arguments

x	The flowDens object returned from density.
type	Either "contour" or "image" to specify the type of plot desired.
nlevels	An integer to specify the number of contour levels or colors shown in the plot.
scale	If "log", the logarithm of the density values will be used to generate the plot; similar interpretation holds for "sqrt". The use of a log or sqrt elicits more information about low density regions.
color	A string containing the name of the function used to generate the desired list of colors.
xlab, ylab	Labels for the x- and y-axes respectively.
...	Other arguments to be passed to contour or image, for example, drawlabels and add. Once an image plot is generated, users may impose a contour plot on it by calling this function with an additional argument add=TRUE.

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

flowClust, density

---

plot,flowFrame,tmixFilterResult-method

Scatterplot / 1-D Density Plot of Filtering (Clustering) Results

---

Description

Depending on the dimensions specified, this method generates either a scatterplot or a one-dimensional density plot (histogram) based on the robust model-based clustering results.

Usage

```
## S4 method for signature 'flowFrame,tmixFilterResult'
plot(x, y, z = NULL, ...)

## S4 method for signature 'flowFrame,tmixFilterResultList'
plot(x, y, z = NULL, ...)
```

Arguments

x	Object of class flowFrame. This is the data object on which filter was performed.
y	Object of class tmixFilterResult or tmixFilterResultList returned from running filter.
z	A character vector of length one or two containing the name(s) of the variable(s) selected for the plot. If it is of length two, a scatterplot will be generated. If it is of length one, a 1-D density plot will be made. If it is unspecified, the first one/two variable(s) listed in y@varNames will be used.
...	All optional arguments passed to the plot or hist method with signature 'flowClust'. Note that arguments x, data and subset have already been provided by y, x and z above respectively.

Note

This plot method is designed such that it resembles the argument list of the plot method defined in the **flowCore** package. The actual implementation is done through the plot or hist method defined for a flowClust object.

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

filter, plot, hist

---

rbox *Reverse Box-Cox Transformation*

Description

This function performs back transformation on Box-Cox transformed data.

Usage

```
rbox(data, lambda)
```

Arguments

data	A numeric vector, matrix or data frame of observations.
lambda	The Box-Cox transformation applied which results in the inputted data matrix.

Value

A numeric vector, matrix or data frame of the same dimension as `data` is returned.

Note

Please refer to the documentation for `box` for details about the Box-Cox transformation in use.

See Also

`box`

`rituximab` *The Rituximab Dataset*

Description

A flow cytometry dataset produced in a drug-screening project to identify agents that would enhance the anti-lymphoma activity of Rituximab, a therapeutic monoclonal antibody. Cells were stained with anti-BrdU FITC and the DNA binding dye 7-AAD.

Format

An object of class `flowFrame` with 1545 cells (rows) and the following eight variables (columns):

FSC.H FSC-Height

SSC.H Side Scatter

FL1.H Anti-BrdU FITC

FL2.H Channel not used

FL3.H 7 AAD

FL1.A Channel not used

FL1.W Channel not used

Time Time

Source

Gasparetto, M., Gentry, T., Sebti, S., O'Bryan, E., Nimmanapalli, R., Blaskovich, M. A., Bhalla, K., Rizzieri, D., Haaland, P., Dunne, J. and Smith, C. (2004) Identification of compounds that enhance the anti-lymphoma activity of rituximab using flow cytometric high-content screening. *J. Immunol. Methods* **292**, 59-71.

ruleOutliers,flowClust-method
Showing or Modifying the Rule used to Identify Outliers

Description

This method shows or modifies the rule used to identify outliers.

Usage

```
ruleOutliers(object)

## S4 method for signature 'flowClust'
ruleOutliers(object)

## S4 method for signature 'flowClustList'
ruleOutliers(object)

ruleOutliers(object) <- value

## S4 replacement method for signature 'flowClust,list'
ruleOutliers(object) <- value
```

Arguments

object	Object returned from flowClust or filter.
value	A list object with one or more of the following named elements: level, u.cutoff and z.cutoff. Their interpretations are the same as those of the corresponding arguments in the flowClust function. Note that when both level and u.cutoff are missing, the rule set by the original value of level or u.cutoff will be unchanged rather than removed. Likewise, when z.cutoff is missing, the rule set by the original value of z.cutoff will be retained.

Value

The replacement method modifies object@ruleOutliers (or object[[k]]@ruleOutliers if object is of class flowClustList or tmixFilterResultList) AND updates the logical vector object@flagOutliers (or object[[k]]@ruleOutliers) according to the new rule.

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

flowClust, filter

---

show,flowClust-method *Show Method for flowClust / tmixFilterResult Object*

Description

This method lists out the slots contained in a flowClust object.

Usage

```
## S4 method for signature 'flowClust'
show(object)

## S4 method for signature 'flowClustList'
show(object)

## S4 method for signature 'tmixFilter'
show(object)

## S4 method for signature 'tmixFilterResult'
show(object)

## S4 method for signature 'tmixFilterResultList'
show(object)
```

Arguments

object	Object returned from flowClust or filter.

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

See Also

flowClust, filter, summary

split,flowClust-method

Splitting Data Based on Clustering Results

Description

This method splits data according to results of the clustering (filtering) operation. Outliers identified will be removed by default.

Usage

```
split(x, f, drop = FALSE, ...)

## S4 method for signature 'data.frame,flowClust'
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'matrix,flowClust'
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'vector,flowClust'
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'flowFrame,flowClust'
```

```
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'flowFrame,tmixFilterResult'
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'flowFrame,flowClustList'
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'data.frame,flowClustList'
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'matrix,flowClustList'
split(
  x,
  f,
  drop = FALSE,
```

```
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'vector,flowClustList'
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)

## S4 method for signature 'flowFrame,tmixFilterResultList'
split(
  x,
  f,
  drop = FALSE,
  population = NULL,
  split = NULL,
  rm.outliers = TRUE,
  ...
)
```

Arguments

x	A numeric vector, matrix, data frame of observations, or object of class flowFrame. This is the object on which flowClust or filter was performed.
f	Object returned from flowClust or filter.
drop	A logical value indicating whether to coerce a column matrix into a vector, if applicable. Default is FALSE, meaning that a single-column matrix will be retained.
...	Further arguments to be passed to or from other methods.
population	An optional argument which specifies how to split the data. If specified, it takes a list object with named or unnamed elements each of which is a numeric vector specifying which clusters are included. If this argument is left unspecified, the data object will be split into K subsets each of which is formed by one out of the K clusters used to model the data. See examples for more details.
split	This argument is deprecated. Should use population instead.
rm.outliers	A logical value indicating whether outliers are removed or not.

Value

A list object with elements each of which is a subset of x and also retains the same class as x. If the split argument is specified with a list of named elements, those names will be used to name the corresponding elements in the resultant list object.

Usage

split(x, f, drop=FALSE, population=NULL, split=NULL, rm.outliers=TRUE, ...)

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

Subset, flowClust, filter

Subset,flowClust-method

Subsetting Data Based on Clustering Results

Description

This method returns a subset of data upon the removal of outliers identified from the clustering (filtering) operations.

Usage

```
## S4 method for signature 'flowFrame,flowClust'
Subset(x, subset, ...)

## S4 method for signature 'flowFrame,tmixFilterResult'
Subset(x, subset, ...)

## S4 method for signature 'data.frame,flowClust'
Subset(x, subset, ...)

## S4 method for signature 'matrix,flowClust'
Subset(x, subset, ...)

## S4 method for signature 'vector,flowClust'
Subset(x, subset, ...)
```

```
## S4 method for signature 'ANY,flowClustList'
Subset(x, subset, ...)

## S4 method for signature 'flowFrame,tmixFilterResultList'
Subset(x, subset, ...)
```

Arguments

x	A numeric vector, matrix, data frame of observations, or object of class flowFrame. This is the object on which flowClust or filter was performed.
subset	Object returned from flowClust or filter.
...	Further arguments to be passed to or from other methods.

Value

An object which is a subset of x. It also retains the same class as x.

Usage

Subset(x, subset, ...)

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

split, flowClust, filter

---

summary,flowClust-method

Summary Method for flowClust Object

Description

This method prints out various characteristics of the model fitted via robust model-based clustering.

Usage

```
summary(object, ...)

## S4 method for signature 'flowClust'
summary(object)

## S4 method for signature 'flowClustList'
summary(object)
```

Arguments

object	Object returned from flowClust or from filter.
...	not used

Details

Various characteristics of the fitted model will be given under the following five categories: Experiment Information, Clustering Summary, Transformation Parameter, Information Criteria, and Data Quality. Under Data Quality, information about data filtering, outliers, and uncertainty is given.

Author(s)

Raphael Gottardo <<raph@stat.ubc.ca>>, Kenneth Lo <<c.lo@stat.ubc.ca>>

See Also

flowClust, filter, show

---

tmixFilter-class *Creating Filters and Filtering Flow Cytometry Data*

Description

The tmixFilter function creates a filter object which is then passed to the filter method that performs filtering on a flowFrame object. This method pair is provided to let **flowClust** integrate with the **flowCore** package.

Usage

```
tmixFilter(filterId = "tmixFilter", parameters = "", ...)

## S4 method for signature 'ANY,flowClust'
x %in% table

## S4 method for signature 'flowFrame,tmixFilterResult'
x %in% table
```

```
## S4 method for signature 'flowFrame,tmixFilter'
x %in% table

## S4 method for signature 'ANY,tmixFilterResult'
x %in% table

## S4 method for signature 'ANY,flowClustList'
x %in% table

## S4 method for signature 'ANY,tmixFilterResultList'
x %in% table

## S4 method for signature 'flowFrame,flowClust'
x[i, j, ..., drop = FALSE]

## S4 method for signature 'flowFrame,tmixFilterResult'
x[i, j, ..., drop = FALSE]

## S4 method for signature 'flowFrame,flowClustList'
x[i, j, ..., drop = FALSE]

## S4 method for signature 'flowFrame,tmixFilterResultList'
x[i, j, ..., drop = FALSE]

## S4 method for signature 'tmixFilterResultList,ANY'
x[[i, j, ..., exact = TRUE]]

## S4 method for signature 'tmixFilterResultList'
length(x)

## S4 method for signature 'tmixFilterResult,tmixFilter'
summarizeFilter(result, filter)
```

Arguments

filterId	A character string that identifies the filter created.
parameters	A character vector specifying the variables to be used in filtering. When it is left unspecified, all the variables of the flowFrame object are used when running filter. Note that its content will be passed to the varNames argument of flowClust when running filter.
...	Other arguments passed to the flowClust function when running filter, namely, expName, K, B, tol, nu, lambda, nu.est, trans, min.count, max.count, min, max, level, u.cutoff, z.cutoff, randomStart, B.init, tol.init, seed and criterion. All arguments are optional except K that specifies the number of clusters. The tmixFilter function returns an object of class tmixFilter that stores all the settings required for performing the filter operations. The filter method is defined in package flowCore and returns an object of

class tmixFilterResult (or tmixFilterResultList if filter has a length >1) that stores the filtering results.

The tmixFilter function returns an object of class tmixFilter that extends the virtual parent filter class in the **flowCore** package. Hence, the filter operators, namely, &, |, ! and subset, also work for the tmixFilter class.

If filter is of length 1, the filter method returns an object of class tmixFilterResult. This class extends both the multipleFilterResult class (in the **flowCore** package) and the flowClust class. Operations defined for the multipleFilterResult class, like %in%, Subset and split, also work for the tmixFilterResult class. Likewise, methods or functions designed to retrieve filtering (clustering) information from a flowClust object can also be applied on a tmixFilterResult object. These include criterion, ruleOutliers, ruleOutliers<-, Map, posterior, importance, uncertainty and getEstimates. Various functionalities for plotting the filtering results are also available (see the links below).

If filter has a length >1, the function returns an object of class tmixFilterResultList. This class extends both the flowClustList class and the multipleFilterResult class. Note that when a tmixFilterResultList object is used in place of a tmixFilterResult object, in most cases the list element corresponding to the best model will be extracted and passed to the method/function call.

x	flowFrame
table	tmixFilterResult
i	tmixFilterResult or tmixFilterResultList
j, drop, exact	not used
result	tmixFilterResult
filter	tmixFilter

References

Lo, K., Brinkman, R. R. and Gottardo, R. (2008) Automated Gating of Flow Cytometry Data via Robust Model-based Clustering. *Cytometry A* **73**, 321-332.

See Also

flowClust, summary, plot, density, hist, Subset, split, ruleOutliers, Map

Examples

```
### The example below largely resembles the one in the flowClust
### man page.  The main purpose here is to demonstrate how the
### entire cluster analysis can be done in a fashion highly
### integrated into flowCore.


data(rituximab)
library(flowCore)

### create a filter object
s1filter <- tmixFilter("s1", c("FSC.H", "SSC.H"), K=1)
```

```
### cluster the data using FSC.H and SSC.H
res1 <- filter(rituximab, s1filter)

### remove outliers before proceeding to the second stage
# %in% operator returns a logical vector indicating whether each
# of the observations lies inside the gate or not
rituximab2 <- rituximab[rituximab %in% res1,]
# a shorthand for the above line
rituximab2 <- rituximab[res1,]
# this can also be done using the Subset method
rituximab2 <- Subset(rituximab, res1)

### cluster the data using FL1.H and FL3.H (with 3 clusters)
s2filter <- tmixFilter("s2", c("FL1.H", "FL3.H"), K=3)
res2 <- filter(rituximab2, s2filter)

show(s2filter)
show(res2)
summary(res2)

# to demonstrate the use of the split method
split(rituximab2, res2)
split(rituximab2, res2, population=list(sc1=c(1,2), sc2=3))

# to show the cluster assignment of observations
table(Map(res2))

# to show the cluster centres (i.e., the mean parameter estimates
# transformed back to the original scale) and proportions
getEstimates(res2)

### demonstrate the use of various plotting methods
# a scatterplot
plot(rituximab2, res2, level=0.8)
plot(rituximab2, res2, level=0.8, include=c(1,2), grayscale=TRUE,
    pch.outliers=2)
# a contour / image plot
res2.den <- density(res2, data=rituximab2)
plot(res2.den)
plot(res2.den, scale="sqrt", drawlabels=FALSE)
plot(res2.den, type="image", nlevels=100)
plot(density(res2, include=c(1,2), from=c(0,0), to=c(400,600)))
# a histogram (1-D density) plot
plot(rituximab2, res2, "FL1.H")

### to demonstrate the use of the ruleOutliers method
summary(res2)
# change the rule to call outliers
ruleOutliers(res2) <- list(level=0.95)
# augmented cluster boundaries lead to fewer outliers
summary(res2)

# the following line illustrates how to select a subset of data
```

```
# to perform cluster analysis through the min and max arguments;
# also note the use of level to specify a rule to call outliers
# other than the default
s2t <- tmixFilter("s2t", c("FL1.H", "FL3.H"), K=3, B=100,
    min=c(0,0), max=c(400,800), level=0.95, z.cutoff=0.5)
filter(rituximab2, s2t)
```

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