Package ‘pbcmc’

April 26, 2017

Type Package

Title Permutation-Based Confidence for Molecular Classification

Version 1.4.0

Date 2016-09-23

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Description The pbcmc package characterizes uncertainty assessment on gene expression classifiers, a. k. a. molecular signatures, based on a permutation test. In order to achieve this goal, synthetic simulated subjects are obtained by permutations of gene labels. Then, each synthetic subject is tested against the corresponding subtype classifier to build the null distribution. Thus, classification confidence measurement can be provided for each subject, to assist physician therapy choice. At present, it is only available for PAM50 implementation in genefu package but it can easily be extend to other molecular signatures.

URL http://www.bdmg.com.ar/

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Depends R (>= 3.4), genefu

Imports Biobase, BiocGenerics, BiocParallel (>= 1.3.13), parallel, reshape2, grid, utils, cowplot, methods, limma, ggplot2, gridExtra, grDevices, stats

Suggests breastCancerUPP, breastCancerNKI, breastCancerVDX, breastCancerTRANSBIG, breastCancerMAINZ, breastCancerUNT

biocViews Classification, GeneExpression, Microarray,
  MultipleComparison, QualityControl, Normalization, Clustering,
  mRNAMicroarray, OneChannel, TwoChannel, RNASeq, KEGG,
  DifferentialExpression

Collate ‘pbcmcPackage.R’ 'MolecularPermutationClassifierClass.R'
  'MolecularPermutationClassifierConstructor.R'
  'MolecularPermutationClassifierGenerics.R'
  'MolecularPermutationClassifierGetseters.R'
  'MolecularPermutationClassifierShow.R' 'PAM50Class.R'
  'PAM50Classify.R' 'PAM50Constructor.R' 'PAM50Filtrate.R'
as PAM50 high level coerce functions

Description

These functions (setAs and as.PAM50) are intended to be used with limma MAList-class in order to coerce its structure into a compatible PAM50 class.

Usage

as(object, Class, strict=TRUE, ext=possibleExtends(thisClass, Class))

as.PAM50(object)

## S4 method for signature 'MAList'
as.PAM50(object)

Arguments

object         MAList object with at least M and genes items, optionally targets.
Class          character with the name of class "PAM50" to be coerced.
strict, ext    see as function.
Details

Basically the $M and $genes items are copied into a MolecularPermutationClassifier’s exprs and annotation slots respectively. In addition, if present, $targets content is also copied to the same named slot.

Value

a PAM50 object with the respective copied data.

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See Also

PAM50 for a complete example.


Examples

```r
##Example 1: Create a PAM50 object ------------------------------
##1) Just an empty object
object<-PAM50()
object
##2) Using Breast Cancer NKI database, if available.
if(requireNamespace("breastCancerNKI")){
  object<-loadBCDataset(Class=PAM50, libname="nki", verbose=TRUE)
  object
  ##Now we can inspect the object
  head(exprs(object))  ##The gene expression
  head(annotation(object))  ##The available annotation
  head(targets(object))  ##The clinical data present in the package
}

##Example 2: Build a PAM50 object with user data ----------------
##Option 1: using PAM50 constructor. The user will only need:
##a) The M gene expression object, i.e., gene in rows and sample in columns
##b) The annotation data.frame which must include the compulsory fields
## "probe", "NCBI.gene.symbol" and "EntrezGene.ID"
M<-pam50$centroids
genes<-pam50$centroids.map
names(genes)<-c("probe", "NCBI.gene.symbol", "EntrezGene.ID")
object<-PAM50(exprs=M, annotation=genes)
object

##Option 2: Two ways to build it from a MAList (as or as.PAM50)----------
##Let's use PAM50 classifier's centroids toy example, i.e., the five subject
##subtypes, which must correctly classify all the subject.
M<-pam50$centroids
genes<-pam50$centroids.map
names(genes)<-c("probe", "NCBI.gene.symbol", "EntrezGene.ID")
maux<-new("MAList", list(M=M, genes=genes))
```
## calling as function

```r
object <- as(maux, "PAM50")
object
```

## same result with as.PAM50 function

```r
object <- as.PAM50(maux)
object
```

---

**classify,PAM50-method**

**classify subjects with PAM50 molecular signature**

### Description

Obtain PAM50 subtype using genefu centroid Spearman’s correlation implementation. If `std`=="median" probes with the same mapping are averaged. Then, the complete database is center normalized using gene median expression. This is done in order to assure selecting the same "gene" to those in "genefu" library, instead of the most variant probe (default in geneid.map), when more than one probe match the same gene. This selection is based on probe population variance that could depends on the number of accounted genes.

### Usage

```r
## S4 method for signature 'PAM50'
classify(object, std = c("none", "scale", "robust", "median")[[1]], verbose = getOption("verbose", default = FALSE))
```

### Arguments

- **object**: a MolecularPermutationClassifier subclass object.
- **std**: character to select standardization alternative "none" (default), "scale" and "robust" as in genefu original implementation, plus the suggested "median" if many subjects are available.
- **verbose**: should the user feedback be displayed? By default value is "verbose" global option parameter, if present, or FALSE otherwise.

### Value

- **a PAM50 object with the updated slots:**
  - @exprs: updated matrix with the used std parameter.
  - @classification:
    - $subtype: subject named factor with all classifier possible levels, i.e, "Basal", "Her2", "LumA", "LumB" and "Normal".
    - $probability: numeric matrix with subtype class probability for each subject, as in genefu, obtained as the positive proportion of correlation explained by each subtype.
    - $correlation: numeric matrix with Spearman’s rho correlation of each subject to the corresponding PAM50 subtypes.
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References


See Also

PAM50 for a complete example.

Other PAM50: as, filtrate, PAM50-method, pam50centroids, permutate, PAM50-method, subjectReport, PAM50-method, subtypes, PAM50-method

Examples

```r
##Using pam50centroids package example data
data(pam50centroids)

##Get the original PAM50 calls using genefu implementation
pam50centroids<-classify(pam50centroids, std="none", verbose=TRUE)
classification(pam50centroids)
```

---

**filtrate**

*Virtual functions for MolecularPermutationClassifier hierarchy*

**Description**

The following functions establish an organized framework for MolecularPermutationClassifier subclasses data processing. In this context, the later are supposed to be implemented with respective responsibilities. In particular, once the class is created the user has to:

- **filtrate**: Removes, from the exprs matrix, subjects not required by the classification algorithm.
- **classify**: Generates subject classification according to subclass implementations (PAM50, etc.).
- **permutate**: Obtains subject classification based on the null correlation distribution by means permutation simulation.
- **subtype**: Obtain the new classification using permutation results.
- **subjectReport**: A friendly report for physician treatment decision support.
- **databaseReport**: A pdf with all subjectReports, if a database is available.
Usage

filtrate(object, verbose = getOption("verbose", default = FALSE))

classify(object, ..., verbose = getOption("verbose", default = FALSE))

permutate(object, nPerm = 10000L, pCutoff = 0.01, where = "fdr",
   keep = FALSE, ..., seed = 1234567890, BPPARAM = bpparam(),
   verbose = getOption("verbose", default = TRUE))

subtypes(object, pCutoff = 0.01, ..., where = c("fdr", "pvalue")[1])

subjectReport(object, subject)

databaseReport(object, fileName, ..., verbose = getOption("verbose", default = TRUE))

Arguments

- **object**: MolecularPermutationClassifier child class object
- **verbose**: should the user feedback be displayed? By default value is "verbose" global option parameter, if present, or FALSE otherwise.
- **...**: additional parameters for future implementations.
- **nPerm**: integer with number of permutations. Default: 1e4L.
- **pCutoff**: numeric with p-value or fdr cutoff used, i.e., variable<pCutoff. Default: 0.01.
- **where**: character with significant value used. Default value is "fdr".
- **keep**: should null distribution simulation values be kept?. Default: FALSE
- **seed**: integer to use as random seed. Default: 1234567890.
- **BPPARAM**: an optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bplapply. Default=bpparam().
- **subject**: integer to select the appropriate subject to report.
- **fileName**: character with the name of the pdf report file to save.

Value

A MolecularPermutationClassifier child according to the actual object class.

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See Also

PAM50 for a complete example.

Other MolecularPermutationClassifier PAM50: PAM50-class, loadBCDataset
Examples

```r
## Using pam50centroids package example data
data(pam50centroids)
pam50centroids
pam50centroids<-filtrate(pam50centroids, verbose=TRUE)
pam50centroids<-classify(pam50centroids, std="none", verbose=TRUE)
## Let's run a quick example with 100 permutations. It is recommended at
## least 10,000
pam50centroids<-permutate(pam50centroids, nPerm=100, pCutoff=0.01,
corCutoff=0.1, verbose=TRUE)
pam50centroids
```

filtrate,PAM50-method  filtrate centroid genes from PAM50 classification

Description

Remove exprs rows not required by MolecularPermutationClassifier subclasses to classify samples, in this case PAM50. This means to only keep genes with valid EntrezGeneID, i.e., not NA and present in PAM50 signature centroids. In addition, annotation slot will only keep "probe", "EntrezGene.ID" and "NCBI.gene.symbol" fields required by genefu's intrinsic.cluster.predict function.

Usage

```r
## S4 method for signature 'PAM50'
filtrate(object, verbose =getOption("verbose", default =
FALSE))
```

Arguments

- `object` a PAM50 object.
- `verbose` should the user feedback be displayed? By default value is "verbose" global option parameter, if present, or FALSE otherwise.

Value

MolecularPermutationClassifier subclass with updated slots:
- `@exprs` only rows required by the classifier.
- `@annotation` consistent with exprs rows and only "probe", "EntrezGene.ID" and "NCBI.gene.symbol" annotation fields.

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See Also

- `PAM50` for a complete example.
- Other PAM50: `as`, `classify,PAM50-method`, `pam50centroids`, `permutate,PAM50-method`, `subjectReport,PAM50-method`, `subtypes,PAM50-method`
loadBCDataset

MolecularPermutationClassifier high level constructor

Description

High level constructor for MolecularPermutationClassifier subclasses using available Bioconductor's Breast Cancer example datasets.

Usage

loadBCDataset(Class, libname = c("upp", "nki", "vdx", "mainz", "transbig", "unt"), verbose = getOption("verbose", default = FALSE))

## S4 method for signature 'classGeneratorFunction'
loadBCDataset(Class, libname = c("upp", "nki", "vdx", "mainz", "transbig", "unt"), verbose = getOption("verbose", default = FALSE))

Arguments

- **Class**: name of MolecularPermutationClassifier child class to use.
- **libname**: lowercase character with the name of the breastCancerXXX database to be loaded. At present, XXX can be "upp", "nki", "vdx", "mainz", "transbig" or "unt". See reference for available breast cancer citations.
- **verbose**: should the user feedback be displayed? By default value is "verbose" global option parameter, if present, or FALSE otherwise.

Value

MolecularPermutationClassifier subclass object with exprs, annotation and targets slots taken from the libname used.

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MolecularPermutationClassifier-class

References


See Also

PAM50 for a complete example.

Other MolecularPermutationClassifier PAM50: PAM50-class, filtrate

Examples

```r
##Using Breast Cancer NKI database, if available, to create a PAM50 class.
if(requireNamespace("breastCancerNKI")){
    object<-loadBCDataset(Class=PAM50, libname="nki", verbose=TRUE)
    object

    ##Now we can inspect the object
    head(exprs(object))    ##The gene expression
    head(annotation(object)) ##The available annotation
    head(targets(object))  ##The clinical data present in the package
}
```

Description

Virtual class to represent gene-based molecular signature classification by means of permutation test.
MolecularPermutationClassifier-class

Slots

parameters named list with at least the following fields:

- **$nPerm** integer with number of permutations. Default: 1e4L
- **$where** character with significant value used. Default value is "fdr".
- **$pCutoff** numeric with p-value or fdr cutoff used, i.e., variable<pCutoff. Default: 0.01
- **$keep** should null distribution simulation values be kept?. Default: FALSE

exprs matrix with gene exprs profile, where genes are in rows and subjects as columns, a.k.a., M matrix.

annotation data.frame with individual annotations (genes, etc). Minimal compulsory fields are:

- **$probe** same characters as in row.names(M).
- **$EntrezGene.ID** integer with NCBI Entrez Data Base.
- **$NCBI.gene.symbol** character with gene mnemonic, a.k.a. gene symbol.

targets data.frame with additional subject data (optional).

classification named list with at least the following fields:

- **$class** factor with all possible class levels.

permutation named list with at least the following fields:

- **$pvalues** numeric matrix with subjects in row and classes in columns.
- **$fdr** numeric matrix with False Discovery Rate correction of p-values by row.

Superclasses

None declared.

Subclasses

- **PAM50** Perou et al. (2000 and 2010) breast cancer subtypes, i. e., Luminal A, Luminal B, Basal, Her2 or Normal-like subtypes as implemented in genefu library (Haibe-Kains et al. 2014).

Functions

MolecularPermutationClassifier S4 class includes the following functions:

- Integrity check:
  - **validity** will check appropriate annotation data.frame minimal required columns, all named parameters and if exprs and annotation dimension matches.
  - **prototype** just for an empty class with default values: nPerm=1e4L, where="fdr", pCutoff=0.01, corCutoff=0.1 and keep=FALSE.

- Generics:
  - **show**, **print** basic class display wrappers.
  - **summary** classifier statistics.

- Constructors (as this class is virtual see subclass’ documentation).

- **setAs** MAList to **PAM50**

- **as.PAM50** wrapper for **PAM50** setAs from MAList.

- **loadBCDataset** wrapper to load BreastCancerXX data (Class, exprs, annotation, clinical data).

- Getters for the corresponding slots (**parameters**, **exprs**, **annotation**, **targets**, **classification** and **permutation**).
PAM50-class

- Setters for the corresponding slots (`parameters<-`, `annotation<-` and `targets<-`).
- Particular (virtual) functions:
  - `filtrate` remove from the `exprs` matrix subjects not required by the classification algorithm.
  - `classify` generate subject classification according to subclasses implementation (PAM50, etc.).
  - `permutate` obtain subject classification based on the null correlation distribution by means permutation simulation.
  - `subtypes` obtain the new classification using permutation results.
  - `subjectReport` a friendly report for Physician treatment decision support.
  - `databaseReport` a pdf with all `subjectReports`, if a database is available.

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References


See Also

- `PAM50` for a complete example, `loadBCDataset` to load BreastCancerXX dataset, `filtrate, classify` and `permutate` to get corresponding Breast Cancer subtype. Getters/Setters for this class are `parameters, exprs, annotation, targets, classification` and `permutation`.
- Other MolecularPermutationClassifier: `parameters, show`

---

**PAM50-class**

**PAM50 S4 implementation in R**

**Description**

This is a concrete MolecularPermutationClassifier based on Perou et al. (2000 & 2010) PAM50 molecular signature, using genefu package implementation (Haibe-Kains et al. 2014).

**Slots**

- `parameters` named list with at least the following fields:
  - `$nPerm` integer with number of permutations. Default: 1e4L
  - `$where` character with significant value used. Default value is "fdr".
  - `$pCutoff` numeric with p-value or fdr cutoff used, i.e., variable<$pCutoff. Default: 0.01
  - `$keep` should null distribution simulation values be kept?. Default: FALSE
**PAM50-class**

**corCutoff**  PAM50 additional numeric parameter with the correlation difference between classes cutoff used, i.e., \(|\rho(\text{profile, class}_A) - \rho(\text{profile, class}_B)| > \text{corCutoff}\)
exprs matrix with gene exprs profile, where genes are in rows and subjects as columns, a.k.a., M matrix.

**annotation**  data.frame with individual annotations (genes, etc). Minimal compulsory fields are:
- **$probe**  same characters as in row.names(M).
- **$EntrezGene.ID**  integer with NCBI Entrez Data Base.
- **$NCBI.gene.symbol**  character with gene mnemonic, a.k.a. gene symbol.

**targets**  data.frame with additional subject data (optional).

**classification**  named list with at least the following fields:
- **$subtype**  factor with PAM50 subtype of each sample.
- **$probability**  matrix with the subtype probability of each subtype per sample, as in genefu library.
- **$correlation**  matrix with the observed correlation of each subtype per sample.

**permutation**  named list with at least the following fields:
- **$correlation**  Only if keep==TRUE is a list of the five subtypes containing a matrix with the permuted null distribution correlations.
- **$pvalues**  matrix with the subject’s p-values of the permutation test per subject.
- **$fdr**  matrix with the corresponding adjusted p-values.
- **$subtype**  data.frame where each subject has the reported ”PAM50” subtype, the ”Permuted” test result i.e. ”Assigned”, ”Not Assigned” or ”Ambiguous”; ”Classes” whether is a single PAM50 subtype or more than one if Ambiguous case; ”Class” if it is needed to assign just one i.e., a single PAM50 subtype or Not Assigned.

**Superclasses**
Direct descendant from MolecularPermutationClassifier-class.

**Subclasses**
None declared.

**Function**
Redefinition from MolecularPermutationClassifier: filtrate, classify, permutate, subjectReporta and databaseReport.

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**References**
See Also

Other MolecularPermutationClassifier PAM50: *filtrate, loadBCDataset*

Examples

```r
##Example 1: Create a PAM50 object -----------------------------------------
##1) Just an empty object
object<-PAM50()
object

##2) Using Breast Cancer NKI database, if available.
if(requireNamespace("breastCancerNKI")){
  object<-loadBCDataset(Class=PAM50, libname="nki", verbose=TRUE)
  object
  ##Now we can inspect the object
  head(exprs(object))  ##The gene expression
  head(annotation(object))  ##The available annotation
  head(targets(object))  ##The clinical data present in the package
}

##Example 2: Build a PAM50 object with user data -------------------------
##Option 1: using PAM50 constructor. The user will only need:
##a) The M gene expression object, i.e., gene in rows and sample in columns
##b) The annotation data.frame which must include the compulsory fields
##   "probe", "NCBI.gene.symbol" and "EntrezGene.ID"
M<-pam50$centroids
genes<-pam50$centroids.map
names(genes)<-c("probe", "NCBI.gene.symbol", "EntrezGene.ID")
object<-PAM50(exprs=M, annotation=genes)
object

##Option 2: Two ways to build it from a MAList (as or as.PAM50)-------------
##Let's use PAM50 classifier's centroids toy example, i.e., the five subject
##subtypes, which must correctly classify all the subject.
M<-pam50$centroids
genes<-pam50$centroids.map
names(genes)<-c("probe", "NCBI.gene.symbol", "EntrezGene.ID")
maux<-new("MAList", list(M=M, genes=genes))
##calling as function
object<-as(maux, "PAM50")
object
##same result with as.PAM50 function
object<-as.PAM50(maux)
object

##Example3: Work with PAM50 object: filtrate, classify and permute--------
##1) Keep only annotated genes presents in PAM50 centroids
object<-filtrate(object, verbose=TRUE)

##2) Get PAM50 subtypes without any normalization
object<-classify(object, std="none", verbose=TRUE)
##Now we can inspect the how the classification went
head(classification(object))

##3) Obtain the permutation subtype
##Let's run a quick example with 100 permutations. It is recommended at
##least 10,000
object<-permutate(object, nPerm=100, pCutoff=0.01, corCutoff=0.1,
keep=TRUE, seed=1234567890, verbose=TRUE)

##Now we can inspect the how the permutation went
head(permutation(object))

##Which parameters were used?
parameters(object)

##Example 4: Obtain summary statistics and reports--------------------------
##1) Let's check if we have a diagonal contingency matrix, i.e., no mistake
##is made in subtype assessment.
summary(object)

##2) Let's take a look at how the patient genes behave according
## to PAM50
subjectReport(object, subject=1)

##3) Just get a pdf with all the used subjects (PAM50 centroids in this
## example).
#databaseReport(object, fileName="PAM50.pdf", verbose=TRUE)

---

### Description

The dataset corresponds to the Permutation-Based Confidence for Molecular Classification package PAM50 example objects, that was filtered, classified and permuted using the following parameters:

- **Permutations**: 10000
- **fdr**: 0.01
- **corCutoff**: 0.1
- **keep**: TRUE

### Usage

data(pam50centroids)

### Format

pam50centroids corresponds with **pam50centroids** dataset available in genefu package.

### Value

a PAM50 object with the results obtained for pam50centroids simulations under the given parameters (see Detail section.)

### Author(s)

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References


See Also

Other PAM50: as, classify,PAM50-method, filtrate,PAM50-method, permutate,PAM50-method, subjectReport,PAM50-method, subtypes,PAM50-method

parameters

Accessors for MolecularPermutationClassifier child class slots

Description

Slot setters/getters for MolecularPermutationClassifier hierarchy classes

Usage

parameters(object)

## S4 method for signature 'MolecularPermutationClassifier'
parameters(object)

parameters(object) <- value

## S4 replacement method for signature 'MolecularPermutationClassifier'
parameters(object) <- value

## S4 method for signature 'MolecularPermutationClassifier'
exprs(object)

## S4 replacement method for signature 'MolecularPermutationClassifier,ANY'
exprs(object) <- value

## S4 method for signature 'MolecularPermutationClassifier'
annotation(object, ...)

## S4 replacement method for signature 'MolecularPermutationClassifier,ANY'
annotation(object) <- value

targets(object)

## S4 method for signature 'MolecularPermutationClassifier'
targets(object)
targets(object) <- value

## S4 replacement method for signature 'MolecularPermutationClassifier'

targets(object) <- value

classification(object)

## S4 method for signature 'MolecularPermutationClassifier'

classification(object)

permutation(object)

## S4 method for signature 'MolecularPermutationClassifier'

permutation(object)

Arguments

object MolecularPermutationClassifier subclass object

value according to the function call:

- parameters: named list with at least the following fields:
  - $nPerm integer with number of permutations. Default: 1e4L
  - $where character with significant value used. Default value is "fdr".
  - $pCutoff numeric with p-value or fdr cutoff used, i.e., variable<$pCutoff. Default: 0.01
  - $corCutoff numeric with correlation difference between classes cutoff used, i.e., \( |\rho(profile, class_A) - \rho(profile, class_B)| \) > $corCutoff
  - $keep should null distribution simulation values be kept?. Default: FALSE
- annotation: data.frame with individual annotations (genes, etc). Minimal compulsory fields are:
  - $probe same characters as in row.names(M).
  - $EntrezGene.ID integer with NCBI Entrez Data Base.
  - $NCBI.gene.symbol character with gene mnemonic, a.k.a. gene symbol.
- exprs: matrix with gene exprs profile, where genes are in rows and subjects as columns, a.k.a., M matrix.
- targets: data.frame with additional subject data.

... additional parameters according to function call.

Value

according to function call one of the following objects:

parameters named list see value parameter

exprs matrix with gene exprs profile, where genes are in rows and subjects as columns, a.k.a., M matrix.

annotation data.frame see value parameter

classification named list with at least the following fields:

$class factor with with all possible class levels.
permutation named list with at least the following fields:

**pvalues** numeric matrix with subjects in row and classes in columns.

**$fdr** numeric matrix with False Discovery Rate correction of pvalues by row.

**parameters**<- MolecularPermutationClassifier object with parameters updated slot.

**exprs**<- MolecularPermutationClassifier object with exprs updated slot.

**annotation**<- MolecularPermutationClassifier object with annotation updated slot.

**targets**<- MolecularPermutationClassifier object with targets updated slot.

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**See Also**

PAM50 for a complete example.

Other MolecularPermutationClassifier: MolecularPermutationClassifier-class, show

**Examples**

```r
##Using pam50centroids package example data
data(pam50centroids)

##Now we can inspect pam50centroids object
ead(exprs(pam50centroids))  ##The gene expression
head(annotation(pam50centroids))  ##The available annotation
head(targets(pam50centroids))  ##The clinical data present in the package

##Work with the parameters
parameters(pam50centroids)  ##Display them
aux<-parameters(pam50centroids)
aux$keep<-TRUE  ##Set keep to FALSE
parameters(pam50centroids)<-aux

##Also exprs<-, annotation<- and targets<- available functions to update
##the respective slots
```

**Description**

Gene expression-based classifiers, known as molecular signatures (MS), are a set of genes coordinately expressed and an algorithm that use these data to predict disease subtypes, response to therapy, disease risk or clinical outcome (Andre et al. 2006). They are especially important in breast cancer (BC) where several MS are currently on the market like PAM50 (Perou et al. 2000 & 2010), Prosigna www.prosigna.com, Oncotype DX www.oncotypedx.com, MammaPrint www.agendia.com, etc. As far as the authors know, these classifiers do not give a real uncertainty of the classification at all. This package characterizes MS classification uncertainty. In order to
achieve this goal, synthetic simulated subjects are obtained by permutations of gene labels. Then, each synthetic subject is tested against the classifier corresponding subtype to build the null distribution, thus, classification confidence measurement can be provided for each subject. In this context, subjects belonging to the null distribution (random or noisy individuals) are not assigned (NA) to any class. On the contrary, if reliable results are obtained, subjects could be either assigned (A) to the more reliably subtype or marked as ambiguous (AMB) if proximal to two or more reliable subtypes. In the later, the combinations of classes are given. At present, it is only implemented for genefu’s PAM50 package (Haibe-Kains et al. 2014) but it can easily be extended to other MS. This package includes the following features:

• Implemented classifier:
  1. PAM50.

• Single subject classification:
  1. No pilot study needs to be carried out to obtain classification uncertainty.
  2. No normalization is required. If required, external database normalization, genefu normalization alternatives (scale/robust) or even gene median can be applied before simulations.

• Classification:
  1. The original PAM50 calls obtained by genefu.
  2. The proposed classification scheme: Assigned (PAM50 call), Not Assigned (NA) or Ambiguous (reliable PAM50 class combinations).
  3. Classification significance p-value or False Discovery Rate (FDR).
  4. Observed subject Spearman’s correlation for each breast cancer subtype.

• Physician treatment decision support:
  1. A friendly subject report is provided which includes summary data such as subtype centroid Spearman’s correlation, p-value and FDR for each subtype, original PAM50 classification and the recommended strategy (assigned, not assigned or ambiguous classes).
  2. Scatter plot of the observed gene-expression (subject) versus PAM50 centroids panel, plus the corresponding linear regression fit.
  3. Null distribution boxplot, plus observed (subject) value.

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References
permutate, PAM50-method

permutate subject gene-expression for PAM50 confidence

Description

Calculate the null Spearman’s $\rho$ distribution of each subtype by means of gene label permutation, in order to evaluate if the observed values could be obtained by random change.

Usage

```r
## S4 method for signature 'PAM50'
permutate(object, nPerm = 10000, pCutoff = 0.01,
          where = "fdr", keep = FALSE, corCutoff = 0.1, seed = 1234567890,
          BPPARAM = bpparam(), verbose = getOption("verbose", default = TRUE))
```

Arguments

- `object`: a MolecularPermutationClassifier subclass object.
- `nPerm`: integer with number of permutations. Default: 1e4L
- `pCutoff`: numeric with p-value or fdr cutoff used, i.e., variable < pCutoff. Default: 0.01
- `where`: character with significant value used. Default value is "fdr".
- `keep`: should null distribution simulation values be kept?. Default: FALSE
- `corCutoff`: numeric with correlation difference between classes cutoff used, i.e., $|\rho(profile, class_A) - \rho(profile, class_B)| > corCutoff$. Default 0.1
- `seed`: integer to use as random seed. Default: 1234567890.
- `BPPARAM`: an optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bplapply. Default=bpparam().
- `verbose`: should the user feedback be displayed? By default value is "verbose" global option parameter, if present, or FALSE otherwise.

Value

a PAM50 object with the following updated slots:

- `@permutation`:
  - `$pvalues`: numeric matrix with subtype pvalues obtained as the number of times the permuted correlation is greater or equal the observed correlation divided the number of permutations.
  - `$fdr`: subtype adjusted pvalues for each subject with False Discovery Rate.
  - `$correlations`: list with subject matrix correlation of each permutation simulation.
  - `$subtype`: data.frame with classification results obtained by subtype function.
- `@parameters`: $nPerm$, $pCutoff$, $where$ and $keep$ updated accordingly.

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References


See Also

PAM50 for a complete example.

Other PAM50: as, classify,PAM50-method, filtrate,PAM50-method, pam50centroids, subjectReport,PAM50-method

Examples

```r
## Using pam50centroids package example data
data(pam50centroids)
pam50centroids
pam50centroids<-filtrate(pam50centroids, verbose=TRUE)
pam50centroids<-classify(pam50centroids, std="none", verbose=TRUE)

## Let's run a quick example with 100 permutations. It is recommended at least 10.000
pam50centroids<-permutate(pam50centroids, nPerm=100, pCutoff=0.01, corCutoff=0.1, verbose=TRUE)
pam50centroids
```

show

Show a MolecularPermutationClassifier subclass object

Description

Basic MolecularPermutationClassifier class information display function (slots, dimensions, etc).

Usage

```r
## S4 method for signature 'MolecularPermutationClassifier'
show(object)
```

Arguments

- `object` an object of MolecularPermutationClassifier class hierarchy

Value

console messages displaying the class content
subjectReport,PAM50-method

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See Also
PAM50 for a complete example.
Other MolecularPermutationClassifier: MolecularPermutationClassifier-class, parameters

Examples

```r
##For an empty object
object<-PAM50()
object

##Using pam50centroids package example data
data(pam50centroids)
pam50centroids
```

Description

subjectReport is basically a grid.arrange object which basically consists of three main parts: a summary table, a two row ggplot2 facet_wrap with scatter ggplots (Wickham 2009) of subject expression and PAM50 centroids (Perou et al. 2000 & 2010) and a textGrob with the simulation parameter used. Particularly:

- **tableGrob** with the following fields:
  - **$Summary** subject name, PAM50 and Permutated subtype
  - **$Fields** for the five PAM50 subtypes:
    - Correlation: PAM50 centroid correlation with observed subject exprs.
    - p-value: permutation p-value obtained using the simulation.
    - FDR: adjusted p-value using False Discovery Rate.

- **ggplot facet_wrap** two rows to display scatter subject exprs vs PAM50 centroids, in addition to a the linear regression fix. If subject, has an unique subtype, then the graph is in red. In addition, if simulated permutations were run with keep=TRUE option, then null distribution boxplots are plotted with observed correlations as a big round point.

- **textGrob** the permutation @parameter slot used in the simulation.

Usage

```r
## S4 method for signature 'PAM50'
subjectReport(object, subject)

## S4 method for signature 'PAM50'
databaseReport(object, fileName, ...)
```
verbose = getOption("verbose", default = TRUE))

## S4 method for signature 'PAM50'
summary(object, ...)

Arguments

- **object**: a PAM50 object.
- **subject**: integer to select the appropriate subject to report.
- **fileName**: character with the name of the pdf report file to save.
- **...**: additional parameters for pdf function call.
- **verbose**: should the user feedback be displayed? By default value is "verbose" global option parameter, if present, or FALSE otherwise.

Details

summary it basically prints descriptive data of PAM50 dataset, the test parameters used, a frequency table of PAM50 Subtypes and a contingency table with Classes vs PAM50 Subtypes.
databaseReport basically is a pdf report where the first page is a global summary of the database, i.e., a summary contingency table of permutation test classes against original PAM50 subtypes results. The following pages are the database respective subjectReport outputs.

Value

depending on function call:
- **subjectReport**: a grid.arrange object.
- **databaseReport**: a pdf file with database summary and subjectReports.
- **summary**: Console summary statistics plus a data.frame

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References


See Also

- **PAM50** for a complete example.
- Other PAM50: `as`, `classify`, `filtrate`, `pam50centroids`, `permutate`, `PAM50-method`, `subtypes`, `PAM50-method`
- Other PAM50: `as`, `classify`, `filtrate`, `pam50centroids`, `permutate`, `PAM50-method`, `subtypes`, `PAM50-method`
- Other PAM50: `as`, `classify`, `filtrate`, `pam50centroids`, `permutate`, `PAM50-method`, `subtypes`, `PAM50-method`
Example

## Using pam50centroids package example data

data(pam50centroids)
pam50centroids

## This object has already run filtrate, classify and permutate. So, now
## we can obtain some reports:
## 1) database summary
summary(pam50centroids)

## 2) Individual subject report. If keep=FALSE boxplot panel is not available
subjectReport(pam50centroids, subject=1) # Basal subtype
subjectReport(pam50centroids, subject=1) # Her2 subtype

## 3) complete database report
# databaseReport(pam50centroids, fileName="PAM50.pdf", verbose=TRUE)

### Description

PAM50 subtypes are obtained using permuted test results. The idea is to give confidence in PAM50 subtype assessment (Perou et al. 2000 & 2010). In this context, the observed Spearman’s ρ correlation is tested against the null distribution obtained for each subtype. Then, only significant correlations are used in accordance to the following scheme:

- **Not assigned**: all subtype have fdr > pcutoff. Hence, there is evidence that the observed ρ can be obtained by random chance.
- **Assigned**: only one fdr <= pcutoff. There is not enough evidence to say that the observed ρ does not belong to the null distribution.
- **Ambiguous**: more than one have fdr <= pcutoff. Then, one of the following alternatives holds given the result of |ρ(profile, class_A) − ρ(profile, class_B)| > corCutoff.
  - **Assigned**: If the statement is TRUE.
  - **Ambiguous**: If the statement is FALSE.

Under the above scheme, the physician has an objective measurement to support the patient treatment decision. Both, with the given permuted subtype and by interpreting the p-value or fdr of each subtype null distribution test.

### Usage

```r
## S4 method for signature 'PAM50'
subtypes(object, pCutoff = 0.01, corCutoff = 0.1,
         where = c("fdr", "pvalue"))[1])
```

### Arguments

- **object**: a MolecularPermutationClassifier subclass object.
- **pCutoff**: numeric with p-value/fdr cutoff used depending on "where" selection. Default: 0.01.
corCutoff numeric with correlation difference between classes cutoff used, i.e., $|\rho(\text{profile, class}_A) - \rho(\text{profile, class}_B)| > \text{corCutoff}$. Default 0.1

where character with significant value used. Default value is "fdr".

Value

a PAM50 object with the updated slots:

@permutation $\text{subtype}$ data.frame with the following fields

$\text{PAM50}$ the original PAM50 subtype

$\text{Permuted}$ factor with the following levels:

- "Not assigned": all subtype have fdr $>$ pcutoff
- "Assigned": only one fdr $\leq$ pcutoff
- "Ambiguous": more than one fdr $\leq$ pcutoff

$\text{Classes}$ a character according to "Permuted" field:

- the unique PAM50 subtype if "Assigned"
- a combination for "Ambiguous" or
- NA if "Not assigned".

$\text{Class}$ idem as Classes but "Ambiguous" is set to PAM50 calls

$\text{Subtype}$ Classes but "Ambiguous" is kept as "Ambiguous" string.

@parameters $\text{SpCutoff, ScorCutoff}$ and $\text{where}$ are updated accordingly.

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References


See Also

PAM50 for a complete example.

Other PAM50: as, classify, PAM50-method, filtrate, PAM50-method, pam50centroids, permutate, PAM50-method, subjectReport, PAM50-method

Examples

```r
##Using pam50centroids package example data, which already had been
##filtrated, classified and permutated.
data(pam50centroids)
summary(pam50centroids)

##Now, let's change pCutoff and corCutoff without the need to run permutate
##again
pam50centroids<-subtypes(pam50centroids, pCutoff=0.01, corCutoff=Inf,
where="fdr")
pam50centroids
summary(pam50centroids)#Note that only Basal is not Ambiguos
```
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