

Package ‘oppar’

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Type Package

Title Outlier profile and pathway analysis in R

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Description The R implementation of mCOPA package published by Wang et al. (2012). Oppar provides methods for Cancer Outlier profile Analysis. Although initially developed to detect outlier genes in cancer studies, methods presented in oppar can be used for outlier profile analysis in general. In addition, tools are provided for gene set enrichment and pathway analysis.

biocViews Pathways, GeneSetEnrichment, SystemsBiology, GeneExpression, Software

Depends R (>= 3.3)

Imports Biobase, methods, GSEABase, GSVA

License GPL-2

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'getSubtypeProbes.R' 'gsva.R' 'opa-generic-methods.R' 'oppar.R'
'utils.R' 'zzz.R'

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Contents

bcm	2
eset	3
getSampleOutlier	4
getSubtypeProbes	5
gsva	6
maupin	9
opa	10
oppar	11
OPPARList-class	12
Index	13

bcm	<i>Breast cancer metastases from different anatomical sites</i>
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Description

An ExpressionSet object containing trimmed GSE46141 data. The object contains gene expression measurements on local breast tumours and liver, lymph node, skin local- regional etc metastatic tumours. Contains 9503 features and 80 samples (ascite, bone, lung and skin samples were removed).

Usage

data(GSE46141)

Format

- Contains gene expression matrix, phenotype (pData) and feature (fData) data:
- ID** In fData(e) – probe IDs
 - EntrezGeneID** In fData(e) – Entrez Ids
 - GeneSymbol** In fData(e) – Gene Symbols
 - geo_accession** In pData(e) – GEO accession IDs
 - source_name_ch1** In pData(e) – Sample information

Value

An ExpressionSet object

Source

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46141>

eset

Tomlins et al. Prostate Cancer data (GEO: GSE6099)

Description

An ExpressionSet object containing microarray gene expression measurements on normal tissue and metastatic prostate cancer tumours, and the corresponding feature and phenotypic meta data.

Usage

```
data(tomlins)
```

Format

An ExpressionSet object with 10945 features and 86 samples.

title In pData(eset) – Sample names

geo_accession In pData(eset) –GEO accession numbers

characteristics_ch1 In pData(eset) – sample description

ID In fData(eset) – probes Ids

Gene.title ...

Gene.symbol In fData(eset) – Gene Symbol

Gene.ID In fData(eset) – Entrez Gene IDs

Value

An ExpressionSet object

Source

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6099>

getSampleOutlier	<i>Retrieving outlier genes in samples</i>
------------------	--

Description

Returns a list of outlier genes for each given sample

Usage

```
getSampleOutlier(profileMatrix, sample.name)
```

```
## S4 method for signature 'matrix,nuchar'
getSampleOutlier(profileMatrix, sample.name)
```

```
## S4 method for signature 'OPPARList,nuchar'
getSampleOutlier(profileMatrix, sample.name)
```

Arguments

profileMatrix	A matrix of 0, -1 and 1 representing outlier genes in samples. Also an object of type OPPARList.
sample.name	A character vector containing one or more sample names, or a numeric vector containing sample indices.

Value

A list of lists. For each given sample, the function returns up-regulated and down-regulated outlier genes.

Methods (by class)

- profileMatrix = matrix, sample.name = nuchar: A method for getSampleOutlier
- profileMatrix = OPPARList, sample.name = nuchar: A method for getSampleOutlier

Examples

```
data(GSE46141)
library(Biobase)
group <- sapply(pData(bcm)$source_name_ch1, function(x){ ifelse(x == "breast",0,1)})
group <- factor(group)
bcm.opa <- opa(bcm,group=group)
# Outlier profile for sample "GSM1124929"
getSampleOutlier(bcm.opa, "GSM1124929")

# Also can use sample index, instead of sample name
getSampleOutlier(bcm.opa, 11)

# A vector of sample names/indices are accepted as well
```

```
getSampleOutlier(bcm.opa, c(1,2))
getSampleOutlier(bcm.opa, c("GSM1124929", "GSM1124941"))
```

getSubtypeProbes	<i>Retrieving outlier genes from a group of related samples</i>
------------------	---

Description

Returns a list of genes that are outlier in a group of samples, such as samples from the same subtype.

Usage

```
getSubtypeProbes(profileMatrix, sample.names)

## S4 method for signature 'matrix,nuchar'
getSubtypeProbes(profileMatrix, sample.names)

## S4 method for signature 'OPPARList,nuchar'
getSubtypeProbes(profileMatrix, sample.names)
```

Arguments

profileMatrix	A matrix of 0,1 and -1, representing outlier genes in samples. Also an object of type <code>OPPARList</code> .
sample.names	A character vector containing sample names, or a numeric vector containing the indices of the samples.

Value

A list of lists. The sub-lists are up-regulated outlier genes, and down-regulated outlier genes.

Methods (by class)

- `profileMatrix = matrix, sample.names = nuchar`: A method for `getSubtypeProbes` with signature `profileMatrix = matrix` and `sample.names = nuchar`
- `profileMatrix = OPPARList, sample.names = nuchar`: A method for `getSubtypeProbes` with signature `profileMatrix = OPPARList` and `sample.names = nuchar`

Examples

```
data(GSE46141)
library(Biobase)
group <- sapply(pData(bcm)$source_name_ch1, function(x){ ifelse(x == "breast",0,1)})
group <- factor(group)
bcm.opa <- opa(bcm,group=group)
# extracting liver samples
index <- which(pData(bcm)$source_name_ch1 == "liver")
samples <- rownames(pData(bcm)[index,])
```

```

samples <- match(samples, colnames(bcm.opa$profileMatrix))
samples <- Reduce(c,samples)
# liver subtype outlier profile
liver.subtype.outlier <- getSubtypeProbes(bcm.opa, samples)

```

gsva

gsva

Description

Gene Set Variation Analysis

Usage

```

gsva(expr, gset.idx.list, ...)

## S4 method for signature 'ExpressionSet,list'
gsva(expr, gset.idx.list, annotation,
      method = c("gsva", "ssgsea", "zscore", "plage"), rnaseq = FALSE,
      abs.ranking = FALSE, min.sz = 1, max.sz = Inf, no.bootstraps = 0,
      bootstrap.percent = 0.632, parallel.sz = 0, parallel.type = "SOCK",
      mx.diff = TRUE, tau = switch(method, gsva = 1, ssgsea = 0.25, NA),
      kernel = TRUE, ssgsea.norm = TRUE, verbose = TRUE,
      is.gset.list.up.down = FALSE)

## S4 method for signature 'ExpressionSet,GeneSetCollection'
gsva(expr, gset.idx.list,
      annotation, method = c("gsva", "ssgsea", "zscore", "plage"),
      rnaseq = FALSE, abs.ranking = FALSE, min.sz = 1, max.sz = Inf,
      no.bootstraps = 0, bootstrap.percent = 0.632, parallel.sz = 0,
      parallel.type = "SOCK", mx.diff = TRUE, tau = switch(method, gsva = 1,
      ssgsea = 0.25, NA), kernel = TRUE, ssgsea.norm = TRUE, verbose = TRUE,
      is.gset.list.up.down = FALSE)

## S4 method for signature 'matrix,GeneSetCollection'
gsva(expr, gset.idx.list, annotation,
      method = c("gsva", "ssgsea", "zscore", "plage"), rnaseq = FALSE,
      abs.ranking = FALSE, min.sz = 1, max.sz = Inf, no.bootstraps = 0,
      bootstrap.percent = 0.632, parallel.sz = 0, parallel.type = "SOCK",
      mx.diff = TRUE, tau = switch(method, gsva = 1, ssgsea = 0.25, NA),
      kernel = TRUE, ssgsea.norm = TRUE, verbose = TRUE,
      is.gset.list.up.down = FALSE)

## S4 method for signature 'matrix,list'
gsva(expr, gset.idx.list, annotation,
      method = c("gsva", "ssgsea", "zscore", "plage"), rnaseq = FALSE,
      abs.ranking = FALSE, min.sz = 1, max.sz = Inf, no.bootstraps = 0,

```

```
bootstrap.percent = 0.632, parallel.sz = 0, parallel.type = "SOCK",
mx.diff = TRUE, tau = switch(method, gsva = 1, ssgsea = 0.25, NA),
kernel = TRUE, ssgsea.norm = TRUE, verbose = TRUE,
is.gset.list.up.down = FALSE)
```

Arguments

<code>expr</code>	Gene expression data which can be given either as an <code>ExpressionSet</code> object or as a matrix of expression values where rows correspond to genes and columns correspond to samples.
<code>gset.idx.list</code>	Gene sets provided either as a list object or as a <code>GeneSetCollection</code> object.
<code>...</code>	other optional arguments.
<code>annotation</code>	In the case of calling <code>gsva()</code> with expression data in a matrix and gene sets as a <code>GeneSetCollection</code> object, the <code>annotation</code> argument can be used to supply the name of the Bioconductor package that contains annotations for the class of gene identifiers occurring in the row names of the expression data matrix. By default <code>gsva()</code> will try to match the identifiers in <code>expr</code> to the identifiers in <code>gset.idx.list</code> just as they are, unless the <code>annotation</code> argument is set.
<code>method</code>	Method to employ in the estimation of gene-set enrichment scores per sample. By default this is set to <code>gsva</code> (Hanzelmann et al, 2013) and other options are <code>ssgsea</code> (Barbie et al, 2009), <code>zscore</code> (Lee et al, 2008) or <code>plage</code> (Tomfohr et al, 2005). The latter two standardize first expression profiles into z-scores over the samples and, in the case of <code>zscore</code> , it combines them together as their sum divided by the square-root of the size of the gene set, while in the case of <code>plage</code> they are used to calculate the singular value decomposition (SVD) over the genes in the gene set and use the coefficients of the first right-singular vector as pathway activity profile.
<code>rnaseq</code>	Flag to inform whether the input gene expression data comes from microarray (<code>rnaseq=FALSE</code> , default) or RNA-Seq (<code>rnaseq=TRUE</code>) experiments.
<code>abs.ranking</code>	Flag to determine whether genes should be ranked according to their sign (<code>abs.ranking=FALSE</code>) or by absolute value (<code>abs.ranking=TRUE</code>). In the latter, pathways with genes enriched on either extreme (high or low) will be regarded as 'highly' activated.
<code>min.sz</code>	Minimum size of the resulting gene sets.
<code>max.sz</code>	Maximum size of the resulting gene sets.
<code>no.bootstraps</code>	Number of bootstrap iterations to perform.
<code>bootstrap.percent</code>	.632 is the ideal percent samples bootstrapped.
<code>parallel.sz</code>	Number of processors to use when doing the calculations in parallel. This requires to previously load either the <code>parallel</code> or the <code>snow</code> library. If <code>parallel</code> is loaded and this argument is left with its default value (<code>parallel.sz=0</code>) then it will use all available core processors unless we set this argument with a smaller number. If <code>snow</code> is loaded then we must set this argument to a positive integer number that specifies the number of processors to employ in the parallel calculation.
<code>parallel.type</code>	Type of cluster architecture when using <code>snow</code> .

<code>mx.diff</code>	Offers two approaches to calculate the enrichment statistic (ES) from the KS random walk statistic. <code>mx.diff=FALSE</code> : ES is calculated as the maximum distance of the random walk from 0. <code>mx.diff=TRUE</code> (default): ES is calculated as the magnitude difference between the largest positive and negative random walk deviations.
<code>tau</code>	Exponent defining the weight of the tail in the random walk performed by both the <code>gsva</code> (Hanzelmann et al., 2013) and the <code>ssgsea</code> (Barbie et al., 2009) methods. By default, this <code>tau=1</code> when <code>method="gsva"</code> and <code>tau=0.25</code> when <code>method="ssgsea"</code> just as specified by Barbie et al. (2009) where this parameter is called alpha.
<code>kernel</code>	Logical, set to <code>TRUE</code> when the GSVA method employs a kernel non-parametric estimation of the empirical cumulative distribution function (default) and <code>FALSE</code> when this function is directly estimated from the observed data. This last option is justified in the limit of the size of the sample by the so-called Glivenko-Cantelli theorem.
<code>ssgsea.norm</code>	Logical, set to <code>TRUE</code> (default) with <code>method="ssgsea"</code> runs the SSGSEA method from Barbie et al. (2009) normalizing the scores by the absolute difference between the minimum and the maximum, as described in their paper. When <code>ssgsea.norm=FALSE</code> this last normalization step is skipped.
<code>verbose</code>	Gives information about each calculation step. Default: <code>FALSE</code> .
<code>is.gset.list.up.down</code>	logical. Is the gene list divided into up/down sublists? Please note that it is important to name the up-regulated gene set list 'up', and the down-regulated gene set list to 'down', if this argument is used (e.g <code>gset = list(up = up_gset, down = down_gset)</code>)

Value

returns gene set enrichment scores for each sample and gene set

Methods (by class)

- `expr = ExpressionSet, gset.idx.list = list`: Method for `ExpressionSet` and `list`
- `expr = ExpressionSet, gset.idx.list = GeneSetCollection`: Method for `ExpressionSet` and `GeneSetCollection`
- `expr = matrix, gset.idx.list = GeneSetCollection`: Method for `matrix` and `GeneSetCollection`
- `expr = matrix, gset.idx.list = list`: Method for `matrix` and `list`

See Also

Hanzelmann, S., Castelo, R., & Guinney, J. (2013). GSVA: gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*, 14, 7. <http://doi.org/10.1186/1471-2105-14-7>

Examples

```
data("Maupin")
names(maupin)
geneSet<- maupin$sig$EntrezID      #Symbol  ##EntrezID # both up and down genes:
up_sig<- maupin$sig[maupin$sig$upDown == "up",]
d_sig<- maupin$sig[maupin$sig$upDown == "down",]
u_geneSet<- up_sig$EntrezID      #Symbol  # up_sig$Symbol  ## EntrezID
d_geneSet<- d_sig$EntrezID
es.dif <- gsva(maupin$data, list(up = u_geneSet, down= d_geneSet), mx.diff=1,
  verbose=TRUE, abs.ranking=FALSE, is.gset.list.up.down=TRUE, parallel.sz = 1 )$es.obs
```

maupin

Maupin's TGFb data and a TGFb gene signature

Description

A list consisting of two components: data: A matrix consisting of gene expression values on 3 control and 3 TGFb treated samples. sig: A TGFb gene signature. A dataframe containing a gene signature and information on whether genes in the signature are up or down regulated.

Usage

```
data(Maupin)
```

Format

A list of two components:

M_Ctrl_R1 In data; Control Sample - Replicate 1

M_Ctrl_R2 In data; Control Sample - Replicate 2

M_Ctrl_R3 In data; Control Sample - Replicate 3

M_TGFb_R1 In data; Control Sample - Replicate 1

M_TGFb_R2 In data; Control Sample - Replicate 2

M_TGFb_R3 In data; Control Sample - Replicate 3

EntrezID In sig; The Entrez IDs

Symbol In sig; Gene symbols

upDown In sig; Direction of regulation e.g. up, down

Value

A list of 2

Source

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23952>

Description

Returns a matrix with 0, -1 and 1 entries that describe outlier profiles in samples. The rows represent genes and the columns represent samples. -1 implies that the gene is a down-regulated outlier, 1 indicates an up-regulate outlier and 0 means that the gene is not an outlier in a sample.

Usage

```
opa(exprs.matrix, ...)

## S4 method for signature 'matrix'
opa(exprs.matrix, group, upper.quantile = 0.95,
     lower.quantile = 0.05)

## S4 method for signature 'ExpressionSet'
opa(exprs.matrix, group, upper.quantile = 0.95,
     lower.quantile = 0.05)
```

Arguments

<code>exprs.matrix</code>	Gene expression data. Can be either a matrix or an object of type <code>ExpressionSet</code> .
<code>...</code>	Numeric. To supply values for <code>upper.quantile</code> and <code>lower.quantile</code> arguments if default values are going to be override.
<code>group</code>	A vector of factors representing the groups to which each sample belong. This can be either a vector of 0s and 1s, or normal and cases.
<code>upper.quantile</code>	Numeric. The cut-off for upper quantile when determining outliers. Default to 0.95
<code>lower.quantile</code>	Numeric. The cut-off for lower quantile when determining outliers. Default to 0.05

Value

`opa` returns an object of type `OPPARList`. The outlier profiles are stored in `profileMatrix` and can be accessed using `$`. It is also possible to retrieve parameters used to run the outlier profile analysis, such as `upper.quantile`, `lower.quantile` via the `$` operator.

Methods (by class)

- `matrix`: `opa(exprs.matrix, group, lower.quantile = 0.05, upper.quantile = 0.95)`
- `ExpressionSet`: `opa(eset, group, lower.quantile = 0.05, upper.quantile = 0.95)`

See Also

Wang, C., Taciroglu, A., Maetschke, S. R., Nelson, C. C., Ragan, M. A., & Davis, M. J. (2012). mCOPA: analysis of heterogeneous features in cancer expression data. *Journal of Clinical Bioinformatics*, 2, 22. <http://doi.org/10.1186/2043-9113-2-22>

Examples

```
# loading bcm object from GSE46141 dataset
data(GSE46141)
library(Biobase)
# defining the group variable. local breast tumors are the controls
# and the rest of the samples are the diseased samples
group <- sapply(pData(bcm)$source_name_ch1, function(x){ ifelse(x == "breast",0,1)})
group <- factor(group)
# running opa with default values (i.e upper.quantile = 0.95, lower.quantile = 0.05)
# the result is an object of type OPPARList
opa(bcm,group = group)
```

oppar

oppar: A package for outlier profile and pathway analysis.

Description

The oppar package provides 3 main function for outlier profile analysis: opa, getSampleOutlier and getSubtypeProbes, and 1 function for pathway and gene set enrichment analysis, based on gsva function implemented in the GSVA package

opa

calculates the outlier profile matrix, using the method proposed in Wang et al. (2012) paper

getSampleOutlier

extracts outlier profile for individual samples

getSubtypeProbes

extracts outlier profile for a group of related samples, such as subtypes

OPPARList-class	<i>A S4 class for the output of OPPAR main function, opa.</i>
-----------------	---

Description

A S4 class for the output of OPPAR main function, opa.

Usage

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

## S4 method for signature 'OPPARList'
x$name
```

Arguments

object	An object of type OPPARList
x	Object of type OPPARList.
name	Name of the slot to access.

Value

returns the number of outlier features detected, the number of samples retained, and the parameters used to run the opa function

extracts slots from an object of type OPPARList.

Methods (by generic)

- show: A show method for objects of class OPPARList
- \$: A method to extract slots in OPPARList

Slots

profileMatrix A matrix of 0, -1 and 1 representing outlier genes in samples

upper.quantile Numeric. The upper quantile cut-off for detection of outliers

lower.quantile Numeric. The lower quantile cut-off for detection of outliers

group A factor vector representing the group to which each sample belong

.Data matrix

Index

- * **datasets**
 - bcm, [2](#)
 - eset, [3](#)
 - maupin, [9](#)
- \$,OPPARList-method (OPPARList-class), [12](#)
- bcm, [2](#)
- eset, [3](#)
- getSampleOutlier, [4](#)
- getSampleOutlier,matrix,nuchar-method
 - (getSampleOutlier), [4](#)
- getSampleOutlier,OPPARList,nuchar-method
 - (getSampleOutlier), [4](#)
- getSubtypeProbes, [5](#)
- getSubtypeProbes,matrix,nuchar-method
 - (getSubtypeProbes), [5](#)
- getSubtypeProbes,OPPARList,nuchar-method
 - (getSubtypeProbes), [5](#)
- gsva, [6](#)
- gsva,ExpressionSet,GeneSetCollection-method
 - (gsva), [6](#)
- gsva,ExpressionSet,list-method (gsva), [6](#)
- gsva,matrix,GeneSetCollection-method
 - (gsva), [6](#)
- gsva,matrix,list-method (gsva), [6](#)
- maupin, [9](#)
- opa, [10](#)
- opa,ExpressionSet-method (opa), [10](#)
- opa,matrix-method (opa), [10](#)
- oppar, [11](#)
- oppar-package (oppar), [11](#)
- OPPARList-class, [12](#)
- show,OPPARList-method
 - (OPPARList-class), [12](#)