

Package ‘ClassDiscovery’

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Title Classes and Methods for “Class Discovery” with Microarrays or Proteomics

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Imports methods, stats, graphics, grDevices, mclust, oompaData, Biobase

Suggests xtable

Description The ClassDiscovery package defines the classes used for “class discovery” problems in the OOMPA project. Class discovery primarily consists of unsupervised clustering methods with attempts to assess their statistical significance.

License Apache License (== 2.0)

LazyLoad yes

biocViews Microarray, Clustering

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aspectHeatmap	<i>Heatmap with control over the aspect ratio</i>
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Description

This function replaces the heatmap function in the stats package with a function with additional features. First, the user can specify an aspect ratio instead of being forced to accept a square image even when the matrix is not square. Second, the user can overlay horizontal or vertical lines to mark out important regions.

Usage

```
aspectHeatmap(x,
              Rowv=NULL,
              Colv=if (symm) "Rowv" else NULL,
              distfun=dist,
              hclustfun=hclust,
              reorderfun=function(d, w) reorder(d, w),
              add.expr,
              symm=FALSE,
              revC=identical(Colv, "Rowv"),
              scale=c("row", "column", "none"),
              na.rm=TRUE,
              margins=c(5, 5),
              ColSideColors,
              RowSideColors,
              cexRow=0.2 + 1/log10(nr),
              cexCol=0.2 + 1/log10(nc),
              labRow=NULL,
              labCol=NULL,
              main=NULL,
              xlab=NULL,
              ylab=NULL,
              keep.dendro=FALSE,
              verbose=getOption("verbose"),
              hExp=1,
              wExp=1,
              vlines=NULL,
              hlines=NULL,
              lasCol=2,
              ...)
```

Arguments

x	See documentation for heatmap .
Rowv	See documentation for heatmap .
Colv	See documentation for heatmap .
distfun	See documentation for heatmap .
hclustfun	See documentation for heatmap .

reorderfun	See documentation for heatmap .
add.expr	See documentation for heatmap .
symm	See documentation for heatmap .
revC	See documentation for heatmap .
scale	See documentation for heatmap .
na.rm	See documentation for heatmap .
margins	See documentation for heatmap .
ColSideColors	See documentation for heatmap .
RowSideColors	See documentation for heatmap .
cexRow	See documentation for heatmap .
cexCol	See documentation for heatmap .
labRow	See documentation for heatmap .
labCol	See documentation for heatmap .
main	See documentation for heatmap .
xlab	See documentation for heatmap .
ylab	See documentation for heatmap .
keep.dendro	See documentation for heatmap .
verbose	See documentation for heatmap .
hExp	height expansion factor (default is 1)
wExp	width expansion factor (default is 1)
vlines	vector of positions at which to draw vertical lines
hlines	vector of positions at which to draw horizontal lines
lasCol	axis label style (las) for columns
...	additional arguments passed along to the image command

Details

The new arguments hExp and wExp are "expansion factors" for the height and width of the figure, respectively. They are used to alter the arguments passed to the layout function internally to heatmap.

The new arguments hlines and vlins are used to specify points at which horizontal or vertical lines, respectively, should be overlaid on the image.

Value

The same as the [heatmap](#) function.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

See Also

[heatmap](#)

Examples

```
nC <- 30
nR <- 1000
fakeData <- matrix(rnorm(nC*nR), ncol=nC, nrow=nR)
aspectHeatmap(fakeData, scale='none', hExp=2, wExp=1.4, margins=c(6,3))
aspectHeatmap(fakeData, scale='none', hExp=2, wExp=1.4, margins=c(6,3),
               vlines=15.5, hlines=c(100, 300))
```

BootstrapClusterTest *Class "BootstrapClusterTest"*

Description

Performs a nonparametric bootstrap (sampling with replacement) test to determine whether the clusters found by an unsupervised method appear to be robust in a given data set.

Usage

```
BootstrapClusterTest(data, FUN, subsetSize, nTimes=100, verbose=TRUE, ...)
```

Arguments

data	A data matrix, numerical data frame, or ExpressionSet object.
FUN	A function that, given a data matrix, returns a vector of cluster assignments. Examples of functions with this behavior are cutHclust , cutKmeans , cutPam , and cutRepeatedKmeans .
...	Additional arguments passed to the classifying function, FUN.
subsetSize	An optional integer argument. If present, each iteration of the bootstrap selects subsetSize rows from the original data matrix. If missing, each bootstrap contains the same number of rows as the original data matrix.
nTimes	The number of bootstrap samples to collect.
verbose	A logical flag

Objects from the Class

Objects should be created using the BootstrapClusterTest function, which performs the requested bootstrap on the clusters. Following the standard R paradigm, the resulting object can be summarized and plotted to determine the results of the test.

Slots

f: A function that, given a data matrix, returns a vector of cluster assignments. Examples of functions with this behavior are [cutHclust](#), [cutKmeans](#), [cutPam](#), and [cutRepeatedKmeans](#).

subsetSize: The number of rows to be included in each bootstrap sample.

nTimes: An integer, the number of bootstrap samples that were collected.

call: An object of class call, which records how the object was produced.

result: Object of class matrix containing, for each pair of columns in the original data, the number of times they belonged to the same cluster of a bootstrap sample.

Extends

Class [ClusterTest](#), directly. See that class for descriptions of the inherited methods `image` and `hist`.

Methods

summary signature(object = BootstrapClusterTest): Write out a summary of the object.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

References

Kerr MK, Churchill GJ.

Bootstrapping cluster analysis: Assessing the reliability of conclusions from microarray experiments.

PNAS 2001; 98:8961-8965.

See Also

[ClusterTest](#), [PerturbationClusterTest](#)

Examples

```
showClass("BootstrapClusterTest")

## simulate data from two different groups
d1 <- matrix(rnorm(100*30, rnorm(100, 0.5)), nrow=100, ncol=30, byrow=FALSE)
d2 <- matrix(rnorm(100*20, rnorm(100, 0.5)), nrow=100, ncol=20, byrow=FALSE)
dd <- cbind(d1, d2)
cols <- rep(c('red', 'green'), times=c(30,20))
colnames(dd) <- paste(cols, c(1:30, 1:20), sep='')
## perform your basic hierarchical clustering...
hc <- hclust(distanceMatrix(dd, 'pearson'), method='complete')

## bootstrap the clusters arising from hclust
bc <- BootstrapClusterTest(dd, cutHclust, nTimes=200, k=3, metric='pearson')
summary(bc)

## look at the distribution of agreement scores
hist(bc, breaks=101)

## let heatmap compute a new dendrogram from the agreement
image(bc, col=blueyellow(64), RowSideColors=cols, ColSideColors=cols)

## plot the agreement matrix with the original dendrogram
image(bc, dendrogram=hc, col=blueyellow(64), RowSideColors=cols, ColSideColors=cols)

## bootstrap the results of PAM
pamc <- BootstrapClusterTest(dd, cutPam, nTimes=200, k=3)
image(pamc, dendrogram=hc, col=blueyellow(64), RowSideColors=cols, ColSideColors=cols)

## contrast the behavior when all the data comes from the same group
xx <- matrix(rnorm(100*50, rnorm(100, 0.5)), nrow=100, ncol=50, byrow=FALSE)
hct <- hclust(distanceMatrix(xx, 'pearson'), method='complete')
```

```

bct <- BootstrapClusterTest(xx, cutHclust, nTimes=200, k=4, metric='pearson')
summary(bct)
image(bct, dendrogram=hct, col=blueyellow(64), RowSideColors=cols, ColSideColors=cols)

## cleanup
rm(d1, d2, dd, cols, hc, bc, pamc, xx, hct, bct)

```

cluster3

Cluster a Dataset Three Ways

Description

Produces and plots dendrograms using three similarity measures: Euclidean distance, Pearson correlation, and Manhattan distance on dichotomized data.

Usage

```
cluster3(data, eps=logb(1, 2), name="", labels=dimnames(data)[[2]])
```

Arguments

data	A matrix, numeric data.frame, or ExpressionSet object.
eps	A numerical value; the threshold at which to dichotomize the data
name	A character string to label the plots
labels	A vector of character strings used to label the items in the dendrograms.

Value

Invisibly returns the data object on which it was invoked.

Author(s)

Kevin R. Coombes emailkrc@silicovore.com

See Also

[hclust](#)

Examples

```

## simulate data from two different classes
d1 <- matrix(rnorm(100*30, rnorm(100, 0.5)), nrow=100, ncol=30, byrow=FALSE)
d2 <- matrix(rnorm(100*20, rnorm(100, 0.5)), nrow=100, ncol=20, byrow=FALSE)
dd <- cbind(d1, d2)
## cluster it 3 ways
par(mfrow=c(2,2))
cluster3(dd)
par(mfrow=c(1,1))

```

ClusterTest-class	Class "ClusterTest"
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Description

This is a base class for tests that attempt to determine whether the groups found by an unsupervised clustering method are statistically significant.

Usage

```
## S4 method for signature 'ClusterTest'
image(x, dendrogram, ...)
```

Arguments

<code>x</code>	An object of the ClusterTest class.
<code>dendrogram</code>	An object with S3 class <code>hclust</code> , as returned by the hclust function.
<code>...</code>	Additional graphical parameters to be passed to the standard heatmap function that is used to implement the <code>image</code> method.

Objects from the Class

Objects can be created by calls of the form `new("ClusterTest", ...)`. Most users, however, will only create objects from one of the derived classes such as [BootstrapClusterTest](#) or [PerturbationClusterTest](#).

Slots

call: An object of class `call`, which shows how the object was constructed.

result: A symmetric matrix containing the results of the cluster reproducibility test. The size of the matrix corresponds to the number of samples (columns) in the data set on which the test was performed. The result matrix should contain "agreement" values between 0 and 1, representing for each pair of samples the fraction of times that they were collected into the same cluster.

Methods

hist signature(`x = "ClusterTest"`): Produces a histogram of the agreement fractions. When a true group structure exists, one expects a multimodal distribution, with one group of agreements near 0 (for pairs belonging to different clusters) and one group of agreements near 1 (for pairs belonging to the same cluster).

image signature(`x = "ClusterTest"`): Uses the heatmap function to display the agreement matrix. The optional `dendrogram` argument should be used to display the extent to which the agreement matrix matches the results of hierarchical clustering using the full data set. This method invisibly returns the result of a call to `heatmap`; thus, you can use `keep.dendro=TRUE` to recover the cluster assignments from the dendrograms.

summary signature(`object = "ClusterTest"`): Write out a summary of the object.

Author(s)

Kevin R. Coombes emailkrc@silicovore.com

References

Kerr MK, Churchill GJ.
Bootstrapping cluster analysis: Assessing the reliability of conclusions from microarray experiments.
 PNAS 2001; 98:8961-8965.

See Also

[BootstrapClusterTest](#), [PerturbationClusterTest](#), [heatmap](#)

Examples

```
showClass("ClusterTest")

## simulate data from two different classes
d1 <- matrix(rnorm(100*30, rnorm(100, 0.5)), nrow=100, ncol=30, byrow=FALSE)
d2 <- matrix(rnorm(100*20, rnorm(100, 0.5)), nrow=100, ncol=20, byrow=FALSE)
dd <- cbind(d1, d2)

## cluster the data
hc <- hclust(distanceMatrix(dd, 'pearson'), method='average')

## make a fake reproducibility matrix
fraud <- function(x) {
  new('ClusterTest', result=abs(cor(x)), call=match.call())
}

fake <- fraud(dd)
summary(fake)

hist(fake)

image(fake) # let heatmap compute a new dendrogram from the agreements

image(fake, dendrogram=hc) # use the actual dendrogram from the data

image(fake, dendrogram=hc, col=blueyellow(64)) # change the colors

## cleanup
rm(fake, fraud, hc, dd, d1, d2)
```

distanceMatrix

Distance Matrix Computation

Description

This function computes and returns the distance matrix determined by using the specified distance metric to compute the distances between the columns of a data matrix.

Usage

```
distanceMatrix(dataset, metric, ...)
```


Arguments

dataset	A numeric matrix or an ExpressionSet
metric	A character string defining the distance metric. This can be pearson, sqrt pearson, spearman, absolute pearson, uncentered correlation, weird or any of the metrics accepted by the dist function. At present, the latter function accepts euclidean, maximum, manhattan, canberra, binary, or minkowski. Any initial substring that uniquely defines one of the metrics will work.
...	Additional parameters to be passed on to dist .

Details

This function differs from [dist](#) in two ways, both of which are motivated by common practice in the analysis of microarray or proteomics data. First, it computes distances between column vectors instead of between row vectors. In a typical microarray experiment, the data is organized so the rows represent genes and the columns represent different biological samples. In many applications, relations between the biological samples are more interesting than relationships between genes. Second, distanceMatrix adds additional distance metrics based on correlation.

- **pearson**The most common metric used in the microarray literature is the pearson distance, which can be computed in terms of the Pearson correlation coefficient as $(1 - \text{cor}(\text{dataset}))/2$.
- **uncentered correlation**This metric was introduced in the Cluster and TreeView software from the Eisen lab at Stanford. It is computed using the formulas for Pearson correlation, but assuming that both vectors have mean zero.
- **spearman**The spearman metric used the same formula, but substitutes the Spearman rank correlation for the Pearson correlation.
- **absolute pearson**The absolute pearson metric used the absolute correlation coefficient; i.e., $(1 - \text{abs}(\text{cor}(\text{dataset})))$.
- **sqrt pearson**The sqrt pearson metric used the square root of the pearson distance metric; i.e., $\text{sqrt}(1 - \text{cor}(\text{dataset}))$.
- **weird**The weird metric uses the Euclidean distance between the vectors of correlation coefficients; i.e., $\text{dist}(\text{cor}(\text{dataset}))$.

Value

A distance matrix in the form of an object of class `dist`, of the sort returned by the `dist` function or the `as.dist` function.

BUGS

It would be good to have a better name for the weird metric.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

See Also

[dist](#), [as.dist](#)

Examples

```
dd <- matrix(rnorm(100*5, rnorm(100)), nrow=100, ncol=5)
distanceMatrix(dd, 'pearson')
distanceMatrix(dd, 'euclid')
distanceMatrix(dd, 'sqrt')
distanceMatrix(dd, 'weird')
rm(dd) # cleanup
```

GenePCA	<i>Class "GenePCA"</i>
---------	------------------------

Description

Perform principal components analysis on the genes (rows) from a microarray or proteomics experiment.

Usage

```
GenePCA(geneData)
## S4 method for signature 'GenePCA,missing'
plot(x, splitter=0)
```

Arguments

geneData	A data matrix, with rows interpreted as genes and columns as samples
x	a GenePCA object
splitter	A logical vector classifying the genes.

Details

This is a preliminary attempt at a class for principal components analysis of genes, parallel to the [SamplePCA](#) class for samples. The interface will (one hopes) improve markedly in the next version of the library.

Value

The GenePCA function constructs and returns a valid object of the GenePCA class.

Objects from the Class

Objects should be created using the GenePCA generator function.

Slots

scores: A matrix of size $P \times N$, where P is the number of rows and N the number of columns in the input, representing the projections of the input rows onto the first N principal components.

variances: A numeric vector of length N ; the amount of the total variance explained by each principal component.

components: A matrix of size $N \times N$ containing each of the first P principal components as columns.

Methods

plot signature(x = GenePCA, y = missing): Plot the genes in the space of the first two principal components.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

See Also

[SamplePCA](#), [princomp](#)

Examples

```
showClass("GenePCA")

## simulate samples from three different groups, with generic genes
d1 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d2 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d3 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
dd <- cbind(d1, d2, d3)

## perform PCA in gene space
gpc <- GenePCA(dd)

## plot the results
plot(gpc)

## cleanup
rm(d1, d2, d3, dd, gpc)
```

hclust

Class "hclust"

Description

Creates an S4 class equivalent to the S3 hclust class returned by the hclust function.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

See Also

[cutree](#), [hclust](#), [heatmap](#)

Description

Unsupervised clustering algorithms, such as partitioning around medoids ([pam](#)), K-means ([kmeans](#)), or hierarchical clustering ([hclust](#)) after cutting the tree, produce a list of class assignments along with other structure. To simplify the interface for the [BootstrapClusterTest](#) and [PerturbationClusterTest](#), we have written these routines that simply extract these cluster assignments.

Usage

```
cutHclust(data, k, method = "average", metric = "pearson")
cutPam(data, k)
cutKmeans(data, k)
cutRepeatedKmeans(data, k, nTimes)

repeatedKmeans(data, k, nTimes)
```

Arguments

data	A numerical data matrix
k	The number of classes desired from the algorithm
method	Any valid linkage method that can be passed to the hclust function
metric	Any valid distance metric that can be passed to the distanceMatrix function
nTimes	An integer; the number of times to repeat the K-means algorithm with a different random starting point

Details

Each of the clustering routines used here has a different structure for storing cluster assignments. The [kmeans](#) function stores the assignments in a ‘cluster’ attribute. The [pam](#) function uses a ‘clustering’ attribute. For [hclust](#), the assignments are produced by a call to the [cutree](#) function.

It has been observed that the K-means algorithm can converge to different solutions depending on the starting values of the group centers. We also include a routine ([repeatedKmeans](#)) that runs the K-means algorithm repeatedly, using different randomly generated starting points each time, saving the best results.

Value

Each of the `cut...` functions returns a vector of integer values representing the cluster assignments found by the algorithm.

The [repeatedKmeans](#) function returns a list `x` with three components. The component `x$kmeans` is the result of the call to the [kmeans](#) function that produced the best fit to the data. The component `x$centers` is a matrix containing the list of group centers that were used in the best call to [kmeans](#). The component `x$withinss` contains the sum of the within-group sums of squares, which is used as the measure of fitness.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

See Also

[cutree](#), [hclust](#), [kmeans](#), [pam](#)

Examples

```
# simulate data from three different groups
d1 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d2 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d3 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
dd <- cbind(d1, d2, d3)

cutKmeans(dd, k=3)
cutKmeans(dd, k=4)

cutHclust(dd, k=3)
cutHclust(dd, k=4)

cutPam(dd, k=3)
cutPam(dd, k=4)

cutRepeatedKmeans(dd, k=3, nTimes=10)
cutRepeatedKmeans(dd, k=4, nTimes=10)

# cleanup
rm(d1, d2, d3, dd)
```

mahalanobisQC

Using Mahalanobis Distance and PCA for Quality Control

Description

Compute the Mahalanobis distance of each sample from the center of an N -dimensional principal component space.

Usage

```
mahalanobisQC(spca, N)
```

Arguments

spca	object of class SamplePCA representing the results of a principal components analysis.
N	integer scalar specifying the number of components to use when assessing QC.

Details

The theory says that, under the null hypothesis that all samples arise from the same multivariate normal distribution, the distance from the center of a D -dimensional principal component space should follow a chi-squared distribution with D degrees of freedom. This theory lets us compute p-values associated with the Mahalanobis distances for each sample. This method can be used for quality control or outlier identification.

Value

Returns a data frame containing two columns, with the rows corresponding to the columns of the original data set on which PCA was performed. First column is the chi-squared statistic, with N degrees of freedom. Second column is the associated p-value.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

References

Coombes KR, et al.
Quality control and peak finding for proteomics data collected from nipple aspirate fluid by surface-enhanced laser desorption and ionization. Clin Chem 2003; 49:1615-23.

Examples

```
library(oompaData)
data(lungData)
spca <- SamplePCA(na.omit(lung.dataset))
mc <- mahalnobisQC(spca, 2)
mc[mc$p.value < 0.01,]
```

Mosaic

Class "Mosaic"

Description

Produce “Eisen” plots of microarray or proteomics data.

Usage

```
Mosaic(data,
        sampleMetric="pearson",
        sampleLinkage="average",
        geneMetric="euclid",
        geneLinkage="average",
        usecor=FALSE,
        center=FALSE,
        name="My mosaic")
## S4 method for signature 'Mosaic'
pltree(x, colors, labels, ...)
## S4 method for signature 'Mosaic,missing'
plot(x, main=x@name, center=FALSE,
```

```
scale=c("none", "row", "column"), limits=NULL,
sampleColors=NULL, sampleClasses=NULL,
geneColors=NULL, geneClasses=NULL, ...)
```

Arguments

data	Either a data frame or matrix with numeric values or an ExpressionSet as defined in the BioConductor tools for analyzing microarray data.
sampleMetric	Any valid distance metric that can be passed to the distanceMatrix function
sampleLinkage	Any valid linkage method that can be passed to the hclust function
geneMetric	Any valid distance metric that can be passed to the distanceMatrix function
geneLinkage	Any valid linkage method that can be passed to the hclust function
center	logical scalar. If TRUE, center the data rows.
usecor	logical scalar. If TRUE, scale the data rows to have standard deviation one.
name	character string specifying the name of this object
x	object of class <code>Mosaic</code>
scale	Same as in heatmap .
colors	An optional vector of character strings containing color names to be used when labeling the trees in the dendrogram. If provided, then the length should equal the number of columns in the original data matrix.
labels	An optional vector of character strings used to label the leaves in the dendrogram. If omitted, the column names are used.
main	character string specifying the main title for the plot
limits	An numeric vector. If provided, the data is truncated for display purposes, both above and below, at the minimum and maximum values of <code>limits</code> .
sampleColors	An optional character vector containing colors that will be used to label different sample types with a color bar across the top of the heat map.
sampleClasses	A logical vector or factor used to classify the samples into groups. Alternatively, an integer specifying the number <code>k</code> of groups into which to cut the sample dendrogram.
geneColors	An optional character vector containing colors that will be used to label different gene types with a color bar along the side of the heat map.
geneClasses	A logical vector or factor used to classify the genes into groups. Alternatively, an integer specifying the number <code>k</code> of groups into which to cut the gene dendrogram.
...	Additional parameters for heatmap .

Details

One of the earliest papers in the microarray literature used independent clustering of the genes (rows) and samples (columns) to produce dendrograms that were plotted along with a red-green heat map of the centered expression values. Since that time, literally thousand of additional papers have published variations on these red-green images. R includes a function, [heatmap](#) that builds such figures. However, that function is general purpose and has numerous optional parameters to tweak the display. The purpose of the `Mosaic` class is to provide a simplified object-oriented wrapper around [heatmap](#), which as a side benefit allows us to keep track of the distance metrics and linkage rules that were used to produce the resulting figure.

Value

The Mosaic function constructs and returns a valid object of the Mosaic class.

Objects from the Class

Objects should be created with the Mosaic function.

Slots

data: The matrix containing the numerical data
samples: A dendrogram of class hclust produced by clustering the biological samples (columns of data).
genes: A dendrogram of class hclust produced by clustering the genes (columns of data).
sampleMetric: A character string; the distance metric used to cluster the samples.
sampleLinkage: A character string; the linkage rule used to cluster the samples.
geneMetric: A character string; the distance metric used to cluster the genes.
geneLinkage: A character string; the linkage rule used to cluster the genes.
call: An object of class call recording how the object was constructed.
name: A character string; the name of this object.

Methods

plot signature(x = Mosaic, y = missing): Produce the “Eisen” plot, using [heatmap](#).
pltree signature(x = Mosaic): Plot the sample class dendrogram in the object.
summary signature(object = Mosaic): Write out a summary of the object.

Author(s)

Kevin R. Coombes <krc@silicovore.com>, P. Roebuck <proebuck@mdanderson.org>

References

Eisen MB, Spellman PT, Brown PO, Botstein D.
Cluster analysis and display of genome-wide expression patterns.
 Proc Natl Acad Sci U S A. 1998 Dec 8;95(25):14863-8.

See Also

[cutree](#), [hclust](#), [heatmap](#)

Examples

```
showClass("Mosaic")

## simulate data from three different sample groups
d1 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d2 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d3 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
dd <- cbind(d1, d2, d3)
kind <- factor(rep(c('red', 'green', 'blue'), each=10))
```



```

## prepare the Mosaic object
m <- Mosaic(dd,
            sampleMetric='pearson',
            geneMetric='spearman',
            center=TRUE,
            usecor=TRUE)
summary(m)

## The default plot with red-green color map
plot(m, col=redgreen(64))

## change to a blue-yellow color map, and mark the four top splits in the
## sample direction with a color bar along the top
plot(m, col=blueyellow(128), sampleClasses=4,
     sampleColors=c('red', 'green', 'blue', 'black'))

## This time, mark the three classes that we know are there
plot(m, col=blueyellow(128), sampleClasses=kind,
     sampleColors=c('red', 'green', 'blue'))

plot(m, col=blueyellow(128),
     geneClasses=3, geneColors=c('red', 'green', 'black'))

## In addition, mark the top 5 splits in the gene dendrogram
plot(m,
     col=blueyellow(128),
     sampleClasses=kind,
     sampleColors=c('red', 'green', 'black'),
     geneClasses=5,
     geneColors=c('cyan', 'magenta', 'royalblue', 'darkgreen', 'orange'))

## plot the sample dendrogram by itself
cols <- as.character(kind)
pltree(m, labels=1:30, colors=cols)

## cleanup
rm(d1, d2, d3, dd, kind, cols, m)

```

PCanova

Class "PCanova"

Description

Implements the PCANOVA method for determining whether a putative group structure is truly reflected in multivariate data set.

Usage

```
PCanova(data, classes, labels, colors, usecor=TRUE)
```

```

## S4 method for signature 'PCanova,missing'
plot(x, tag='', mscale=1, cex=1, ...)

```

Arguments

data	either data frame or matrix with numeric values, or an ExpressionSet as defined in the BioConductor tools for analyzing microarray data.
labels	character vector used to label points in plots. The length of the labels vector should equal the number of columns (samples) in the data matrix. Since only the first character of each label is used in the plots, these should be unique.
classes	A subset of the labels used to indicate distinct classes. Again, the method truncates each class indicator to a single letter.
colors	character vector containing color names; this should be the same length as the vector of labels.
usecor	logical scalar. If TRUE, standardize the rows of the data matrix before use.
x	object of class PCanova
tag	character string to name the object, used as part of the plot title.
mscale	A real number. This is a hack; for some reason, the projection of the sample vectors into the principal component space computed from the matrix of group means seems to be off by a factor approximately equal to the square root of the average number of samples per group. Until we sort out the correct formula, this term can be adjusted until the group means appear to be in the correct place in the plots.
cex	Character expansion factor used only in the plot legend on the plot of PC correlations.
...	additional graphical parameters passed on to plot when displaying the principal components plots.

Details

The PCANOVA method was developed as part of the submission that won the award for best presentation at the 2001 conference on the Critical Assessment of Microarray Data Analysis (CAMDA; <http://www.camda.duke.edu>). The idea is to perform the equivalent of an analysis of variance (ANOVA) in principal component (PC) space. Let $X(i,j)$ denote the j th column vector belonging to the i th group of samples. We can model this as $X(i,j) = \mu + \tau(i) + E(i,j)$, where μ is the overall mean vector, $\tau(i)$ is the “effects” vector for the i th group, and $E(i,j)$ is the vector of residual errors. We can perform principal components analysis on the full matrix X containing all the columns $X(i,j)$, on the matrix containing all the group mean vectors $\mu + \tau(i)$, and on the residual matrix containing all the $E(i,j)$ vectors. PCANOVA develops a measure (“PC correlation”) for comparing these three sets of principal components. If the PC correlation is close to 1, then two principal component bases are close together; if the PC correlation is close to zero, then two principal components bases are dissimilar. Strong group structures are recognizable because the PC correlation between the total-matrix PC space and the group-means PC space is much larger than the PC correlation between the total-matrix PC space and the residual PC space. Weak or nonexistent group structures are recognizable because the relative sizes of the PC correlations is reversed.

Value

The PCanova function returns an object of the PCanova class.

Objects from the Class

Objects should be created by calling the PCanova generator function.

Slots

orig.pca: A matrix containing the scores component from PCA performed on the total matrix. All principal components analyses are performed using the `SamplePCA` class.

class.pca: A matrix containing the scores component from PCA performed on the matrix of group-mean vectors.

resid.pca: A matrix containing the scores component from PCA performed on the matrix of residuals.

mixed.pca: A matrix containing the projections of all the original vectors into the principal component space computed from the matrix of group mean vectors.

xc: An object produced by performing hierarchical clustering on the total data matrix, using `hclust` with pearson distance and average linkage.

hc: An object produced by performing hierarchical clustering on the matrix of group means, using `hclust` with pearson distance and average linkage.

rc: An object produced by performing hierarchical clustering on the matrix of residuals, using `hclust` with pearson distance and average linkage.

n: An integer; the number of samples.

class2orig: The numeric vector of PC correlations relating the total-matrix PCA to the group-means PCA.

class2resid: The numeric vector of PC correlations relating the residual PCA to the group-means PCA.

orig2resid: The numeric vector of PC correlations relating the total-matrix PCA to the residual PCA.

labels: A character vector of plot labels to indicate the group membership of samples.

classes: A character vector of labels identifying the distinct groups.

colors: A character vector of color names used to indicate the group membership for samples in plots.

call: An object of class `call` that records how the object was constructed.

Methods

plot `signature(x = PCanova, y = missing)`: Plot the results of the PCANOVA test on the data. This uses `par` to set up a 2x2 layout of plots. The first three plots show the sample vectors (color-coded and labeled) in the space spanned by the first two principal components for each of the three PCAs. The final plot shows the three sets of PC correlations. Colors in the first three plots are determined by the `colors` slot of the object, which was set when the object was created. Colors in the PC correlation plot are determined by the current values of `oompaColor$OBSERVED`, `oompaColor$EXPECTED`, and `oompaColor$PERMTEST`.

pltree `signature(x = PCanova)`: Produce dendrograms of the three hierarchical clusters of the samples, based on all the data, the group means, and the residuals. Since this method uses `par` to put all three dendrograms in the same window, it cannot be combined with other plots.

summary `signature(object = PCanova)`: Write out a summary of the object.

BUGS

- 1 The projection of the sample vectors into the principal component space of the group-means is off by a scale factor. The `mscale` parameter provides a work-around.
- [2] The `pltree` method fails if you only supply two groups; this may be a failure in `hclust` if you only provide two objects to cluster.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

References

Examples of the output of PCANOVA applied to the NCI60 data set can be found at <http://bioinformatics.mdanderson.org/camda01.html>. The full description has not been published (out of laziness on the part of the author of this code). The only description that has appeared in print is an extremely brief description that can be found in the proceedings of the CAMDA 2001 conference.

See Also

[SamplePCA](#)

Examples

```
showClass("PCanova")

## simulate data from three groups
d1 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d2 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d3 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
dd <- cbind(d1, d2, d3)
## colors that match the groups
cols <- rep(c('red', 'green', 'blue'), each=10)

## compute the PCanova object
pan <- PCanova(dd, c('red', 'green', 'blue'), cols, cols)
summary(pan)

## view the PC plots
plot(pan)

## view the dendrograms
pltree(pan, line=-0.5)

## compare the results when there is no underlying group structure
dd <- matrix(rnorm(100*50, rnorm(100, 0.5)), nrow=100, ncol=50, byrow=FALSE)
cols <- rep(c('red', 'green', 'blue', 'orange', 'cyan'), each=10)
pan <- PCanova(dd, unique(cols), cols, cols)
plot(pan, mscale=1/sqrt(10))

pltree(pan, line=-0.5)

## cleanup
rm(d1, d2, d3, dd, cols, pan)
```

Description

Performs a parametric bootstrap test (by adding independent Gaussian noise) to determine whether the clusters found by an unsupervised method appear to be robust in a given data set.

Usage

```
PerturbationClusterTest(data, FUN, nTimes=100, noise=1, verbose=TRUE, ...)
```

Arguments

data	A data matrix, numerical data frame, or ExpressionSet object.
FUN	A function that, given a data matrix, returns a vector of cluster assignments. Examples of functions with this behavior are cutHclust , cutKmeans , cutPam , and cutRepeatedKmeans .
...	Additional arguments passed to the classifying function, FUN.
noise	An optional numeric argument; the standard deviation of the mean zero Gaussian noise added to each measurement during each bootstrap. Defaults to 1.
nTimes	The number of bootstrap samples to collect.
verbose	A logical flag

Objects from the Class

Objects should be created using the `PerturbationClusterTest` function, which performs the requested bootstrap on the clusters. Following the standard R paradigm, the resulting object can be summarized and plotted to determine the results of the test.

Slots

f: A function that, given a data matrix, returns a vector of cluster assignments. Examples of functions with this behavior are [cutHclust](#), [cutKmeans](#), [cutPam](#), and [cutRepeatedKmeans](#).

noise: The standard deviation of the Gaussian noise added during each bootstrap sample.

nTimes: An integer, the number of bootstrap samples that were collected.

call: An object of class `call`, which records how the object was produced.

result: Object of class `matrix` containing, for each pair of columns in the original data, the number of times they belonged to the same cluster of a bootstrap sample.

Extends

Class [ClusterTest](#), directly. See that class for descriptions of the inherited methods `image` and `hist`.

Methods

summary signature(object = PerturbationClusterTest): Write out a summary of the object.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

References

Kerr MK, Churchill GJ.
Bootstrapping cluster analysis: Assessing the reliability of conclusions from microarray experiments.
 PNAS 2001; 98:8961-8965.

See Also

[BootstrapClusterTest](#), [ClusterTest](#)

Examples

```
showClass("PerturbationClusterTest")

## simulate data from two different groups
d1 <- matrix(rnorm(100*30, rnorm(100, 0.5)), nrow=100, ncol=30, byrow=FALSE)
d2 <- matrix(rnorm(100*20, rnorm(100, 0.5)), nrow=100, ncol=20, byrow=FALSE)
dd <- cbind(d1, d2)
cols <- rep(c('red', 'green'), times=c(30,20))
colnames(dd) <- paste(cols, c(1:30, 1:20), sep='')
## perform your basic hierarchical clustering...
hc <- hclust(distanceMatrix(dd, 'pearson'), method='complete')

## bootstrap the clusters arising from hclust
bc <- PerturbationClusterTest(dd, cutHclust, nTimes=200, k=3, metric='pearson')
summary(bc)

## look at the distribution of agreement scores
hist(bc, breaks=101)

## let heatmap compute a new dendrogram from the agreement
image(bc, col=blueyellow(64), RowSideColors=cols, ColSideColors=cols)

## plot the agreement matrix with the original dendrogram
image(bc, dendrogram=hc, col=blueyellow(64), RowSideColors=cols, ColSideColors=cols)

## bootstrap the results of K-means
kmc <- PerturbationClusterTest(dd, cutKmeans, nTimes=200, k=3)
image(kmc, dendrogram=hc, col=blueyellow(64), RowSideColors=cols, ColSideColors=cols)

## contrast the behavior when all the data comes from the same group
xx <- matrix(rnorm(100*50, rnorm(100, 0.5)), nrow=100, ncol=50, byrow=FALSE)
hct <- hclust(distanceMatrix(xx, 'pearson'), method='complete')
bct <- PerturbationClusterTest(xx, cutHclust, nTimes=200, k=4, metric='pearson')
summary(bct)
image(bct, dendrogram=hct, col=blueyellow(64), RowSideColors=cols, ColSideColors=cols)

## cleanup
rm(d1, d2, dd, cols, hc, bc, kmc, xx, hct, bct)
```

Description

Provides an interface to the plot method for [hclust](#) that makes it easier to plot dendrograms with labels that are color-coded, usually to indicate the different levels of a factor.

Usage

```
plotColoredClusters(hd, labs, cols, cex = 0.8, main = "", line = 0, ...)
pcc(hd, colors=NULL, ...)
```

Arguments

hd	An object with S3 class <code>hclust</code> , as produced by the <code>hclust</code> function.
labs	A vector of character strings used to label the leaves in the dendrogram.
cols	A vector of color names suitable for passing to the <code>col</code> argument of graphics routines.
cex	A numeric value; the character expansion parameter of <code>par</code> .
main	A character string; the plot title
line	An integer determining how far away to plot the labels; see mtext for details.
colors	A list; see details.
...	Any additional graphical parameters that can be supplied when plotting an <code>hclust</code> object.

Details

The `plotColoredClusters` function is used to implement the `pltree` methods of the `Mosaic` class and the `PCanova` class. It simply bundles a two step process (first plotting the dendrogram with no labels, followed by writing the labels in the right places with the desired colors) into a single unit.

The `pcc` function also produces dendrograms with colored annotations. However, instead of coloring the labels based on a single factor, it produces color bars for any number of factors. The `colors` argument should be a list with named components, where each component should correspond to a factor and a color scheme. Specifically, the components must themselves be lists with two components named `fac` (and containing the factor) and `col` (containing a named vector specifying colors for each level of the factor).

Value

The function has no useful return value; it merely produces a plot.

See Also

[hclust](#), [Mosaic](#), [PCanova](#), [par](#)

Examples

```
# simulate data from three different groups
d1 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d2 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d3 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
dd <- cbind(d1, d2, d3)

# perform hierarchical clustering using correlation
```

```

hc <- hclust(distanceMatrix(dd, 'pearson'), method='average')
cols <- rep(c('red', 'green', 'blue'), each=10)
labs <- paste('X', 1:30, sep='')

# plot the dendrogram with color-coded groups
plotColoredClusters(hc, labs=labs, cols=cols)

# simulate another dataset
fakedata <- matrix(rnorm(200*30), ncol=30)
colnames(fakedata) <- paste("P", 1:30, sep='')
# define two basic factors, with colors
faccol <- list(fac=factor(rep(c("A", "B"), each=15)),
              col=c(A='red', B='green'))
fac2col <- list(fac=factor(rep(c("X", "Y", "Z"), times=10)),
              col=c(X='cyan', Y='orange', Z='purple'))
# add another factor that reverses the colors
BA <- faccol
BA$col <- c(A='blue', B='yellow')
# assemble the list of factors
colors <- list(AB=faccol, XYZ=fac2col, "tricky long name"=fac2col,
              another=BA)
# cluster the samples
hc <- hclust(distanceMatrix(fakedata, "pearson"), "ward")
# plot the results
pcc(hc, colors)

#cleanup
rm(d1, d2, d3, dd, hc, cols, labs)
rm(fakedata, faccol, fac2col, BA, colors)

```

SamplePCA

Class "SamplePCA"

Description

Perform principal components analysis on the samples (columns) from a microarray or proteomics experiment.

Usage

```

SamplePCA(data, splitter=0, usecor=FALSE, center=TRUE)
## S4 method for signature 'SamplePCA,missing'
plot(x, splitter=x@splitter, col, main='', which=1:2, ...)

```

Arguments

data	Either a data frame or matrix with numeric values or an ExpressionSet as defined in the BioConductor tools for analyzing microarray data.
splitter	If data is a data frame or matrix, then splitter must be either a logical vector or a factor. If data is an ExpressionSet, then splitter can be a character string that names one of the factor columns in the associated phenoData subobject.
center	A logical value; should the rows of the data matrix be centered first?

<code>usecor</code>	A logical value; should the rows of the data matrix be scaled to have standard deviation 1?
<code>x</code>	A <code>SamplePCA</code> object
<code>col</code>	A list of colors to represent each level of the <code>splitter</code> in the plot. If this parameter is missing, the function will select colors automatically.
<code>main</code>	A character string; the plot title
<code>which</code>	A numeric vector of length two specifying which two principal components should be included in the plot.
<code>...</code>	Additional graphical parameters for plot.

Details

The main reason for developing the `SamplePCA` class is that the `princomp` function is very inefficient when the number of variables (in the microarray setting, genes) far exceeds the number of observations (in the microarray setting, biological samples). The `princomp` function begins by computing the full covariance matrix, which gets rather large in a study involving tens of thousands of genes. The `SamplePCA` class, by contrast, uses singular value decomposition (`svd`) on the original data matrix to compute the principal components.

The base functions `screeplot`, which produces a barplot of the percentage of variance explained by each component, and `plot`, which produces a scatter plot comparing two selected components (defaulting to the first two), have been generalized as methods for the `SamplePCA` class. You can add sample labels to the scatter plot using either the `text` or `identify` methods. One should, however, note that the current implementation of these methods only works when plotting the first two components.

Value

The `SamplePCA` function constructs and returns an object of the `SamplePCA` class. We assume that the input data matrix has N columns (of biological samples) and P rows (of genes).

The `predict` method returns a matrix whose size is the number of columns in the input by the number of principal components.

Objects from the Class

Objects should be created using the `SamplePCA` function. In the simplest case, you simply pass in a data matrix and a logical vector, `splitter`, assigning classes to the columns, and the constructor performs principal components analysis on the column. The `splitter` is ignored by the constructor and is simply saved to be used by the plotting routines. If you omit the `splitter`, then no grouping structure is used in the plots.

If you pass `splitter` as a factor instead of a logical vector, then the plotting routine will distinguish all levels of the factor. The code is likely to fail, however, if one of the levels of the factor has zero representatives among the data columns.

As with the class comparison functions (see, for example, `MultiTtest`) that are part of OOMPA, we can also perform PCA on `ExpressionSet` objects from the BioConductor libraries. In this case, we pass in an `ExpressionSet` object along with a character string containing the name of a factor to use for splitting the data.

Slots

scores: A matrix of size $N \times N$, where N is the number of columns in the input, representing the projections of the input columns onto the first N principal components.

variances: A numeric vector of length N ; the amount of the total variance explained by each principal component.

components: A matrix of size $P \times N$ (the same size as the input matrix) containing each of the first P principal components as columns.

splitter: A logical vector or factor of length N classifying the columns into known groups.

usecor: A logical value; was the data standardized?

shift: A numeric vector of length P ; the mean vector of the input data, which is used for centering by the predict method.

scale: A numeric vector of length P ; the standard deviation of the input data, which is used for scaling by the predict method.

call: An object of class call that records how the object was created.

Methods

plot signature(x = SamplePCA, y = missing): Plot the samples in a two-dimensional principal component space.

predict signature(object = SamplePCA): Project new data into the principal component space.

screepplot signature(x = SamplePCA): Produce a bar chart of the variances explained by each principal component.

summary signature(object = SamplePCA): Write out a summary of the object.

identify signature(object = SamplePCA): interactively identify points in the plot of a SamplePCA object.

text signature(object = SamplePCA): Add sample identifiers to the scatter plot of a SamplePCA object, using the base text function.

Author(s)

Kevin R. Coombes <krc@silicovore.com>

See Also

[princomp](#), [GenePCA](#)

Examples

```
showClass("SamplePCA")

## simulate data from three different groups
d1 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d2 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
d3 <- matrix(rnorm(100*10, rnorm(100, 0.5)), nrow=100, ncol=10, byrow=FALSE)
dd <- cbind(d1, d2, d3)
kind <- factor(rep(c('red', 'green', 'blue'), each=10))
colnames(dd) <- paste(kind, rep(1:10, 3), sep='')

## perform PCA
spc <- SamplePCA(dd, splitter=kind)
```

```
## plot the results
plot(spc, col=levels(kind))

## mark the group centers
x1 <- predict(spc, matrix(apply(d1, 1, mean), ncol=1))
points(x1[1], x1[2], col='red', cex=2)
x2 <- predict(spc, matrix(apply(d2, 1, mean), ncol=1))
points(x2[1], x2[2], col='green', cex=2)
x3 <- predict(spc, matrix(apply(d3, 1, mean), ncol=1))
points(x3[1], x3[2], col='blue', cex=2)

## check out the variances
screeplot(spc)

## cleanup
rm(d1, d2, d3, dd, kind, spc, x1, x2, x3)
```

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