Package 'phenoTest'

December 8, 2025

Type Package

Title Tools to test association between gene expression and phenotype in a way that is efficient, structured, fast and scalable. We also provide tools to do GSEA (Gene set enrichment analysis) and copy number variation.

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Author Evarist Planet

Maintainer Evarist Planet <evarist.planet@epfl.ch>

Description Tools to test correlation between gene expression and phenotype in a way that is efficient, structured, fast and scalable. GSEA is also provided.

License GPL (>=2)

Depends R (>= 3.6.0), Biobase, methods, annotate, Heatplus, BMA, ggplot2, Hmisc

Imports survival, limma, gplots, Category, AnnotationDbi, hopach, biomaRt, GSEABase, genefilter, xtable, annotate, mgcv, hgu133a.db, ellipse

Suggests GSEABase, GO.db

Enhances parallel, org.Ce.eg.db, org.Mm.eg.db, org.Rn.eg.db, org.Hs.eg.db, org.Dm.eg.db

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3 phenoTest-package

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Test correlation between gene expression and phenotype.

Description

Test correlation between gene expression and phenotype.

Details

Package: phenoTest Type: Package Version: 1.0

Date: 2010-04-28

License: What license is it under?

LazyLoad:

Author(s)

Evarist Planet Maintainer: Evarist Planet <evarist.planet@irbbarcelona.org>

barplotSignatures

Summary plots for gene signature vs phenotype association

Description

Summarizes the univariate relationships between genes in one or more signatures and several phenotype variables, as summarized in epheno objects (which can be created with the ExpressionPhenoTest function).

By default barplotSignifSignatures performs a binomial test (binom. test from package stats) for each signature to see if the number of up up regulated and down regulated genes is different enough to be statistically different. When a reference gene set is provided we test if the proportions of up and down regulated genes of each gene set is different from the proportions in the reference gene set. This has been done with a chi-square test. When a reference gene set is provided and parameter testUpDown is TRUE (by default its FALSE) the number of genes corresponding to up and down regulated are compared with those of the reference gene set separately.

Usage

```
barplotSignatures(x, signatures, referenceSignature, alpha=.05,
p.adjust.method='none', ylab, cex.text=1, ...)
barplotSignifSignatures(x, signatures, referenceSignature, testUpDown=FALSE,
simulate.p.value = FALSE, B = 10<sup>4</sup>, p.adjust.method='none', alpha=.05,
ylab, ylim=ylim, cex.text=1, ...)
```

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Arguments

x epheno object, as returned by ExpressionPhenoTest.

signatures List with each element corresponding to a signature. The gene names in each

signature must match those in epheno.

referenceSignature

If specified, the average fold change in each signature is compared to the average

fold change in the signature referenceSignature.

testUpDown If set to TRUE, bars corresponding to up and down-regulated genes are compared

with those of referenceSignature separately. This argument is ignored if

referenceSignature is not specified.

cex.text Character expansion for the text indicating the P-values. Ignored if referenceS-

ignature is missing.

alpha Confidence levels for barplot error bars.

p.adjust.method

P-value adjustment method, passed on to p. adjust.

simulate.p.value

A logical indicating whether chi-square p-values should be computed by Monte

Carlo simulation (passed on to chisq. test).

B Integer specifying the number of replicates in the Monte Carlo simulation (passed

on to chisq. test).

ylab y-axis labels ylim y-axis limits

. . . Other arguments to be passed on to boxplot.

Value

When a single signature is provided as input, a single plot assessing the association of that signature with all phenotype variables is created. If several signatures are provided, one separate plot is created for each phenotype variable.

Author(s)

Evarist Planet

Examples

```
#create epheno
data(epheno)

#construct two signatures
sign1 <- sample(featureNames(epheno))[1:20]
sign2 <- sample(featureNames(epheno))[1:15]
mySignature <- list(sign1,sign2)
names(mySignature) <- c('My first signature','My preferred signature')

#plot
barplotSignifSignatures(epheno[,'Relapse'],mySignature,alpha=0.05)</pre>
```

barplotSignatures-methods

Methods for Function barplotSignatures in Package 'phenoTest'

Description

Methods for function barplotSignatures in Package 'phenoTest'. For more information read the function's manual.

Methods

- signature(x = "epheno", signatures = "character") Method for an epheno object and one signature stored in an object of class character.
- signature(x = "epheno", signatures = "GeneSetCollection") Method for an epheno object
 and several signatures stored in an object of class GeneSetCollection.
- signature(x = "epheno", signatures = "GeneSet") Method for an epheno object and one signature stored in an object of class GeneSet.
- signature(x = "epheno", signatures = "list") Method for an epheno object and several signatures stored in an object of class list.

barplotSignifSignatures-methods

Methods for Function barplotSignifSignatures in Package 'phenoTest'

Description

Methods for function barplotSignifSignatures in Package 'phenoTest'. For more information read the function's manual.

Methods

- signature(x = "epheno", signatures = "character") Method for an epheno object and one signature stored in an object of class character.
- signature(x = "epheno", signatures = "list") Method for an epheno object and several signatures stored in an object of class list.
- signature(x = "epheno", signatures = "GeneSet") Method for an epheno object and one signature stored in an object of class GeneSet.
- signature(x = "epheno", signatures = "GeneSetCollection") Method for an epheno object and several signatures stored in an object of class GeneSetCollection.

6 ClusterPhenoTest

ClusterPhenoTest	Test association of clusters with phenotype.

Description

Test the associations between clusters that each sample belongs to (based on gene expression) and each phenotype.

Usage

```
ClusterPhenoTest(x,cluster,vars2test,B=10^4,p.adjust.method='none')
```

Arguments

X	ExpressionSet with phenotype information stored in pData(x).
cluster	variable of class character or factor telling at which cluster each sample be-

longs to.

vars2test list with components 'continuous', 'categorical', 'ordinal' and 'survival' indi-

cating which phenotype variables should be tested. 'continuous', 'categorical' and 'ordinal' must be character vectors, 'survival' a matrix with columns named 'time' and 'event'. The names must match names in names(pData(x)).

B An integer specifying the number of replicates used in the chi-square Monte

Carlo test (passed on to chisq. test).

p.adjust.method

Method for P-value adjustment, passed on to p.adjust.

Details

Test association between the provided clusters and each phenotype.

For variables in vars2test\\$continuous and vars2test\\$ordinal a Kruskal-Wallis Rank Sum test is used; for vars2test\\$categorical a chi-square test (with exact p-value if simulate.p.value is set to TRUE); for var2test\\$survival a Cox proportional hazards likelihood-ratio test.

Author(s)

David Rossell

Examples

```
#load data
data(eset)
eset

#construct vars2test
survival <- matrix(c("Relapse", "Months2Relapse"), ncol=2, byrow=TRUE)
colnames(survival) <- c('event', 'time')
#add positive to have more than one category</pre>
```

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```
pData(eset)[1:20,'lymph.node.status'] <- 'positive'
vars2test <- list(survival=survival,categorical='lymph.node.status')
vars2test

#first half of the samples will be one cluster and the rest the other cluster
cluster <- c(rep('Cluster1',floor(ncol(eset)/2)),rep('Cluster2',ncol(eset)-floor(ncol(eset)/2)))
#test association
ClusterPhenoTest(eset,cluster,vars2test=vars2test)</pre>
```

epheno

epheno object.

Description

Object obatined with ExpressionPhenoTest function.

Usage

data(epheno)

Format

The format is: Formal class 'epheno' [package "phenoTest"] with 8 slots ..@ p.adjust.method : chr "none" ..@ assayData :<environment: 0x1050d5a78> ..@ phenoData :Formal class 'Annotated-DataFrame' [package "Biobase"] with 4 slots@ varMetadata :'data.frame': 5 obs. of 1 variable: \$ labelDescription: chr [1:5] NA NA NA NA @ data :'data.frame': 12 obs. of 5 variables: \$ phenoName : Factor w/ 3 levels "lymph.node.status",..: 3 3 3 3 3 1 1 1 2 3 \$ phenoClass: Factor w/ 3 levels "categorical",..: 2 2 2 2 2 1 1 1 3 2 \$ phenoType : Factor w/ 3 levels "mean", "pval", ... 1 1 1 3 3 1 1 3 3 2 survTime: Factor w/ 1 level "Months2Relapse": NA@ dimLabels : chr [1:2] "sampleNames" "sampleColumns"@ .__classVersion__:Formal [1:3] 1 1 0 ..@ featureData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots@ varMetadata: 'data.frame': 0 obs. of 1 variable:\$ labelDescription: chr(0)@ data: 'data.frame': 1000 obs. of 0 variables@ dimLabels: chr [1:2] "featureNames" "featureColumns"@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 class 'MIAME' [package "Biobase"] with 13 slots@ name : chr ""@ lab : chr ""@ contact : chr ""@ title : chr ""@ abstract : chr ""@ url : chr ""@ pubMedIds: chr "" @ samples: list() @ hybridizations: list() @ normControls : list() @ preprocessing : list() @ other : list() @ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots @ .Data:List of 1 \$: int [1:3] 1 0 0 ..@ annotation : chr "hgu133a" ..@ protocolData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots@ varMetadata :'data.frame': 0 obs. of 1 variable:\$ labelDescription: chr(0)@ data :'data.frame': 12 obs. of 0 variables@ dimLabels : chr [1:2] "sampleNames" "sampleColumns"@ .__classVersion__:Formal class 'Versions'

8 epheno-class

Examples

```
data(epheno)
## maybe str(epheno); plot(epheno) ...

epheno-class Class "epheno"
```

Description

Object obtained with the ExpressionPhenoTest function. Contains FC, HR and pvals from testing expression values of each gene against phenotypic variables.

Objects from the Class

Objects can be created by calls of the form new("epheno", assayData, phenoData, featureData, exprs, ...).

Slots

- p. adjust.method: Object of class "character" containing the multiple testing adjustment method used (if one was used).
- approach: Object of class "character" containing 'frequentist' or 'bayesian' depending on the user's selecion.
- assayData: Object of class "AssayData" that is inherited from the ExpressionSet object used to create the epheno object.
- phenoData: Object of class "AnnotatedDataFrame" that contains information about the variables stored in the experimentData slot such as their class (continuous, categorical, etc) or type (mean, summaryDif, pval, etc).
- featureData: Object of class "AnnotatedDataFrame" that is inherited from the ExpressionSet object used to create the epheno object.
- experimentData: Object of class "MIAME" that is inherited from the ExpressionSet object used to create the epheno object.
- annotation: Object of class "character" that is inherited from the ExpressionSet object used to create the epheno object.
- protocolData: Object of class "AnnotatedDataFrame" that is inherited from the ExpressionSet object used to create the epheno object.
- .__classVersion__: Object of class "Versions" that is inherited from the ExpressionSet object used to create the epheno object.

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Extends

Class "ExpressionSet", directly. Class "eSet", by class "ExpressionSet", distance 2. Class "VersionedBiobase", by class "ExpressionSet", distance 3. Class "Versioned", by class "ExpressionSet", distance 4.

Methods

```
[ signature(x = "epheno", i = "ANY", j = "ANY"): inherited from the ExpressionSet class.
dim signature(x = "epheno"): inherited from the ExpressionSet class.
export2CSV signature(x = "epheno"): ...
getFc signature(x = "epheno"): getter for the fold changes.
getHr signature(x = "epheno"): getter for the hazard ratios.
getMeans signature(x = "epheno"): getter for the means.
getSignif signature(x = "epheno"): getter for the pvalues or posterior probabilities.
getPvals signature(x = "epheno"): getter for the pvalues.
getPostProbs signature(x = "epheno"): getter for the posterior probabilities.
getSummaryDif signature(x = "epheno"): getter that returns hazard ratios, fold changes and
     pvalues.
gseaSignatures signature(x = "epheno", signatures = "list"): Used to compute GSEA. Please
     read the gseaSignatures manual.
logFcHr signature(x = "epheno"): getter for the log of fold changes and hazard ratios.
p.adjust.method signature(x = "epheno"): getter for the p value adjustment method that has
     been used.
phenoClass signature(x = "epheno"): Returns the class off all variables.
phenoNames signature(x = "epheno"): Returns the names of the tested phenotypes.
show signature(object = "epheno"): Shows a brief overview of the object.
```

Author(s)

Evarist Planet

Examples

```
showClass("epheno")
```

10 epheno2html

epheno2html	Create html files and plots from an epheno object.
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Description

Creates html files and plots using an epheno object, which stores the association between a list of variables and gene expression.

Usage

```
epheno2html(x, epheno, outputdir, prefix = "", genelimit = 50, categories = 3, withPlots = TRUE, mc.core
```

Arguments

X	An object of class ExpressionSet (used to generate the epheno object) containing expression levels in $exprs(x)$, phenotype information in $pData(x)$ and annotation in annotation(x).
epheno	an object produced by ExpressionPhenoTest. this object will contain univariate association between a list of phenotype variables and gene expression as weel as p-values.
outputdir	where to place files.
prefix	will be used to add a text to the beginning of the files that will be created.
genelimit	maximum number of genes on the list.
categories	Number of categories used for continuous variables. It has to be the same as the one used for ExpressionPhenoTest.
withPlots	when FALSE no plots will be produced. Makes the process faster.
mc.cores	number of cores that will be used to run the process.

Author(s)

Evarist Planet

Examples

```
#Example on building homology tables for human.
#mart <- useMart("ensembl", "hsapiens_gene_ensembl")
#homol.symbol <- getLDS(attributes = c("entrezgene"),
# mart = mart, attributesL = c("external_gene_id"),
# martL = mart, filters = "entrezgene", values = entrezid)
#mart <- useMart("ensembl", "hsapiens_gene_ensembl")
#homol.genename <- getLDS(attributes = c("entrezgene"),
# mart = mart, attributesL = c("description"), martL = mart,
# filters = "entrezgene", values = entrezid)</pre>
```

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eset

Example data.

Description

Example data of class ExpressionSet.

Usage

data(eset)

Format

The format is: Formal class 'ExpressionSet' [package "Biobase"] with 7 slots ..@ assayData :<environment: 0x1050d9390> ..@ phenoData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots@ varMetadata: 'data.frame': 7 obs. of 1 variable:\$ labelDescription: chr [1:7] NA NA NA NA@ data :'data.frame': 286 obs. of 7 variables:\$ PID: int [1:286] 3 5 6 7 8 9 11 14 15 17 GEOaccession: Factor w/ 286 levels "GSM36777", "GSM36778",..: 17 20 21 22 24 25 58 59 60 61 lymph.node.status: chr [1:286] "negative" "negative" "negative" "negative" \$ Months2Relapse : int [1:286] 101 118 9 106 37 125 109 14 99 137 Relapse: int [1:286] 0 0 1 0 1 0 0 1 0 0 ER.Status : num [1:286] 0 1 0 0 0 1 1 0 1 1 BrainRelapse : int [1:286] 0 0 0 0 0 0 0 0 0 0@ dimLabels : chr [1:2] "sampleNames" "sampleColumns"@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots@ .Data:List of 1 \$: int [1:3] 1 1 0 ..@ featureData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots@ varMetadata: 'data.frame': 16 obs. of 3 variables:\$ Column : chr [1:16] "ID" "GB_ACC" "SPOT_ID" "Species Scientific Name" \$\text{Description} : Factor w/ 15 levels "","A gene symbol, when one is available (from UniGene).",..: 3 5 15 13 12 1 11 1 10 14 labelDescription: chr [1:16] NA NA NA NA @ data :'data.frame': 1000 obs. of 16 variables: ID: Factor w/ 22284 levels "1007_s_at","1053_at",..: 1 2 3 4 5 6 7 8 9 10 \$ GB ACC : Factor w/ 21129 levels "AF052179", "AF061832",...: 93 30 95 97 25 24 96 99 28 20 \$ SPOT_ID : chr [1:1000] NA NA NA NA\$ Species.Scientific.Name: Factor w/ 2 levels "Homo sapiens",..: 1 1 1 1 1 1 1 1 1 1\$ Annotation.Date : Factor w/ 2 levels "Jul 11, 2007",..: 1 1 1 1 1 1 1 1 1 1 1\$ Sequence. Source: Factor w/3 levels "Affymetrix Proprietary Database",..: 1 2 1 2 1 2 1 1 2 1\$ Target.Description: Factor w/ 21363 levels "Consensus includes gb:AI656011 /FEA=EST DB_XREF=gi:4739990 /DB_XREF=est:tt42e08.x1 /CLONE=IMAGE:2243462 /UG=Hs.116875 KIAA0156"| __truncated__,..: 16 13 18 20 8 7 19 22 11 4\$ Representative.Public.ID: Factor w/ 21197 levels "AF052179", "AF061832", ...: 93 30 95 97 25 24 96 99 28 20 Gene.Title: Factor w/ 14208 levels "ADP-ribosylation factor 1",..: 35 66 46 60 44 97 96 64 26 33\$ Gene.Symbol : Factor w/ 13293 levels "ABCF1","ARF1",..: 20 59 40 53 33 96 94 58 15 18 \$ ENTREZ_GENE_ID : chr [1:1000] "780" "5982" "3310" "7849" RefSeq.Transcript.ID: Factor w/ 13074 levels "NM 000409", "NM 000661 /// NM 001024921",...: 37 45 41 52 1 50 49 82 47 4 \$ Gene.Ontology.Biological.Process: Factor w/ 7245 levels "","0000074 // regulation of progression through cell cycle // traceable author statement /// 0006139 // nucleobase, nucleoside, nu"| __truncated__,..: 61 22 78 32 79 60 14 63 72 20

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..\$ Gene.Ontology.Cellular.Component: Factor w/ 4148 levels "","0000502 // proteasome complex (sensu Eukaryota) // traceable author statement /// 0005634 // nucleus // inferred from electroni"| truncated ...: 72 45 1 44 1 1 42 71 6 68 \$ Gene.Ontology.Molecular.Function: Factor w/ 7314 levels "","0000049 // tRNA binding // non-traceable author statement /// 0000166 // nucleotide binding // inferred from electronic annotat"| __truncated__,..: 23 26 27 40 81 18 39 71 74 69@ dimLabels : chr [1:2] "featureNames" "featureColumns"@ .__classVer-with 13 slots @ name : chr "" @ lab : chr "" @ contact : chr "" @ title : chr ""@ abstract : chr ""@ url : chr ""@ pubMedIds : chr ""@ samples: list()@ hybridizations: list()@ normControls: list()@ preprocessing: list()@ other: list()@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots @ .Data:List of 1 \$: int [1:3] 1 0 0 .. @ annotation : chr "hgu133a" ..@ protocolData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots@ varMetadata: 'data.frame': 0 obs. of 1 variable:\$ labelDescription: chr(0)@ data:'data.frame': 286 obs. of 0 variables@ dimLabels: chr [1:2] "sampleNames" "sampleColumns"@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 'Versions' [package "Biobase"] with 1 slots@ .Data:List of 4 \$: int [1:3] 2 12 0\$: int [1:3] 2 10 0\$: int [1:3] 1 3 0\$: int [1:3] 1 0 0

References

Has been obtained from GEO (GSE2034). Only first 1000 probesets where stored (the rest has been removed).

Examples

```
data(eset)
## maybe str(eset) ; plot(eset) ...
```

eset.genelevel

Example data.

Description

Example data of class ExpressionSet with one probeset per gene.

Usage

```
data(eset.genelevel)
```

Format

The format is: Formal class 'ExpressionSet' [package "Biobase"] with 7 slots ..@ assayData :<environment: 0x1050d9390> ..@ phenoData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots@ varMetadata :'data.frame': 7 obs. of 1 variable:\$ labelDescription: chr [1:7] NA NA NA NA@ data :'data.frame': 286 obs. of 7 variables:\$

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PID: int [1:286] 3 5 6 7 8 9 11 14 15 17 GEOaccession: Factor w/ 286 levels "GSM36777", "GSM36778",..: 17 20 21 22 24 25 58 59 60 61 lymph.node.status: chr [1:286] "negative" "negative" "negative" "negative" \$ Months2Relapse : int [1:286] 101 118 9 106 37 125 109 14 99 137 Relapse: int [1:286] 0 0 1 0 1 0 0 1 0 0 \$ ER.Status : num [1:286] 0 1 0 0 0 1 1 0 1 1 \$ BrainRelapse : int [1:286] 0 0 0 0 0 0 0 0 0 0@ dimLabels : chr [1:2] "sampleNames" "sampleColumns"@ _classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots @ .Data:List of 1 \$: int [1:3] 1 1 0 ..@ featureData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots@ varMetadata: 'data.frame': 16 obs. of 3 variables:\$ Column : chr [1:16] "ID" "GB_ACC" "SPOT_ID" "Species Scientific Name" \$ Description : Factor w/ 15 levels ""," A gene symbol, when one is available (from UniGene).",..: 3 5 15 13 12 1 11 1 10 14 labelDescription: chr [1:16] NA NA NA NA @ data :'data.frame': 1000 obs. of 16 variables: \$ ID: Factor w/ 22284 levels "1007_s_at","1053_at",..: 1 2 3 4 5 6 7 8 9 10 \$GB_ACC : Factor w/ 21129 levels "AF052179","AF061832",..: 93 30 95 97 25 24 96 99 28 20 \$ SPOT_ID : chr [1:1000] NA NA NA NA\$ Species.Scientific.Name: Factor w/ 2 levels "Homo sapiens",..: 1 1 1 1 1 1 1 1 1 1\$ Annotation.Date : Factor w/ 2 levels "Jul 11, 2007",..: 1 1 1 1 1 1 1 1 1 1 1 Sequence. Source: Factor w/ 3 levels "Affymetrix Proprietary Database",..: 1 2 1 2 1 2 1 1 2 1\$ Target.Description: Factor w/ 21363 levels "Consensus includes gb:AI656011 /FEA=EST DB_XREF=gi:4739990 /DB_XREF=est:tt42e08.x1 /CLONE=IMAGE:2243462 /UG=Hs.116875 KIAA0156" truncated ...: 16 13 18 20 8 7 19 22 11 4 Representative.Public.ID: Factor w/ 21197 levels "AF052179", "AF061832", ..: 93 30 95 97 25 24 96 99 28 20 \$ Gene.Title: Factor w/ 14208 levels "ADP-ribosylation factor 1",..: 35 66 46 60 44 97 96 64 26 33 \$ Gene.Symbol : Factor w/ 13293 levels "ABCF1", "ARF1",...: 20 59 40 53 33 96 94 58 15 18\$ ENTREZ GENE ID : chr [1:1000] "780" "5982" "3310" "7849" RefSeq.Transcript.ID: Factor w/ 13074 levels "NM_000409", "NM_000661 /// NM_001024921",...: 37 45 41 52 1 50 49 82 47 4 \$ Gene.Ontology.Biological.Process: Factor w/ 7245 levels "","0000074 // regulation of progression through cell cycle // traceable author statement /// 0006139 // nucleobase, nucleoside, nu" __truncated__,..: 61 22 78 32 79 60 14 63 72 20\$ Gene.Ontology.Cellular.Component: Factor w/ 4148 levels "","0000502 // proteasome complex (sensu Eukaryota) // traceable author statement /// 0005634 // nucleus // inferred from electroni" _truncated__,..: 72 45 1 44 1 1 42 71 6 68 Gene.Ontology.Molecular.Function: Factor w/7314 levels "","0000049 // tRNA binding // non-traceable author statement /// 0000166 // nucleotide binding // inferred from electronic annotat" | __truncated__,..: 23 26 27 40 81 18 39 71 74 69@ dimLabels : chr [1:2] "featureNames" "featureColumns"@ .__classVer-with 13 slots@ name : chr ""@ lab : chr ""@ contact : chr ""@ title : chr ""@ abstract : chr ""@ url : chr ""@ pubMedIds : chr ""@ samples: list() @ hybridizations: list() @ normControls: list() @ preprocessing: list()@ other: list() @ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots @ .Data:List of 1 \$: int [1:3] 1 0 0 .. @ annotation : chr "hgu133a" ..@ protocolData :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots@ varMetadata:'data.frame': 0 obs. of 1 variable:\$ labelDescription: chr(0)@ data:'data.frame': 286 obs. of 0 variables@ dimLabels : chr [1:2] "sampleNames" "sampleColumns"@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slots@ .Data:List of 1\$: int [1:3] 1 1 0 .. .@ .__classVersion__:Formal class

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```
'Versions' [package "Biobase"] with 1 slots .....@ .Data:List of 4 ......$: int [1:3] 2 12 0 .......
..$: int [1:3] 2 10 0 .......$: int [1:3] 1 3 0 ......$: int [1:3] 1 0 0
```

References

Has been obtained from GEO (GSE2034). Only first 1000 probesets where stored (the rest has been removed). After that the expressionSet was filtered to keep only one probeset per gene. We used the nsFilter function from package genefilter to accomplish this task.

Examples

```
data(eset.genelevel)
## maybe str(eset.genelevel) ; plot(eset.genelevel) ...
```

eset2genelevel

Filter ExpressionSet to keep one probeset per gene.

Description

Only one probeset per gene will be kept and entrezid will be used as gene identifier. nsFilter from package genefilter is used to select the probeset. The selected probeset is the one with higher interquartilic range.

Usage

```
eset2genelevel(x)
```

Arguments

X

an object of class ExpressionSet.

Author(s)

Evarist Planet

See Also

genefilter::nsFilter

Examples

```
#data(eset)
#library(hgu133a.db)
#x <- eset2genelevel(eset)
#x
#head(featureNames(x))</pre>
```

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export2CSV Export object to comma-separated text file.	export2CSV	Export object to comma-separated text file.	
--	------------	---	--

Description

Saves object as comma-separated text file (CSV), using write.csv.

Usage

```
export2CSV(x, file, row.names=FALSE, ...)
```

Arguments

X	object to be exported. Currently methods for objects of class epheno (produced
	with ExpressionPhenoTest function) are implemented.

file Name of the file where the results are to be saved

row.names Passed on to write.csv

... Other arguments to be passed on to write.csv

export2CSV-methods Methods for Function export2CSV in Package 'phenoTest'

Description

Methods for function export2CSV in Package 'phenoTest'

Methods

signature(x = "epheno") Exports summary differences (fold changes, hazard ratios), p-values and gene annotation (when available) to a CSV (comma separated value) file

16 ExpressionPhenoTest

ExpressionPhenoTest	Tests univariate association between a list of phenotype variables and
	gene expression.

Description

Tests univariate association between a list of phenotype variables and gene expression.

Usage

```
ExpressionPhenoTest(x, vars2test, adjustVars,
p.adjust.method='BH',continuousCategories=3,mc.cores,approach='frequentist')
```

Arguments

X	ExpressionSet containing expression levels in $exprs(x)$ and phenotype information in pData(x).	
vars2test	list with components 'continuous', 'categorical', 'ordinal' and 'survival' indicating which phenotype variables should be tested. 'continuous', 'categorical' and 'ordinal' must be character vectors, 'survival' a matrix with columns named 'time' and 'event'. The names must match names in names(pData(x)).	
adjustVars	variables that will be used as adjustment variables when fitting linear models and/or cox models. This variables have to exist in colnames(pData(x)).	
p.adjust.method		
	method for p-value adjustment, passed on to p. adjust. Valid values are c("holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none").	
continuousCategories		
	number of categories used for continuous variables.	
mc.cores	the number of cores to use, i.e. how many processes will be spawned (at most).	

Details

approach

If approach is 'frequentist': -The effect of both continuous, categorical and ordinal phenotype variables on gene expression levels are tested via lmFit. -For ordinal variables a single coefficient is used to test its effect on gene expression (trend test), which is then used to obtain a P-value (means for each category are reported in the output). -Gene expression effects on survival are tested via Cox proportional hazards model, as implemented in function 'coxph'.

this can be either 'frequentist' or 'bayesian'. With frequentist pvalues will be

computed. With 'bayesian' posterior probabilities will be computed.

If approach is bayesian posterior probabilities are computed comparing the BIC of a model with the variable of interest as explanatory variable against the BIC of the same model without the variable of interest as explanatory variable.

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Value

The output is an epheno object, which basically extends an ExpressionSet object. The means, fold changes, standarized hazard ratios and pvalues are stored in the experimentData slot which is accessible with the exprs method. Information about the kind of information of each variable can be found in the phenoData slot which is accessible with the pData method.

There are several methods that can be used to access the information stored in an epheno object. For more information please type one of the following: getFc(x), getHr(x), getMeans(x), getSignif,getPvals(x), getPostProbs, getSummaryDif(x), logFcHr(x), p.adjust.method(x), phenoClass(x), phenoNames(x).

Author(s)

David Rossell

References

Kass R.E. and Wasserman L. A Reference Bayesian Test for Nested Hypotheses and its Relationship to the Schwarz Criterion. Journal of the American Statistical Association, 90, pp. 928-934.

Examples

```
#load eset
data(eset)
eset

#prepare vars2test
survival <- matrix(c("Relapse","Months2Relapse"),ncol=2,byrow=TRUE)
colnames(survival) <- c('event','time')
vars2test <- list(survival=survival)

#run ExpressionPhenoTest
epheno <- ExpressionPhenoTest(eset,vars2test,p.adjust.method='none')
epheno</pre>
```

findCopyNumber

Find copy number regions using expression data in a similar way ACE does.

Description

Given enrichment scores between two groups of samples and the chromosomical positions of those enrichment scores this function finds areas where the enrichemnt is bigger/lower than expected if the positions where assigned at random. Plots of the regions and positions of the enriched regions are provided.

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Usage

```
findCopyNumber(x, minGenes = 15, B = 100, p.adjust.method = "BH",
pvalcutoff = 0.05, exprScorecutoff = NA, mc.cores = 1, useAllPerm = F,
genome = "hg19", chrLengths, sampleGenome = TRUE, useOneChr = FALSE,
useIntegrate = TRUE,plot=TRUE,minGenesPerChr=100)
```

Arguments

x An object of class data. frame with gene or probe identifiers as row names and

the following columns: es (the enrichment score), chr (the chromosome where the gene or probe belong to) and pos (position in the chromosome in megabases). It can be obtained (from an epheno object) with the function getEsPositions.

minGenes Minimum number of genes in a row that have to be enriched to mark the region

as enriched. Has to be bigger than 2.

B Number of permuations that will be computed to calculate pvalues. If useAllPerm

is FALSE this value has to be bigger than 100. If useAllPerm is TRUE the computations are much more expensive, therefore it is not recommended to use a B

bigger than 100.

p.adjust.method

P value adjustment method to be used. p.adjust.methods provides a list of avail-

able methods.

pvalcutoff All genes with an adjusted p value lower than this parameter will be considered

enriched.

exprScorecutoff

Genes with a smoothed score that is not bigger (lower if the given number is

negative) than the specified value will not be considered significant.

mc.cores Number of cores to be used in the computation. If mc.cores is bigger than 1 the

multicore library has to be loaded.

useAllPerm If FALSE for each gene only permutations of genes that are in an area with

similar density (similar number of genes close to them) are used to compute

pvalues. If TRUE all permutations are used for each gene.

We recommend to use the option FALSE after having observed that the enrich-

ment can depend on the number of genes that are in the area.

We recommend to use the option TRUE if the positions of the enrichment score are equidistant. Take into account that this option is much slower and needs less

permutations, therefore a smaller B is preferred.

See details for more info.

genome Genome that will be used to draw cytobands.

chrLengths An object of class numeric containing chromosome names as names. This

names have to be the same as the ones used in x\$chr If missing the last po-

sition is used.

sampleGenome If positions are sampled over the hole genome (across chromosomes) or within

each chromosome. This is TRUE by default.

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useOneChr Use only one chromosome to build the distribution under the null hypothesis

that genes/probes are not enriched. By default this is FALSE. The chromosome that is used is chosen as follows: after removing small chromosomes we select the one closest to the median quadratic distance to 0. Setting this parameter to

TRUE decreases processing time.

assume any distribution for the distribution under the null hypothesis, the second

assumes it is normally distributed.

plot If FALSE the function will make no plots.

minGenesPerChr Chromosomes with less than minGenesPerChr will be removed from the analy-

sis.

Details

Enrichemnt scores can be either log fold changes, log hazard ratios, log variability ratios or any other score.

Within each chromosome a smoothed score for each gene is obtained via generalized additive models, the smoothing parameter for each chromosome being chosen via cross-validation. The obtained smoothing parameter of each chromosome will be used in permutations.

We assessed statistical significance by permuting the positions thrue the hole genome. If useAllPerm is FALSE for each gene the permutations of genes that are in an area with similar density (distance to tenth gene) are used to compute pvalues. We observed that genes with similar densities tend to have similar smoothed scores. If we set 1000 permutations (B=1000) scores are permuted thrue the hole genome 10 times (1000/100). For each smoothed scored the permutations of the 100 smoothed scores with most similar density (distance to tenth gene) are used. Therefore each smoothed score will be compared to 1000 smoothed scores obtained from permutations.

If scores are at the same distance in the genome from each other (for instance when we have a score every fixed certain bases) the option useAllPerm=TRUE is recommended. In this case every smoothed score is compared to all smoothed scores obtained via permutations. In this case having 20,000 genes and setting the paramter B=10 would mean that the scores are permuted 10 times times thrue the hole genome, obtaining 200,000 permuted smoothed scores. Each observed smoothed score will be tested against the distribution of the 200,000 permuted smoothed scores.

Only regions with as many genes as told in minGenes being statistically significant (pvalue lower than parameter pvalcutoff) after adjusting pvalues with the method specified in p. adjust.method will be selected as enriched. If exprScorecutoff is different form NA, a gene to be statistically significant will need (aditionally to the pvalue cutoff) to have a smoothed score bigger (lower if exprScorecutoff is negative) than the specified value.

Value

Plots all chromomes and marks the enriched regions. Also returns a data.frame containing the positions of the enriched regions. This output can be passed by to the genesInArea function to obtain the names of the genes that are in each region.

Author(s)

Evarist Planet

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See Also

```
getEsPositions, genesInArea
```

Examples

genesInArea

Find genes that are in given areas.

Description

Combine the output of getEsPositions and findCopyNumber to see which genes are in the enriched areas.

Given areas of enrichment (obtained with findCopyNumber) and a set of genes or probes and their positions in the genome (obtained with getEsPositions) the function tells which genes fall in each area.

Usage

```
genesInArea(x, regions)
```

Arguments

Х

An object of class data.frame with gene or probe identifiers as row names and the following columns: es (the enrichment score), chr (the chromosome where the gene or probe belong to) and pos (position in the chromosome in megabases). It can be obtained with the function getEsPositions.

regions

This is usually the output of findCopyNumber function.

Author(s)

Evarist Planet

See Also

getEsPositions, findCopyNumber

Examples

```
data(epheno)
phenoNames(epheno)
mypos <- getEsPositions(epheno,'Relapse')
head(mypos)
#regions <- findCopyNumber(mypos)
#head(regions)
#genes <- genesInArea(mypos,regions)
#head(genes)</pre>
```

```
get gseaSignatures' elements
```

Substract element's of a gseaSignaturesSign or gseaSignaturesVar object (obtained using the gseaSignatures function).

Description

getEs returns ES (enrichment scores) getEsSim returns simulated ES (needed to compute pvals), getNes returns NES (normalized enrichment scores) and getFcHr returns the fold changes or hazard used to compute the ES, simulated ES and NES.

Usage

```
getEs(x)
getEsSim(x)
getNes(x)
getFcHr(x)
```

Arguments

Χ

an gseaSignaturesSign or gseaSignaturesVar object. Those objects are obtained using the gseaSignatures function.

Author(s)

Evarist Planet

getEsPositions

Obtain chromosome positions for each gene.

Description

Given an object of class epheno obtain the gene positions on the genome.

Usage

```
getEsPositions(epheno, phenoName, organism = "human", logEs = T, center = FALSE)
```

Arguments

epheno An object of class epheno usually obtained with ExpressionPhenoTest
phenoName The phenotype that we want to use. Has to be in phenoNames(epheno)

organism Has to be 'human' or 'mouse'. The default is 'human'.

logEs If the values have to be log scaled.

center If the values have to be genome centered. If TRUE the genome average will be

substracted to every value.

Details

The output will usually be passed to findCopyNumber.

Value

An object of class data. frame will be returned containing 3 variables: es (enichment score for fold change or hazard ratio), chr (chromosome), pos (position in Mb). epheno's featureNames will be used as row names.

Author(s)

Evarist Planet

Examples

```
data(epheno)
phenoNames(epheno)
mypos <- getEsPositions(epheno,'Relapse')
head(mypos)</pre>
```

```
getters for the epheno object
```

Getters for the epheno object:

Description

getFc gets the fold changes. getHr gets the hazard ratios. getMeans gets the means. getPvals gets the p values. getPostProbs get the posterior probabilities. getSignif gets the pvalues or the posterior probabilities depending on the approach (frequentist or bayesian) that was used when the epheno object was created. getSummaryDif gets fold changes and hazard ratios. lofFcHr gets the fold changes and hazard ratios after log scaling. p.adjust.method gets the p value adjustment method that was used when creating the object. phenoClass returns a data.frame telling the class (ordinal, continuous, categorical or survival) of each phenotype. phenoNames gets the phenotype names. approach gets the approach that was used (either frequentist or bayesian).

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Usage

```
getFc(x)
getHr(x)
getMeans(x)
getSignif(x)
getPvals(x)
getPostProbs(x)
getSummaryDif(x)
logFcHr(x)
p.adjust.method(x)
phenoClass(x)
phenoNames(x)
approach(x)
```

Arguments

Χ

epheno object

Author(s)

Evarist Planet

getVars2test

Get phenotypic variables that were tested.

Description

Returns an object containing the names of the variables that were tested when the epheno object was created. Will return an object of class list. Variables of the same type (categorical, survival, etc) will be in the same slot of the list. The slot names are the types of the variables.

Author(s)

Evarist Planet

Examples

```
data(epheno)
getVars2test(epheno)
```

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getVars2test-methods Methods for Function getVars2test in Package 'phenoTest'

Description

Methods for function getVars2test in Package 'phenoTest'. For more information read the funcion's manual.

Methods

signature(x = "epheno") Method for an object of class epheno.

gsea

GSEA (Gene Set Enrichment Analysis).

Description

Computes the enrichment scores and simulated enrichment scores for each variable and signature. An important parameter of the function is logScale. Its default value is TRUE which means that by default the provided scores (i.e. fold changes, hazard ratios) will be log scaled. Remember to change this parameter to FALSE if your scores are already log scaled. The getEs, getEsSim, getFc, getHr and getFcHr methods can be used to acces each subobject. For more information please visit the man pages of each method.

It also computes the NES (normalized enrichment score), p values and fdr (false discovery rate) for all variables and signatures. For an overview of the output use the summary method.

In case of providing gene sets which have more than 10 distinct lengths an approximation of the calculation of the enrichment score simulations (ESM) will be computed. The value of the ESM only depends on the length of the gene set. Therefore we compute the ESM over a grid of possible gene set lengths which are representative of the lengths of the provided gene sets. Then we fit a generalized additive model model with cubic splines to predict the NES value based on the length of every gene set. This provides a much faster approach that can be very useful when we need to run the software over a huge number of gene sets.

Usage

```
gsea(x,gsets,logScale=TRUE, absVals=FALSE, averageRepeats=FALSE, B=1000,
    mc.cores=1, test="perm",p.adjust.method="none",
    pval.comp.method="original",pval.smooth.tail=TRUE,minGenes=10,
    maxGenes=500,center=FALSE)
```

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Arguments

x ePhenoTest, numeric or matrix object containing scores (hazard ratios or fold

changes).

gsets character or list object containing the names of the genes that belong to each

signature.

logScale if values should be log scaled.

absVals if TRUE fold changes and hazard ratios that are negative will be turned into

positive before starting the process. This is useful when genes can go in both

directions.

averageRepeats if x is of class numeric and has repeated names (several measures for some

indivdual names) we can average the measures of the same names.

B number of simulations to perform.

mc.cores number of processors to use.

test the test that will be used. 'perm' stands for the permutation based method,

'wilcox' stands for the wilcoxon test (this is the fastest one) and 'ttperm' stands

for permutation t test.

p.adjust.method

p adjustment method to be used. Common options are 'BH', 'BY', 'bonferroni' or 'none'. All available options and their explanations can be found on the

p.adjust function manual.

pval.comp.method

the p value computation method. Has to be one of 'signed' or 'original'. The

default one is 'original'. See details for more information.

pval.smooth.tail

if we want to estimate the tail of the ditribution where the pvalues will be gen-

erated.

minGenes gene sets with less than minGenes genes will be removed from the analysis.

maxGenes gene sets with more than maxGenes genes will be removed from the analysis.

center if we want to center scores (fold changes or hazard ratios). The following is will

be done: x = x-mean(x).

Details

The following preprocessing was done on the provided scores (i.e. fold changes, hazard ratios) to avoid errors during the enrichment score computation: -When having two scores with the same name its average was used. -Zeros were removed. -Scores without names (which can not be in any signature) removed. -Non complete cases (i.e. NAs, NaNs) were removed. ES score was calculated for each signature and variable (see references). If parameter test is 'perm' the signature was permutted and the ES score was recalculated (this happened B times for each variable, 1000 by default). If test is 'wilcox' a wilcoxon test in which we test the fact that the average value of the genes that do belong to our signature is different from the average value of the genes that do not belong to our signature will be performed. If test is 'ttperm' a permutation t-test will be used. Take into account that the final plot will be different when 'wilcox' is used.

The simulated enrichment scores and the calculated one are used to find the p value.

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P value calculation depends on the parameter pval.comp.method. The default value is 'original'. In 'original' we are simply computing the proportion of anbolute simulated ES which are larger than the observed absolute ES. In 'signed' we are computing the proportion of simulated ES which are larger than the observed ES (in case of having positive enrichment score) and the proportion of simulated ES which are smaller than the observed ES (in case of having negative enrichment score).

Author(s)

Evarist Planet

References

Aravind Subramanian, (October 25, 2005) *Gene Set Enrichment Analysis*. www.pnas.org/cgi/doi/10.1073/pnas.0506580102

C.A. Tsai and J.J. Chen. *Kernel estimation for adjusted p-values in multiple testing. Computational Statistics & Data Analysis* http://econpapers.repec.org/article/eeecsdana/v_3a51_3ay_3a2007_3ai_3a8_3ap_3a3885-3897.htm

See Also

gsea.go

Examples

```
#load epheno object
data(epheno)
epheno

#we construct two signatures
sign1 <- sample(featureNames(epheno))[1:20]
sign2 <- sample(featureNames(epheno))[50:75]
mySignature <- list(sign1,sign2)
names(mySignature) <- c('My first signature','My preferred signature')

#run gsea functions
gseaData <- gsea(x=epheno,gsets=mySignature,B=100,mc.cores=1)
my.summary <- summary(gseaData)
my.summary
#plot(gseaData)</pre>
```

gsea.selGsets

Subset an object of class gseaData.

Description

gsea.selGsets to select a subgroup of gene sets from an object of class gseaData. gsea.selVars to select a subgroup of variables from an object of class gseaData.

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Usage

```
gsea.selGsets(x, selGsets)
gsea.selVars(x, selVars)
```

Arguments

x an object of class gseaData.

selGsets names of the gene sets that we want to keep.
selVars names of the variables that we want to keep.

Value

Returns an object of class gseaData.

Author(s)

Evarist Planet

gsea2html Export an object of class gseaData to an html file.

Description

Exports gseaData objects to html files with plots and links to online databases.

Usage

```
gsea2html(gseaData, epheno, variable, title = "", path, file, digits = 3, plotEs = FALSE, limit=100)
```

Arguments

gseaData an object of class gseaData.

epheno the object of class epheno that was used to create gseaData.

variable varible that we are interested in.

title title that will be shown on top of the table.

path directory where we want to store the html files.

file filename.

digits Number of decimal digits that will be shown on the table.

plotEs if this is TRUE enrichment score plots will be plotted instead of normalized

enrichment score plots.

limit maximum number of gene sets that will be exported.

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Details

This function produces a browseable version of the table that we can obtain with summary(gseaData). We will obtain one plot per NES (or ES) and we will be able to see which genes belong to each gene set and the values they have in the epheno object.

Author(s)

Evarist Planet

Examples

```
#WITH PROBESET AS IDENTIFIER
data(eset)
data(epheno)
set.seed(777)
sign1 <- sample(featureNames(eset))[1:20]</pre>
sign2 <- sample(featureNames(eset))[1:50]</pre>
mySignature <- list(sign1,sign2)</pre>
names(mySignature) <- c('My first signature', 'Another signature')</pre>
mySignature
mygsea <- gsea(x=epheno[,1],gsets=mySignature,B=100,p.adjust='BH')</pre>
summary(mygsea)
#following line has been commented to prevent the creation of files
#gsea2html(gseaData=mygsea,epheno=epheno,variable=phenoNames(epheno)[1],title='My test',path='~/Desktop',file=
#WITH ENTREZID AS IDENTIFIER
data(eset.genelevel)
eset.genelevel
set.seed(777)
sign1 <- sample(featureNames(eset.genelevel))[1:20]</pre>
sign2 <- sample(featureNames(eset.genelevel))[1:50]</pre>
mySignature.genelevel <- list(sign1,sign2)</pre>
names(mySignature.genelevel) <- c('My first signature', 'Another signature')</pre>
mySignature.genelevel
epheno.genelevel <- ExpressionPhenoTest(eset.genelevel,vars2test=list(categorical='lymph.node.status'))</pre>
\label{lem:mygsea} mygsea.genelevel <- gsea(x=epheno.genelevel,gsets=mySignature.genelevel,B=100,p.adjust='BH')
summary(mygsea.genelevel)
#following line has been commented to prevent the creation of files
#gsea2html(gseaData=mygsea.genelevel,epheno=epheno.genelevel,variable=phenoNames(epheno.genelevel),title='My to
```

gseaData-class

Class "gseaData"

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Description

This class is an ES (enrichment score) and ES.sim (simulated enrichment score) container that will be used in the GSEA (Gene Set Enrichment Analysis) process. There is one container for every gene signature.

Objects from the Class

Objects can be created by calls of the form new("gseaData", ...).

Slots

```
.Data: Object of class "list".
gseaSignaturesSign: Object of class "gseaSignaturesSign" or "gseaSignaturesVar".
gseaSignificanceSign: Object of class "gseaSignificanceSign" or "gseaSignificanceVar".
```

Extends

```
Class "list", from data part. Class "vector", by class "list", distance 2. Class "AssayData", by class "list", distance 2.
```

Methods

```
getEs signature(x = "gseaData"): Returns the enrichment scores.
```

getEsSim signature(x = "gseaData"): Returns the simulated enrichemt scores (the ones obtained after permutations).

getFcHr signature(x = "gseaData"): Returns the fold change and/or the hazard ratio that were used to compute the enrichment scores.

Author(s)

Evarist Planet

Examples

```
showClass("gseaSignaturesSign")
```

gseaSignatures

Compute ES (enrichment scores) and es.sim (simulated enrichment scores) for given phenotipic variable(s) and signature(s).

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Description

This function has been deprecated. You could better use gsea instead.

This function computes the first step in the process of obtaining a GSEA-like plot. It computes the enrichment scores and simulated enrichment scores for each variable and signature. The output will usually be used as input for the gseaSignificance function. An important parameter of the function is logScale. Its default value is TRUE which means that by default the provided scores (i.e. fold changes, hazard ratios) will be log scaled. Remember to change this parameter to FALSE if your scores are already log scaled. The getEs, getEsSim, getFc, getHr and getFcHr methods can be used to access each subobject. For more information please visit the man pages of each method.

Usage

```
gseaSignatures(x,gsets,logScale=TRUE,absVals=FALSE,averageRepeats=FALSE,
B=1000,mc.cores=1,test='perm', minGenes=10,maxGenes=500,center=FALSE)
```

Arguments

Х	${\tt ePhenoTest}, {\tt numeric}\ or\ {\tt matrix}\ object\ containing\ hazard\ ratios\ or\ fold\ changes.$
gsets	character or list object containing the names of the genes that belong to each signature.
logScale	if values should be log scaled.
absVals	if TRUE fold changes and hazard ratios that are negative will be turned into positive before starting the process. This is useful when genes can go in both directions.
averageRepeats	if x is of class numeric and has repeated names (several measures for some indivdual names) we can average the measures of the same names.
В	number of simulations to perform.
mc.cores	number of processors to use.
test	the test that will be used. 'perm' stands for the permutation based method, 'wilcox' stands for the wilcoxon test (this is the fastest one) and 'ttperm' stands for permutation t test.
minGenes	gene sets with less than minGenes genes will be removed from the analysis.

Details

maxGenes

center

The following preprocessing was done on the provided scores (i.e. fold changes, hazard ratios) to avoid errors during the enrichment score computation: -When having two scores with the same name its average was used. -Zeros were removed. -Scores without names (which can not be in any signature) removed. -Non complete cases (i.e. NAs, NaNs) were removed. ES score was calculated for each signature and variable (see references). If parameter test is 'perm' the signature was permutted and the ES score was recalculated (this happened B times for each variable, 1000 by

be done: x = x-mean(x).

gene sets with more than maxGenes genes will be removed from the analysis.

if we want to center scores (fold changes or hazard ratios). The following is will

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default). If test is 'wilcox' a wilcoxon test in which we test the fact that the average value of the genes that do belong to our signature is different from the average value of the genes that do not belong to our signature will be performed. If test is 'ttperm' a permutation t-test will be used. Take into account that the final plot will be different when 'wilcox' is used.

Author(s)

Evarist Planet

References

Aravind Subramanian, (October 25, 2005) *Gene Set Enrichment Analysis*. www.pnas.org/cgi/doi/10.1073/pnas.0506580102

Examples

```
#load epheno object
data(epheno)
epheno

#we construct two signatures
sign1 <- sample(featureNames(epheno))[1:20]
sign2 <- sample(featureNames(epheno))[50:75]
mySignature <- list(sign1,sign2)
names(mySignature) <- c('My first signature','My preferred signature')

#run gsea functions
#my.gseaSignatures <- gseaSignatures(x=epheno,signatures=mySignature,B=100,mc.cores=1)
#my.gseaSignificance <- gseaSignificance(my.gseaSignatures)
#my.summary <- summary(my.gseaSignificance)
#my.summary
#plot(my.gseaSignatures,my.gseaSignificance)</pre>
```

gseaSignatures-class Class "gseaSignatures" ES and EsSim container.

Description

This object contains de ES (enrichment scores) and simulated ES that will be used in the GSEA (Gene Set Enrichment Analysis) process.

Objects from the Class

Objects can be created by calls of the form new("gseaSignatures", ...).

Slots

.Data: Object of class "list".

- es: Object of class "numeric" Contains the observed enrichment scores. The ones that were compted from the data without permuting anything.
- es.sim: Object of class "numeric" Contains the enrichment score that were obtained after permutations.
- signature: Object of class "numeric" The subset of genes we are interested in.

Extends

Class "list", from data part. Class "vector", by class "list", distance 2. Class "AssayData", by class "list", distance 2.

Methods

No methods defined with class "gseaSignatures" in the signature.

Author(s)

Evarist Planet

Examples

```
showClass("gseaSignatures")
```

gseaSignatures-methods

Methods for Function gseaSignatures in Package 'phenoTest'

Description

Methods for function gseaSignatures in Package 'phenoTest'. For more information read the function's manual.

Methods

```
signature(x = "ANY", signatures = "character") Method for signature of class character.
```

signature(x = "ANY", signatures = "GeneSet") Method for signature of class character.

signature(x = "epheno", signatures = "list") Method for an epheno object and several signatures stored in an object of class list.

signature(x = "matrix", signatures = "GeneSetCollection") Method for an matrix object
and several signatures stored in an object of class GeneSetCollection.

signature(x = "epheno", signatures = "GeneSetCollection") Method for an epheno object
and several signatures stored in an object of class GeneSetCollection.

- signature(x = "numeric", signatures = "GeneSetCollection") Method for an numeric object and several signatures stored in an object of class GeneSetCollection.
- signature(x = "matrix", signatures = "list") Method for an matrix object and several signatures stored in an object of class list.
- signature(x = "numeric", signatures = "list") Method for an numeric object and several
 signatures stored in an object of class list.

gseaSignaturesSign-class

Class "gseaSignaturesSign"

Description

This class is an ES (enrichment score) and ES.sim (simulated enrichment score) container that will be used in the GSEA (Gene Set Enrichment Analysis) process. There is one container for every gene signature.

Objects from the Class

Objects can be created by calls of the form new("gseaSignaturesSign", ...).

Slots

.Data: Object of class "list".

gseaSignatures: Object of class "gseaSignatures" This is the object that will contain the ES and ES.sim.

es.sim.gam: Object of class "matrix" enrichment scores computed with the gam method.

fc.hr: Object of class "character" fold change or hazard ratio used to compute the enrichment scores.

s: Object of class "logical" The subset of genes we are interested in.

test: Object of class "character" The statistical test that will be used.

Extends

Class "list", from data part. Class "vector", by class "list", distance 2. Class "AssayData", by class "list", distance 2.

Methods

getEs signature(x = "gseaSignaturesSign"): Returns the enrichment scores.

getEsSim signature(x = "gseaSignaturesSign"): Returns the simulated enrichemt scores (the ones obtained after permutations).

getFcHr signature(x = "gseaSignaturesSign"): Returns the fold change and/or the hazard ratio that were used to compute the enrichment scores.

gseaSignificance signature(x = "gseaSignaturesSign"): This is the next step in the process of performing GSEA. This funtion will test if the gene sets are enriched.

Author(s)

Evarist Planet

Examples

```
showClass("gseaSignaturesSign")
```

```
gseaSignaturesVar-class
```

Class "gseaSignaturesVar"

Description

This class is an ES (enrichment score) and ES.sim (simulated enrichment score) container that will be used in the GSEA (Gene Set Enrichment Analysis) process. There is one container for every phenotype. Every one of this containers (of class gseaSignaturesSign) is a container itsef and has the enrichment scores of all signatures. GseaSignaturesVar contains one element per phenotype (phenotypic variable). Every one of this elements is of class gseaSignaturesSign and contains one element per signature.

Objects from the Class

Objects can be created by calls of the form new("gseaSignaturesVar", ...).

Slots

```
.Data: Object of class "list".
```

gseaSignatures: Object of class "gseaSignaturesSign" This object contains the enrichment scores and other elements that will be used in the GSEA process.

Extends

```
Class "list", from data part. Class "vector", by class "list", distance 2. Class "AssayData", by class "list", distance 2.
```

Methods

```
getEs signature(x = "gseaSignaturesVar"): Returns the enrichment scores.
```

getEsSim signature(x = "gseaSignaturesVar"): Returns the simulated enrichemt scores (the ones obtained after permutations).

getFcHr signature(x = "gseaSignaturesVar"): Returns the fold change and/or the hazard ratio that were used to compute the enrichment scores.

gseaSignificance signature(x = "gseaSignaturesVar"): This is the next step in the process of performing GSEA. This funtion will test if the gene sets are enriched.

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Author(s)

Evarist Planet

Examples

showClass("gseaSignaturesVar")

gseaSignificance

ES' (enrichment scores) sifgnificance.

Description

This function has been deprecated. You could better use gsea instead.

This function performs the second step in the process of obtaining a GSEA-like plot. It computes the NES (normalized enrichment score), p values and fdr (false discovery rate) for all variables and signatures. A gseaSignaturesSign or gseaSignaturesVar object will be needed as input (these objects can be obtained with the gseaSignatures function). For an overview of the output use the summary method. The next step after using the gseaSignificance function would be using the plot method.

Usage

gseaSignificance(x,p.adjust.method='none',pval.comp.method='original',pval.smooth.tail=TRUE)

Arguments

X

gseaSignaturesSign or gseaSignaturesVar object obtained with the gseaSignatures method. This object contains the enrichment scores ,the simulated enrichment scores and the fold changes or hazard ratios.

p.adjust.method

p adjustment method to be used. Common options are 'BH', 'BY', 'bonferroni' or 'none'. All available options and their explanations can be found on the p.adjust function manual.

pval.comp.method

the p value computation method. Has to be one of 'signed' or 'original'. The default one is 'original'. See details for more information.

pval.smooth.tail

if we want to estimate the tail of the ditribution where the pvalues will be generated.

Details

The simulated enrichment scores and the calculated one are used to find the p value.

P value calculation depends on the parameter pval.comp.method. The default value is 'original'. In 'original' we are simply computing the proportion of anbolute simulated ES which are larger than the observed absolute ES. In 'signed' we are computing the proportion of simulated ES which are larger than the observed ES (in case of having positive enrichment score) and the proportion of simulated ES which are smaller than the observed ES (in case of having negative enrichment score).

Author(s)

Evarist Planet

References

Aravind Subramanian, (October 25, 2005) *Gene Set Enrichment Analysis*. www.pnas.org/cgi/doi/10.1073/pnas.0506580102

C.A. Tsai and J.J. Chen. *Kernel estimation for adjusted p-values in multiple testing. Computational Statistics & Data Analysis* http://econpapers.repec.org/article/eeecsdana/v_3a51_3ay_3a2007_3ai_3a8_3ap_3a3885-3897.htm

Examples

```
#for examples see the help file of gseaSigntaures: ?gseaSignatures
```

```
gseaSignificanceSign-class
```

Class "gseaSignificanceSign"

Description

This object contains the results of the test of enrichment that was performed on each gene set. There is one container for every gene signature.

Objects from the Class

Objects can be created by calls of the form new("gseaSignificanceSign", ...).

Slots

```
.Data: Object of class "list".
```

gseaSignificance: Object of class "matrix" Contains the statistics. Use the summary method to access this information.

 $\verb"p.adjust.method: Object of class" character" The p-value adjustment method that was used.$

Extends

```
Class "list", from data part. Class "vector", by class "list", distance 2. Class "AssayData", by class "list", distance 2.
```

Methods

No methods defined with class "gseaSignificanceSign" in the signature.

Author(s)

Evarist Planet

Examples

```
showClass("gseaSignificanceSign")
```

```
gseaSignificanceVar-class
```

Class "gseaSignificanceVar"

Description

This object contains the results of the test of enrichment that was performed on each gene set and phenotype. There is one container for every phenotype. Every one of this containers (of class gseaSignificanceSign) is a container itsef and has the results of the tests for all signatures. GseaSignificanceVar contains one element per phenotype (phenotypic variable). Every one of this elements is of class gseaSignificanceSign and contains one element per signature.

Objects from the Class

Objects can be created by calls of the form new("gseaSignificanceVar", ...).

Slots

```
.Data: Object of class "list" .
gseaSignificance: Object of class "gseaSignificanceSign" This object contains the results of the tests.
```

Extends

```
Class "list", from data part. Class "vector", by class "list", distance 2. Class "AssayData", by class "list", distance 2.
```

Methods

No methods defined with class "gseaSignificanceVar" in the signature.

Author(s)

Evarist Planet

```
showClass("gseaSignificanceVar")
```

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heatmapPhenoTest Produce heatmap from phenotype data.

Description

Show the associations between clusters that each sample belongs to and each phenotype in a heatmap and/or a Kaplan-Meier plot.

Usage

```
heatmapPhenoTest(x, signatures, vars2test, probes2genes = FALSE,
filterVar, filteralpha = 0.05, distCol = "pearson", nClust = 2, distRow
= "cor", p.adjust.method = "none", simulate.p.value = FALSE, B = 10^5,
linkage = "average", equalize = FALSE, center = TRUE, col, survCol,
heat.kaplan="both", ...)
```

Arguments

X	ExpressionSet with phenotype information stored in pData(x).	
signatures	Either character vector or list of character vectors with gene sets to be used to draw heatmaps (gene names should match those in featureNames(x)). A separate heatmap will be produced for each element in the list.	
vars2test	list with components 'continuous', 'categorical', 'ordinal' and 'survival' indicating which phenotype variables should be tested. 'continuous', 'categorical' and 'ordinal' must be character vectors, 'survival' a matrix with columns named 'time' and 'event'. The names must match names in names(pData(x)).	
probes2genes	If set to TRUE a single probe is selected for each gene. nsFilter is used to select the probe with highest inter-quartile range.	
filterVar	If specified, only genes with significant differences in the variable filterVar will be displayed in the heatmap. Note that this option will not affec the sample clustering, as this is obtained using both significant and non-significant genes.	
filteralpha	Significance level for the filtering based on filterVar.	
distCol	Distance metric used to cluster columns (e.g. patients/samples). Can take any value accepted by dist. Pearson and Spearman correlations are also allowed. Write 'spearman' or 'pearson' to use them.	
nClust	Number of desired clusters.	
distRow	Distance metric used to cluster rows (e.g. genes). Can take any value accepted by distancematrix.	
p.adjust.method		
	Method for P-value adjustment, passed on to p.adjust.	
simulate.p.value		
	If set to FALSE the chi-square test p-values are computed using asymptotics,	

otherwise a simulation is used (see chisq.test for details).

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В	An integer specifying the number of replicates used in the chi-square Monte Carlo test (passed on to chisq.test).
linkage	Linkage used for clustering. Must be either 'complete', 'average' or 'minimum'.
equalize	Should color codes be equalized between genes, i.e. all genes present the same range of colors. Passed on to heatmap_plus.
center	centering is done by subtracting the column means (omitting NAs).
col	Color scheme to be used for heatmap. Defaults to a green/red scheme designed to look nice for microarray data.
survCol	Colors for the Kaplan-Meier survival curves.
heat.kaplan	can be "heat" if we want to plot a heatmap, "kaplan" if we want to plot a kaplanmeier or "both" if we want both of them.
	Other arguments for the survival plot, e.g. lty etc.

Details

Makes two clusters of samples based on the expression levels of the genes from the given signature and plots a heatmap and/or a Kaplan-Meier showing the association between belonging to one cluster or the other and each phenotype.

For variables in vars2test\\$continuous and vars2test\\$ordinal a Kruskal-Wallis Rank Sum test is used; for vars2test\\$categorical a chi-square test (with exact p-value if simulate.p.value is set to TRUE); for var2test\\$survival a Cox proportional hazards likelihood-ratio test.

Author(s)

David Rossell

```
#load data
data(eset)
eset

#construct vars2test
survival <- matrix(c("Relapse","Months2Relapse"),ncol=2,byrow=TRUE)
colnames(survival) <- c('event','time')
vars2test <- list(survival=survival)
vars2test

#construct a signature
sign <- sample(featureNames(eset))[1:20]

#make plot
heatmapPhenoTest(eset,sign,vars2test=vars2test,heat.kaplan='heat')
heatmapPhenoTest(eset,sign,vars2test=vars2test,heat.kaplan='kaplan')</pre>
```

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heatmapPhenoTest-methods

Methods for Function heatmapPhenoTest in Package 'phenoTest'

Description

Methods for function heatmapPhenoTest in Package 'phenoTest'. For more information read the function's manual.

Methods

signature(x = "ExpressionSet", signatures = "character") Method for an ExpressionSet
 object and one signature stored in an object of class character.

signature(x = "ExpressionSet", signatures = "list") Method for an ExpressionSet object and several signatures stored in an object of class list.

signature(x = "ExpressionSet", signatures = "missing") Method for an ExpressionSet object and no signatures.

signature(x = "ExpressionSet", signatures = "GeneSet") Method for an ExpressionSet object and one signature stored in an object of class GeneSet.

signature(x = "ExpressionSet", signatures = "GeneSetCollection") Method for an ExpressionSet
 object and several signatures stored in an object of class GeneSetCollection.

pAdjust

Adjust p values of an epheno object.

Description

Adjusts the p values of an epheno object. The p. adjust function will be used. For more information read the p. adjust function's help.

Usage

```
pAdjust(x, method = "BH")
```

Arguments

x an epheno object.

method the correction method that will be used. Se the p.adjust help for more info

about the methods.

Author(s)

Evarist Planet

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Examples

```
#load epheno object
data(epheno)
epheno

#Adjust pvalue
p.adjust.method(epheno)
epheno <- pAdjust(epheno,method='BH')
p.adjust.method(epheno)</pre>
```

pAdjust-methods

Methods for Function pAdjust in Package 'phenoTest'

Description

Methods for function pAdjust in Package 'phenoTest'. This function adjusts the p-values of an epheno object. For more informe read the function's manual.

Methods

```
signature(x = "epheno") Adjusts the pvalues of an epheno object.
```

Author(s)

Evarist Planet

рса

Principal components plot.

Description

Creates a Principal Components plot where we can show paired samples, and confidence intervals for the mean of every group of interest. We can also choose the component or components we want to plot.

Usage

```
pca(x, group, group2, pair, names, ellipse = FALSE, main = "", components = c(1, 2))
```

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Arguments

X	An object of class ExpressionSet.
group	Variable in $pData(x)$ that contains the groups of interest. Samples of the same group will be plotted with the same color.
group2	Variable in pData(x) that contains secondary groups of interest. Sample of the same secondary group of interest will be plotted with the same symbol.
pair	Variable in $pData(x)$ that contains the information about the pairs of data. Those pairs will be joined by a line.
names	Variable in $pData(x)$ that contains the information about the names of the samples.
ellipse	If we want to plot ellipses with the 95 percent confidence intervals for every group.
main	A title for the plot.

components Which components we want to plot. By default the first principal component

will be plotted on the x axis and the second principal component will be plotted on the y axis. More than two components may be specified. If so multiple plots

will be produced.

Author(s)

Evarist Planet

See Also

prcomp.

Examples

```
data(eset)
pca(x=eset, group='Relapse', names='GEOaccession')
#pca(x=eset, group='Relapse', names='GEOaccession', components=1:3)
```

plot.gseaData

GSEA-like Plot.

Description

Builds a GSEA plot using a gseaData object. gseaData object can be obtained with the gsea function.

Usage

```
plot.gseaData(x,selGsets,selVars,...)
```

plot.gseaSignatures 43

Arguments

x this has to be of class gseaData
 selGsets object of class character containing the names of the gene sets that we want to plot.
 selVars object of class character containing the names of the variables that we want to plot.

... Arguments to be passed to plot.

Author(s)

Evarist Planet

References

Aravind Subramanian, (October 25, 2005) Gene Set Enrichment Analysis. www.pnas.org/cgi/doi/10.1073/pnas.0506580102

Examples

```
#for examples see the help file of gseaSigntaures: ?gseaSignatures
```

plot.gseaSignatures GSEA-like Plot.

Description

Builds a GSEA plot using a gseaSignature object (one of gseaSignaturesSign or gseaSignaturesVar obtained with the gseaSignatures function) and a gseaSignificance object (one of gseaSignificanceSign or gseaSignificanceVar obtained with the gseaSignificance function).

Usage

```
plot.gsea Signatures Sign(x,gsea Significance,es.ylim,nes.ylim,es.nes="both",\dots)
```

Arguments

x object of class gseaSignaturesSign or gseaSignaturesVar.

gseaSignificance

object of class gseaSignificanceSign or gseaSignificanceVar.

es.ylim ylim values for the ES plot. nes.ylim ylim values for the NES plot.

es.nes can be "es" if we want to plot enrichment score, "nes" if we want to plot nor-

malised enrichment scores or "both"if we want to plot them both.

... Arguments to be passed to plot.

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Author(s)

Evarist Planet

References

Aravind Subramanian, (October 25, 2005) *Gene Set Enrichment Analysis*. www.pnas.org/cgi/doi/10.1073/pnas.0506580102

See Also

plot.gseaSignaturesSign, plot.gseaSignaturesVar

Examples

```
#for examples see the help file of gseaSigntaures: ?gseaSignatures
```

show-methods

Methods for Function show in Package 'methods'.

Description

Methods for function show in Package 'methods'.

Methods

```
signature(object = "AnnotatedDataFrame") Will show an object of class AnnotatedDataFrame.
signature(object = "ANY") Will show an object of class ANY.
signature(object = "classRepresentation") Will show an object of class classRepresentation.
signature(object = "container") Will show an object of class container.
signature(object = "epheno") Will show an object of class epheno.
signature(object = "eSet") Will show an object of class eSet.
signature(object = "genericFunction") Will show an object of class genericFunction.
signature(object = "gseaSignaturesSign") Will show an object of class gseaSignaturesSign.
signature(object = "gseaSignaturesVar") Will show an object of class gseaSignaturesVar.
signature(object = "gseaSignificanceSign") Will show an object of class gseaSignificanceSign.
signature(object = "gseaSignificanceVar") Will show an object of class gseaSignificanceVar.
signature(object = "LargeDataObject") Will show an object of class LargeDataObject.
signature(object = "MethodDefinition") Will show an object of class MethodDefinition.
signature(object = "MethodSelectionReport") Will show an object of class MethodSelectionReport.
signature(object = "MethodWithNext") Will show an object of class MethodWithNext.
signature(object = "MIAME") Will show an object of class MIAME.
signature(object = "namedList") Will show an object of class namedList.
```

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```
signature(object = "ObjectsWithPackage") Will show an object of class ObjectsWithPackage.
signature(object = "oldClass") Will show an object of class oldClass.
signature(object = "ScalarCharacter") Will show an object of class ScalarCharacter.
signature(object = "ScalarObject") Will show an object of class ScalarObject.
signature(object = "signature") Will show an object of class signature.
signature(object = "TestResults") Will show an object of class TestResults.
signature(object = "traceable") Will show an object of class traceable.
signature(object = "Versioned") Will show an object of class Versioned.
signature(object = "Versions") Will show an object of class Versions.
signature(object = "VersionsNull") Will show an object of class VersionsNull.
```

smoothCoxph

Plots the Cox proportional hazard smoothed by gene expression level.

Description

Builds a plot showing how hazard behaves over different levels of expression of a given gene. Confidence intervals are also provided.

Usage

```
smoothCoxph(time, event, x, xlim, ylim, xlab, ylab, logrisk=TRUE, ...)
```

Arguments

time	variable where time to survival is stored.
event	variable where survival event is stored.
x	numeric containing the expression levels of a given gene.
xlim	xlim for the plot.
ylim	ylim for the plot.
xlab	xlab for the plot.
ylab	ylab for the plot.
logrisk	logrisk if we want to compute risk or logrisk estimates. By default this is TRUE, which has a better behaviour under small sample sizes.
	other arguments that will be passed to plot.

Author(s)

David Rossell.

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Examples

```
#load eset
data(eset)

#make plot
smoothCoxph(pData(eset)$Months2Relapse,pData(eset)$Relapse,exprs(eset)[25,])
```

summary.gseaData

Obtain a data.frame with the pvalues and fdr for all signatures and variables of a gseaData object.

Description

Builds a data.frame object that can easily be written to a csv file containing the ES, NES, pval.ES, pval.NES and FDR.

Usage

```
summary.gseaData(object,...)
```

Arguments

object of class gseaData,

... Arguments to be passed to summary.

Author(s)

Evarist Planet

References

Aravind Subramanian, (October 25, 2005) *Gene Set Enrichment Analysis*. www.pnas.org/cgi/doi/10.1073/pnas.0506580102

See Also

summary.gseaSignificanceSign, summary.gseaSignificanceVar

```
#for examples see the help file of gseaSigntaures: ?gsea
```

summary.gseaSignificance

Obtain a data.frame with the pvalues and fdr for all signatures and variables of a gseaSignificanceSign or gseaSignificanceVar object.

Description

Builds a data.frame object that can easily be written to a csv file containing the ES, NES, pval.ES, pval.NES and FDR.

Usage

```
summary.gseaSignificanceSign(object,...)
```

Arguments

object of class gseaSignificanceSign or gseaSignificanceVar.

... Arguments to be passed to summary.

Author(s)

Evarist Planet

References

Aravind Subramanian, (October 25, 2005) *Gene Set Enrichment Analysis*. www.pnas.org/cgi/doi/10.1073/pnas.0506580102

Examples

```
#for examples see the help file of gseaSigntaures: ?gseaSignatures
```

write.html

Write a data.frame to an html file.

Description

Creates an html file with links and plots from a table.

Usage

```
write.html(x, links, tiny.pic, tiny.pic.size = 100, title = "", file, digits = 3)
```

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Arguments

x Object of class data. frame.

links Object of class list with one item per column in x. If we want the ith column

of x to have links to a site or local file we will have to write those links into the

ith element of links.

tiny.pic Object of class list with one item per column in x. If we want the ith column

of x to show plots instead of text we will have to write the path to the plots into

the ith element of links.

tiny.pic.size size of the pictures if any.

title Title that will be shown on top of the html file.

file path and name of the file that will be created.

digits number of digits that will be shown in numeric columns of x.

Author(s)

Evarist Planet

See Also

write.csv, write.table, htmlpage

```
##
##Code has been commented to avoid the creation of files
##
#(x <- data.frame(gene.symbol=c('AARS','ABCF1','ABLIM1'),value=c(2.054,30.024,5.0221),plot=rep('Open',3)))
#tiny.pic <- links <- vector('list',length=ncol(x))
#links[[1]] <- paste('http://www.genecards.org/index.php?path=/Search/keyword/',x[,1])
#for (i in 1:nrow(x)) {
# png(paste('~/Desktop/',x[i,1],'.png',sep=''))
# plot(1:3,log(1:3))
# dev.off()
#}
#tiny.pic[[3]] <- links[[3]] <- paste(x[,1],'.png',sep='')
#write.html(x,links=links,tiny.pic=tiny.pic,file='~/Desktop/x.html',title='My html test')</pre>
```

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