Package 'flowBin'

April 23, 2025

Type Package

Title Combining multitube flow cytometry data by binning

Version 1.44.0

Date 2013-5-11

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Description Software to combine flow cytometry data that has been multiplexed into multiple tubes with common markers between them, by establishing common bins across tubes in terms of the common markers, then determining expression within each tube for each bin in terms of the tube-specific markers.

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LazyLoad yes

Imports class, limma, snow, BiocGenerics

Depends methods, flowCore, flowFP, R (>= 2.10)

Suggests parallel

biocViews ImmunoOncology, CellBasedAssays, FlowCytometry

Collate 'AllClasses.R' 'AllGenerics.R' 'BinnedFlowSample-accessors.R' 'checkQNorm.R' 'flowBin.R' 'flowFPBin.R' 'FlowSample-accessors.R' 'getBinExpr.R' 'kMeansBin.R' 'mapClustersKNN.R' 'quantileNormalise.R' 'removeSparseBins.R'

git_url https://git.bioconductor.org/packages/flowBin

git_branch RELEASE_3_21

git_last_commit 49acac2

git_last_commit_date 2025-04-15

Repository Bioconductor 3.21

Date/Publication 2025-04-23

Contents

`amlsample` *Multitube AML sample as example data for flowBin*

Description

Multitube AML sample as example data for flowBin

Format

a `flowSample` containing 7 tubes with 3 common parameters and 4 measure parameters per tube.

Source

FlowRepository.org accession FR-FCM-ZZYA

`BinnedFlowSample` *A* `FlowSample`*, but with binning information for each tube*

Description

`clust.labels`**:** `list` of cluster label vectors, one for each tubeNote: all slots can be get and set using accessor methods, for example `bin.pars(myFlowSet) <- c(1,2,5)`

checkQNorm *Function to check the quantile normalisation of a FlowSample using flowFP*

Description

Function to check the quantile normalisation of a FlowSample using flowFP

Details

object and normed.object are compared using `flowFP` binning, to assess the deviation in bin counts between the two.

Value

`list` containing two matrices of standard deviations across bins (rows) vs tubes (columns) for before (`sd.before`) and after (`sd.after`).

Examples

```
data(amlsample)
normed.sample <- quantileNormalise(aml.sample)
qnorm.check <- checkQNorm(aml.sample, normed.sample, do.plot=FALSE)
show(qnorm.check)
```

eventsInBins *Count number of events for each tube in each bin*

Description

Useful for QA of bin mapping

flowBin *function to run the entire flowBin pipeline*

Description

Takes a list of flowFrames representing tubes from a single flow cytometry sample, and combines them using binning of events in terms of common markers across tubes.

Usage

```
flowBin(tube.list, bin.pars, control.tubes = vector(),
  measure.pars = NULL,
  sample.name = "Unnamed Flow Expr Set",
  bin.method = "kmeans", expr.method = "medianFI",
  sparse.bin.thresh = 0.001, dequantize = T,
  snow.cluster = NULL, n.bins = 128, scale.expr = F,
  do.qnorm = T, return.bins = F)
```

Arguments

`tube.list`	a list of flowFrames, one for each tube to combine
`bin.pars`	a numerical vector indicating which flow parameters in the each flowFrame to use for combining tubes. These should be the same markers assayed across every tube.
`control.tubes`	a vector indicating which tubes in tube.list to use for negative controls. May be empty.
`measure.pars`	a list of which parameters to measure expression for, with one vector for each tube. If left NULL, this defaults to all parameters other than those specified as `bin.pars`
`sample.name`	name of this flowSample, for convenience (defaults to 'Unnamed Flow Expr Set')
`bin.method`	The method to use for creating bins. The two options are "kmeans" for k-means clustering and nearest-neighbour mapping of bins. or "flowFP" for flowFP binning and direct mapping of bin boundaries across tubes.
`expr.method`	The method to use to compute bin expression across tubes. This defaults to MFI of the cells belonging to that bin in each tube. Other options are
`sparse.bin.thresh`	Bins which contain fewer than this proportion of total events in any tube will be excluded as outliers. Defaults to 0.001
`dequantize`	If TRUE, adds a small (region of 1e-8) value to flow data to help break ties when binning.
`snow.cluster`	A cluster created using the `snow` package, which flowBin will use to speed up computation. If NULL, flowBin will execute in serial mode.
`n.bins`	Number of bins to use. Note that this must be a power of 2 if flowFP is selected as binning method.
`scale.expr`	If TRUE, the resulting expression values will be scaled to (0,1) using the ranges specified in the flowFrames in `tube.list`.
`do.qnorm`	If TRUE, the binning markers will be quantile normalized prior to binning.
`return.bins`	If TRUE, return a `BinnedFlowExprSet` containing the bins themselves as well as the expression for each bin.

Value

A matrix containing expression values for each bin in terms of each marker across all tubes. If `return.bins` is set TRUE, then a list containing a `BinnedFlowExprSet` followed by the expression matrix is returned.

Examples

```
data(amlsample)
tube.combined <- flowBin(aml.sample@tube.set,
bin.pars=aml.sample@bin.pars,
bin.method='flowFP',
control.tubes=aml.sample@control.tubes,
expr.method='medianFIDist', scale.expr=TRUE)
heatmap(tube.combined, scale='none')
```

`flowFPBin` *Bin sample using flowFP binning*

Description

Bin sample using flowFP binning

Arguments

`object`	flowSample to bin
`n.bins=128`	number of bins to use. This should be a power of 2, and will be rounded down to the nearest power of 2 if not.
`snow.cluster=NULL`	Optional snow cluster to use for parallel execution.
`dequantize=T`	If TRUE, adds a small (region of 1e-8) value to flow data to help break ties when binning.

Value

a `BinnedFlowSample`

Examples

```
data(amlsample)
normed.sample <- quantileNormalise(aml.sample)
res <- flowFPBin(normed.sample)
```

FlowSample — *A class similar to flowSet, but with extra information needed by flowBin*

Description

name: character string - name of the object

tube.set: list of flowFrames containing raw flow data.

control.tubes: Integer vector indicating which tubes in the list (if any) to use as negative controls. May be empty.

bin.pars: Integer vector indicating which parameters to use for binning. These must be in the same position in all tubes.

measure.pars: list of integer vectors indicating which parameters to use for measurement. These must be specified per tube.Note: all slots can be get and set using accessor methods, for example `bin.pars(myFlowSet) <- c(1,2,5)`

getBinExpr — *Calculate the expression of each bin in a BinnedFlowSample in terms of the measurement markers*

Description

getBinExpr main function definition

Arguments

method	Method to use to compute expression, passed as a string. Defaults to medianFI, which takes the simple median of each bin, and does not require control tubes. Other options available are medianFIDist, which uses medians with the median of the negative control subtracted out, and propPos which sets a threshold at the 98th percentile of the negative control and determines what proportion of cells lie above that.
include.bin.medians	logical, specifies whether to compute the medians of each bin in terms of the binning markers and include them in the result or not. Defaults to T.
scale	logical specifying whether to scale the results to the interval (0,1). If T (default), then all medians will be divided by the range for that marker as specified in the flowFrame.

Value

A numeric matrix containing expression values, with bins as rows and markers as columns

Examples

```
data(amlsample)
normed.sample <- quantileNormalise(aml.sample)
binned.sample <- flowFPBin(normed.sample)
binned.sample <- removeSparseBins(binned.sample, 0.001)
bin.expr <- getBinExpr(binned.sample)
heatmap(bin.expr, scale='none')
```

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kMeansBin *Bin sample using K-means binning*

Description

Bin sample using K-means binning

Arguments

object	flowSample to bin
n.bins=128	number of bins to use. This should be a power of 2, and will be rounded down to the nearest power of 2 if not.
n.neighbours=1	number of neighbours to use for KNN mapping of bins from clustered tube
snow.cluster=NULL	Optional snow cluster to use for parallel execution.
random.seed=101	Random seed to set to make K-means clustering deterministic.
dequantize=T	If TRUE, adds a small (region of 1e-8) value to flow data to help break ties when binning.

Details

Runs K-means clustering on the binning markers in the first tube of the data set. These clusters are then mapped to the other tubes using K-nearest neighbours.

Value

a BinnedFlowSample

Examples

```
data(amlsample)
normed.sample <- quantileNormalise(aml.sample)
res <- kMeansBin(normed.sample)
```

mapBinsKNN *Internal function to map bins by KNN*

Description

Internal function to map bins by KNN

Arguments

object	flowSample to map the bins of
tube.1.labels	integer vector of bin labels for the events in tube 1
n.neighbours=1	number of neighbours to use for KNN mapping of bins from clustered tube
snow.cluster=NULL	Optional snow cluster to use for parallel execution.
dequant=T	If TRUE, adds a small (region of 1e-8) value to flow data to help break ties when binning.

Details

Takes a FlowSample and labels for the events in tube 1, and maps these to all other tubes.

Value

a BinnedFlowSample

Examples

```
data(amlsample)
tube1.expr <- exprs(tube.set(aml.sample)[[1]])
kmeans.res <- kmeans(tube1.expr, 100)
kmeans.labels <- kmeans.res$cluster

#Now create a binnedFlowExprSet using the cluster labels for tube 1
clustered.sample <- mapBinsKNN(aml.sample, kmeans.labels)
sort(table(bin.labels(clustered.sample)[[3]]))
```

quantileNormalise *quantileNormalise normalise binning paramaters across all tubes of a flowSample*

Description

Since the binning parameters are the same across tubes, and samples each tube is an aliquot from the same sample, these should have the same underlying distribution. Hence, quantile normalisation can be used to force this to be so, removing technical variation.

Examples

```
data(amlsample)
normed.sample <- quantileNormalise(aml.sample)
qnorm.check <- checkQNorm(aml.sample, normed.sample, do.plot=FALSE)
show(qnorm.check)
```

`removeSparseBins` *Remove bins from a BinnedFlowSample with few events in them*

Description

Remove bins from a BinnedFlowSample with few events in them

Arguments

`object`	the BinnedFlowSample to act on
`cutoff.prop=NULL`	the minimum proportion that a bin must contain to be kept. If NULL, only bins with no events in at least one tube will be removed.

Details

This is important to do prior to calculating bin expression, as bins containing 2 or less events, for, example, cannot have their median computed.

Value

a BinnedFlowSample with sparse bins removed

`show-methods` *Methods to view flowBin objects*

Description

Methods for function `show` in Package flowBin

Methods

`signature(object = "BinnedFlowExprSet")` Show number of bins and samples for a BinnedFlowExprSet.

`signature(object = "CVResult")` Show various statistics stored in a CVResult object.

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