

Package ‘roastgsa’

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Type Package

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Title Rotation based gene set analysis

BugReports <https://github.com/adricaba/roastgsa/issues>

Description This package implements a variety of functions useful for gene set analysis using rotations to approximate the null distribution. It contributes with the implementation of seven test statistic scores that can be used with different goals and interpretations. Several functions are available to complement the statistical results with graphical representations.

Encoding UTF-8

VignetteBuilder knitr

biocViews Microarray, Preprocessing, Normalization, GeneExpression, Survival, Transcription, Sequencing, Transcriptomics, Bayesian, Clustering, Regression, RNASeq, MicroRNAArray, mRNAMicroarray, FunctionalGenomics, SystemsBiology, ImmunoOncology, DifferentialExpression, GeneSetEnrichment, BatchEffect, MultipleComparison, QualityControl, TimeCourse, Metabolomics, Proteomics, Epigenetics, Cheminformatics, ExonArray, OneChannel, TwoChannel, ProprietaryPlatforms, CellBiology, BiomedicalInformatics, AlternativeSplicing, DifferentialSplicing, DataImport, Pathways

Depends R (>= 4.3.0)

Imports parallel, grDevices, graphics, utils, stats, methods, grid, RColorBrewer, gplots, ggplot2, limma, Biobase

Suggests BiocStyle, knitr, rmarkdown, GSEABenchmarkR, EnrichmentBrowser, preprocessCore, DESeq2

License GPL-3

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| | |
|-----------|------------------------------------|
| dragtable | <i>dragtable for html writings</i> |
|-----------|------------------------------------|

Description

from dragtable v1.0 of Dan Vanderkam.

Usage

dragtable

Format

character vector

Value

Character vector with dragtable

Source

<http://danvk.org/dragtable/>

References

kryogenix.org/code/browser/sorttable

`expr.tcga`*Tumor Bladder TCGA data*

Description

Counts matrix of RNA-seq study with 19 tumor Bladder Urothelial Carcinoma samples and 19 adjacent healthy tissues

Usage`expr.tcga`**Format**

matrix

Value

Matrix with expression matrix

Source<https://bioconductor.org/packages/release/bioc/html/GSEABenchmarkR.html>**References**

Geistlinger L, Csaba G, Santarelli M, Ramos M, Schiffer L, Law C, Turaga N, Davis S, Carey V, Morgan M, Zimmer R, Waldron L (2020). Toward a gold standard for benchmarking gene set enrichment analysis. *Briefings in Bioinformatics*. doi:10.1093/bib/bbz158.

`fd.tcga`*Tumor Bladder TCGA data*

Description

Gene information of RNA-seq study with 19 tumor Bladder Urothelial Carcinoma samples and 19 adjacent healthy tissues

Usage`fd.tcga`**Format**

DFrame

Value

Data frame with gene symbols

Source

<https://bioconductor.org/packages/release/bioc/html/GSEABenchmarkR.html>

References

Geistlinger L, Csaba G, Santarelli M, Ramos M, Schiffer L, Law C, Turaga N, Davis S, Carey V, Morgan M, Zimmer R, Waldron L (2020). Toward a gold standard for benchmarking gene set enrichment analysis. *Briefings in Bioinformatics*. doi:10.1093/bib/bbz158.

| | |
|--------------|---|
| hallmarks.hs | <i>Hallmarks homo sapiens gene symbol</i> |
|--------------|---|

Description

Hallmark geneset collection from msigdb

Usage

hallmarks.hs

Format

character list

Value

List with hallmark genes

Source

<https://www.gsea-msigdb.org/gsea/downloads.jsp>

References

Liberzon, A. et al.: The Molecular Signatures Database Hallmark Gene Set Collection. *Cell Systems* 1(6), 417-425 (2015). doi:10.1016/j.cels.2015.12.004

| | |
|----------------|------------------------------------|
| heatmaprgsa_hm | <i>Heatmap of roastgsa results</i> |
|----------------|------------------------------------|

Description

Heatmap showing sample variation for either genes (in a particular gene set) or summarized gene signatures.

Usage

```
heatmaprgsa_hm(obj, y, intvar, adj.var = NULL, whplot = 1, toplot = TRUE,
  pathwaylevel = FALSE, mycol = c("black", "orange", "green", "white"),
  sample2zero = FALSE, rgsa.like=FALSE, psel = NULL,
  dendrogram = "n", col= bluered(100), trace='none',
  notecol='black', notecex=1, keysize=.9,
  cexCol=1.5, Rowv = NULL, Colv = FALSE, las =2, fdrkey = FALSE,
  quantile.sat = 0.95, order1= NULL, order2 = NULL, sizex =8, sizey =5, ...)
```

Arguments

| | |
|--------------|--|
| obj | an object of class 'roastgsa' |
| y | data used for roastgsa |
| intvar | name of variable of interest in obj\$formula. If missing, last term of obj\$formula is used |
| adj.var | name of covariates in obj\$design to adjust using a linear model in the heatmap representation. If NULL no prior adjustment applies |
| whplot | selected pathway. If integer vector, the pathways are selected in the same order as the table in obj\$res |
| toplot | whether to plot the heatmap or just return the adjusted expression matrix |
| pathwaylevel | If TRUE, the heatmap shows the variation at the pathway level. Otherwise, the heatmap shows the variation of all genes in the selected pathways. |
| mycol | color for heatmap columns defining the groups of the variable of interest |
| sample2zero | Only applicable for obj\$statistic = "maxmean". If TRUE, expression of genes, whose moderated-t sign is contrary to the roastgsa score, is set to zero for all samples (as part of the maxmean strategy) |
| rgsa.like | apply roastgsa transformations of data (restandardization and set.statistic operations) samplewise (see details below). |
| psel | character vector with probesets (one per gene) to be used for roastgsa statistic in a microarray experiment |
| dendrogram | heatmap.2 parameter. Character string indicating whether to draw 'none', 'row', 'column' or 'both' dendrograms. Defaults to 'n' |
| col | heatmap.2 parameter. Colors used for the image |
| trace | heatmap.2 parameter. Character string indicating whether a solid "trace" line should be drawn across 'row's or down 'column's, 'both' or 'none'. The distance of the line from the center of each color-cell is proportional to the size of the measurement. |
| notecol | heatmap.2 parameter. Color of note |
| notecex | heatmap.2 parameter. Size of note |
| keysize | heatmap.2 parameter. Numeric value indicating the size of the key |
| cexCol | heatmap.2 parameter. Cex.axis in for the column axis labeling |
| Rowv | heatmap.2 parameter. Determines if and how the row dendrogram should be reordered |
| Colv | heatmap.2 parameter. Determines if and how the col dendrogram should be reordered |
| las | orientation of x axis |

| | |
|--------------|---|
| fdrkey | if TRUE, the BH adjusted p-value for every pathway tested is printed in the plot. Only considered when pathwaylevel = TRUE |
| quantile.sat | numeric between 0.5 and 1 used to saturate high values at such specified quantile (used to avoid extreme values in the visualization) |
| order1 | genes order. If NULL its ordered based on the moderated-t statistics |
| order2 | samples order. If NULL its ordered using the information of the variable of interest. |
| sizeX | size of x axis |
| sizeY | size of y axis |
| ... | Arguments passed to or from other methods to the low level. |

Details

This heatmap considers $n + 1$ columns (n being the sample size). The first column represents the moderated-t statistic (or a restandarization of the same in case of competitive testing). The other columns confine the expression data scaled by the standard error of the estimated coefficient in the model and centered (if `rgsa.like = TRUE`). In such case, the cross product of all data columns and the design matrix equals the first column of the heatmap, and the average of the first column of the heatmap equals the observed roastgsa test statistic (at least when the `set.statistic` used is either `mean` or `maxmean`).

Value

a data.frame object with source data for heatmap representation

Author(s)

Adria Caballe Mestres

References

[1] A comparison of rotation-based scores for gene set analysis Adria Caballe Mestres, Antonio Berenguer Llergo, Camille Stephan-Otto Attolini bioRxiv 2021.03.23.436604;

See Also

[roastgsa](#) and [plotStats](#) and [plotGSEA](#)

Examples

```

y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)

roastgsa1 <- roastgsa(y, covar, form = form, self.contained = TRUE,
  set.statistic = "maxmean", index = index, nrot = 200,
  mcores = 1, execution.info = FALSE)

```

```

heatmaprgsa_hm(roastgsa1, y, intvar = "voi", whplot = 1, topplot = TRUE,
  pathwaylevel = FALSE, mycol = c("black","orange","green","white"),
  sample2zero = FALSE)

heatmaprgsa_hm(roastgsa1, y, intvar = "voi", whplot = 1:10, topplot = TRUE,
  pathwaylevel = TRUE, mycol = c("black","orange","green","white"),
  sample2zero = FALSE)

```

htmlrgsa

roastgsa results in html form

Description

Writing html document with roastgsa output

Usage

```

htmlrgsa(obj, htmlpath = "", htmlname = "file.html", plotpath = "",
  plotstats = TRUE, plotgsea = TRUE, indheatmap = TRUE, ploteffsize = TRUE,
  links_plots = list(stats= NULL, gsea = NULL, heatmap = NULL, effsize
  = NULL), y, whplots = NULL, geneDEhtmlfiles = NULL, tit = "",
  margins = c(5,16), sizesHeatmap = c(1200, 800), typeheatmap =
  c("heatmap.2","ggplot2"), intvar, adj.var = NULL, mycol, varrot,
  psel = NULL, sorttable, dragtable, ...)

```

Arguments

| | |
|-----------------|---|
| obj | an object of class 'roastgsa' |
| htmlpath | path for html file to be placed |
| htmlname | name of html file |
| plotpath | added path from argument htmlpath where plots should be saved |
| plotstats | plots using plotStats are created |
| plotgsea | plots using plotGSEA are created |
| indheatmap | plots using heatmaprgsa_hm at the gene level are created |
| ploteffsize | plots using ploteffsignaturesize are created |
| links_plots | list with 4 elements (stats, gsea, heatmap and effsize) specifying the path of all plots (paths set from htmlpath) in case these were already created. If NULL, links are obtained from plotpath if any of plotstats, plotGSEA, indheatmap or ploteffsize is TRUE |
| y | data used for roastgsa |
| whplots | selected pathways. If integer vector, the pathways are selected in the same order as the table in obj\$res. If null all tested pathways are selected |
| geneDEhtmlfiles | vector with links to html-tables showing the differential expression results for the subsets of genes determined by whplots |
| tit | title of the html file |

| | |
|--------------|---|
| margins | margins for the heatmap plots |
| sizesHeatmap | vector with two elements providing png sizes (width, height) |
| typeheatmap | either ggplot2 type or heatmap.2 type |
| intvar | for heatmaprgsa_hm . Name of variable of interest in obj\$formula. If missing, last term of obj\$formula is used |
| adj.var | for heatmaprgsa_hm . Name of covariates in obj\$design to adjust using a linear model in the heatmap representation. If NULL no prior adjustment applies |
| mycol | color for heatmap columns defining the groups of the variable of interest |
| varrot | an object of class 'varrotrand' (see varrotrand) with estimated rotation score variances for randomly selected genesets of several sizes. Cannot be missing if <code>ploteffsize = TRUE</code> |
| pset | character vector with probesets (one per gene) to be used for roastgsa statistic in a microarray experiment |
| sorttable | internal data loaded with roasgsa package. Permits sorting columns in html tables. |
| dragtable | internal data loaded with roasgsa package. Permits dragging elements in html tables. |
| ... | Arguments passed to or from other methods to the low level. |

Details

This function permits to explore a html-table with the statistical results and graphical representation of the top gene sets obtained from an object of class `roastgsa`.

By default four plots are considered for each gene set of interest: [plotStats](#), [plotGSEA](#), [heatmaprgsa_hm](#) and [ploteffsignaturesize](#). The first three can be computed from the 'roastgsa' object, whereas for [ploteffsignaturesize](#), an object of class 'varrotrand' (see [varrotrand](#)) with the estimated rotation score variances for randomly selected gene sets of several sizes has to be defined at first.

Value

It saves an html table with the main results of the roastgsa hypothesis testing.

Author(s)

Adria Caballe Mestres

References

[1] A comparison of rotation-based scores for gene set analysis Adria Caballe Mestres, Antonio Berenguer Llergo, Camille Stephan-Otto Attolini *bioRxiv* 2021.03.23.436604;

See Also

[roastgsa](#)

Examples

```
data(sorttable)
data(dragtable)

y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)

roastgsa1 <- roastgsa(y, covar, form = form, self.contained = TRUE,
  set.statistic = "maxmean", index = index, nrot = 200,
  mcores = 1, execution.info = FALSE)

htmlrgsa(roastgsa1, htmlpath = "", htmlname = "test.html", plotpath = "plots/",
  plotstats = FALSE, plotgsea = FALSE, indheatmap = FALSE,
  ploteffsize = FALSE, links_plots = list(stats= NULL, gsea = NULL,
  heatmap = NULL, effsize = NULL), y = y, sorttable = sorttable,
  dragtable = dragtable)
```

kegg.hs

KEGG genesets homo sapiens entrez

Description

KEGG genesets obtained with limma function `getGeneKEGGLinks`

Usage

```
kegg.hs
```

Format

character list

Value

List with KEGG genes

Source

<https://www.kegg.jp/kegg/rest/keggapi.html>

References

Kanehisa, M. and Goto, S.; KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* 28, 27-30 (2000).

pd.tcga

Tumor Bladder TCGA data

Description

Sample information of RNA-seq study with 19 tumor Bladder Urothelial Carcinoma samples and 19 adjacent healthy tissues

Usage

```
pd.tcga
```

Format

DFrame

Value

Data frame with sample info

Source

<https://bioconductor.org/packages/release/bioc/html/GSEABenchmarkR.html>

References

Geistlinger L, Csaba G, Santarelli M, Ramos M, Schiffer L, Law C, Turaga N, Davis S, Carey V, Morgan M, Zimmer R, Waldron L (2020). Toward a gold standard for benchmarking gene set enrichment analysis. Briefings in Bioinformatics. doi:10.1093/bib/bbz158.

plot.roastgsa

roastgsa plot

Description

Plot for roastgsa objects

Usage

```
## S3 method for class 'roastgsa'  
plot(x, type = c("stats", "GSEA"), whplot = 1,  
      maintitle = "", gsainfo = TRUE, cex.sub = 0.8, lwd = 2, ...)
```

Arguments

| | |
|-----------|--|
| x | an object of class 'roastgsa' |
| type | plot type, either 'stats' or 'GSEA' |
| whplot | selected pathway. If integer vector, the pathways are selected in the same order as observed in the obj\$res table |
| maintitle | plot main title. If maintitle == "", the name of the pathway in obj is printed |
| gsainfo | if TRUE, the subtitle shows the GSA main results |
| cex.sub | cex for subtitle |
| lwd | line width |
| ... | Arguments passed to or from other methods to the low level. |

Details

Details for using 'type = stats' in the plot are given in [plotStats](#). Details for using 'type = GSEA' in the plot are given in [plotGSEA](#).

Value

plot object with the graphical representation of roastgsa results.

Author(s)

Adria Caballe Mestres

References

[1] A comparison of rotation-based scores for gene set analysis Adria Caballe Mestres, Antonio Berenguer Llergo, Camille Stephan-Otto Attolini *bioRxiv* 2021.03.23.436604;

See Also

[roastgsa](#) and [plotStats](#)

Examples

```

y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)

roastgsa1 <- roastgsa(y, covar, form = form, self.contained = TRUE,
  set.statistic = "maxmean", index = index, nrot = 200,
  mcores = 1, execution.info = FALSE)
plot(roastgsa1, type = "stats", whplot = 1, gsainfo =TRUE, maintitle =
  "", statistic = "mean")

```

plot.ssGSA

Plot single sample Gene Set Analysis

Description

Scatter plot of single sample z-score summarized data

Usage

```
## S3 method for class 'ssGSA'
plot(x, orderby, whplot = 1, col = "black", samplename = FALSE,
      maintitle = "", ssgsaInfo = TRUE, cex.sub = 0.8, ...)
```

Arguments

| | |
|------------|--|
| x | object of class 'ssGSA' |
| orderby | numeric or factor vector of the same size and order of data columns used for ssGSA. It sets the x-axis of the plot |
| whplot | selected pathway. If integer vector, the pathways are selected in the same order as the table in x\$res |
| col | color of scatterplot points |
| samplename | whether to show or not the names of the samples instead of points |
| maintitle | plot main title. If maintitle = "", the name of the pathway in obj is printed |
| ssgsaInfo | if TRUE, the subtitle shows the ssGSA results |
| cex.sub | cex for subtitle |
| ... | Arguments passed to or from other methods to the low level. |

Details

This graphic is a great alternative to explore gene set variation at sample level. This is sometimes ignored when doing GSEA, where classic representations (e.g., [plotGSEA](#)) show gene variation after averaging out the sample differences within each experimental condition.

Value

plot object with the graphical representation of ssGSA results

Author(s)

Adria Caballe Mestres

References

[1] Caballe Mestres A, Berenguer Llergo A and Stephan-Otto Attolini C. Adjusting for systematic technical biases in risk assessment of gene signatures in transcriptomic cancer cohorts. *bioRxiv* (2018).

See Also[ssGSA](#)**Examples**

```

y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)
design <- model.matrix(form, covar)

ssgsa1 <- ssGSA(y, obj=NULL, design = design, contrast = 2, index = index,
  method = c("GScor"))
plot(ssgsa1, orderby = covar$voi, whplot = 1 )

```

ploteffsignaturesize *roastgsa effective signature size*

Description

Approximation of effective signature size under gene randomization

Usage

```
ploteffsignaturesize(obj, varrot, whplot = 1, ...)
```

Arguments

| | |
|--------|--|
| obj | an object of class 'roastgsa' |
| varrot | an object of class 'varrotrand' (see varrotrand) with estimated rotation score variances for randomly selected genesets of several sizes. |
| whplot | selected pathway. If integer vector, the pathways are selected in the same order as the table in obj\$res |
| ... | Arguments passed to or from other methods to the low level. |

Details

The plot shows the approximated probability of obtaining a test statistic variance (under rotations of the residual space of the data) as extreme as the observed when generating randomly gene sets of several sizes.

Value

plot object with the effective signature size representation of roastgsa results

Author(s)

Adria Caballe Mestres

References

[1] A comparison of rotation-based scores for gene set analysis Adria Caballe Mestres, Antonio Berenguer Llergo, Camille Stephan-Otto Attolini bioRxiv 2021.03.23.436604;

See Also

[varrotrand](#) and [roastgsa](#)

Examples

```
y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)

roastgsa1 <- roastgsa(y, covar, form = form, self.contained = TRUE,
  set.statistic = "maxmean", index = index, nrot = 100,
  mcores = 1, execution.info = FALSE)

varrot <- varrotrand(roastgsa1, y,
  testedsizes = c(seq(5,50, by=5), seq(55,200,by=10)),
  nrep = 50)

ploteffsignaturesize(roastgsa1, varrot, whplot = 2)
```

plotGSEA

GSEA plot

Description

GSEA plot for roastgsa objects

Usage

```
plotGSEA(obj, whplot = 1, maintitle = "", gsainfo = TRUE, cex.sub = 0.8,
  lwd = 2, ...)
```

Arguments

| | |
|-----------|--|
| obj | an object of class 'roastgsa' |
| whplot | selected pathway. If integer vector, the pathways are selected in the same order as observed in the obj\$res table |
| maintitle | plot main title. If maintitle == "", the name of the pathway in obj is printed |
| gsainfo | if TRUE, the subtitle shows the GSA main results |
| cex.sub | cex for subtitle |
| lwd | line width |
| ... | Arguments passed to or from other methods to the low level. |

Details

Standard representation of Kolmogorov-Smirnov GSEA enrichment score.

Value

plot object with the GSEA representation of roastgsa results

Author(s)

Adria Caballe Mestres

References

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *PNAS*. 2005;102(43):15545-15550.

See Also

[roastgsa](#) and [plotStats](#)

Examples

```
y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)

roastgsa1 <- roastgsa(y, covar, form = form, self.contained = TRUE,
  set.statistic = "maxmean", index = index, nrot = 200,
  mcores = 1, execution.info = FALSE)
plotGSEA(roastgsa1, whplot = 1, gsainfo = TRUE, maintitle =
  "", statistic = "mean")
```

plotStats

General GSA plot

Description

General gene set analysis plot showing the ordered moderated-t statistics for the selected pathway

Usage

```
plotStats(obj, whplot = 1, maintitle = "", statistic = "mean",
  ylimAll = TRUE, ylim = NULL, minpointsDens = 20,
  gsainfo = TRUE, cex.sub = 0.8, lwd = 2, ...)
```

Arguments

| | |
|---------------|---|
| obj | an object of class 'roastgsa' |
| whplot | selected pathway. If integer vector, the pathways are selected in the same order as the table in obj\$res |
| maintitle | plot main title. If maintitle = "", the name of the pathway in obj is printed |
| statistic | to be selected from 'mean' or 'median' |
| ylimAll | y limits are found using data from all genesets (if TRUE) or using data from only the plotted geneset (if FALSE). Only if ylim = NULL |
| ylim | vector of size two with y limits |
| minpointsDens | minum number of genes needed to draw the density plot |
| gsainfo | if TRUE, the subtitle shows the enrichment results |
| cex.sub | cex for subtitle |
| lwd | line width |
| ... | Arguments passed to or from other methods to the low level. |

Details

The `statistic` argument is used for competitive testing computations of restandardized moderated-t statistics. If "median", the median of all stats is used for centering and the median absolute deviation is used for scaling. If "mean", standard normalization applies.

It shows the ordered moderated t-statistics in various formats, area for up- and down- expressed genes, barcode plot for these ordered values and density.

Value

plot object with a general representation of roastgsa results

Author(s)

Adria Caballe Mestres

References

[1] A comparison of rotation-based scores for gene set analysis Adria Caballe Mestres, Antonio Berenguer Llergo, Camille Stephan-Otto Attolini bioRxiv 2021.03.23.436604;

See Also

[roastgsa](#) and [plotGSEA](#)

Examples

```
y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)
```



```

roastgsa1 <- roastgsa(y, covar, form = form, self.contained = TRUE,
  set.statistic = "maxmean", index = index, nrot = 200,
  mcores = 1, execution.info = FALSE)
plotStats(roastgsa1, whplot = 1, maintitle = "general plot", statistic =
"mean")

```

roastgsa

*Rotation-based Gene Set Analysis***Description**

Gene set analysis using rotations for hypothesis testing. Test statistic options include KS-based statistics used in GSEA or GSVA as well as summary statistics such as mean, maxmean, median, absmean and mean.rank

Usage

```

roastgsa(y, covar, form, contrast = NA, design = NULL, gsetsel,
  gspath, index = NULL, self.contained = FALSE,
  set.statistic = "maxmean", psel = NULL, nrot = 9999,
  minsize = 10, maxsize = 500, mcores = 1,
  execution.info = TRUE, weights = NULL, shrink.resid = TRUE,
  normalizeScores = TRUE, ...)

```

Arguments

| | |
|----------------|---|
| y | expression matrix with columns indicating samples and rows indicating genes |
| covar | data frame with the covariates |
| form | description of the model to be fitted |
| contrast | comparison to consider in the model. If NA, the last column of the design matrix is used |
| design | the design matrix of the experiment. If null, this is calculated using the form and the covar arguments |
| gsetsel | character string with gene set database to be used in format .gmt. If missing, index argument has to be provided |
| gspath | path for the gene set database |
| index | list with index vectors specifying which rows of y are in the testing sets. Either integer indexes with row positions or gene identifiers can be stated. If NULL, the index is computed using information in the gsetsel and gspath arguments |
| self.contained | competitive test (FALSE) or self contained test (TRUE) |
| set.statistic | to be chosen from "maxmean" (default), "mean", "mean.rank", "median", "absmean", "GSEA" and "GSVA" |
| psel | character vector with probesets (one per gene) to be used for roastgsa statistic in a microarray experiment |
| nrot | number of rotations used for hypothesis testing |
| minsize | minimum size of the testing sets allowed for hypothesis testing |

| | |
|------------------|--|
| maxsize | maximum size of the testing sets allowed for hypothesis testing |
| mccores | the number of cores to use for parallel executions |
| executation.info | Show (if set to TRUE) the progress-bar of the iterative process |
| weights | list with the gene weights in each testing set. Only for set.statistic = "maxmean" and "mean". If NULL, weights are assumed to be constant |
| shrink.resid | if TRUE, the coefficients of the linear model are shrunk towards zero for rotations to increase the power |
| normalizeScores | transform the moderated t-statistics to z-scores |
| ... | Arguments passed to or from other methods to the low level. |

Details

We consider 7 different enrichment score functions which we refer by the names of mean, maxmean, median, absmean, mean.rank, GSEA and GSVA. The first four functions (mean, maxmean, median, absmean) are formulated for the two type of testing problems (self-contained and competitive). The mean.rank, GSEA and GSVA are exclusive scores for the competitive approach. The absmean is a non-directional score that can be used to give priority to gene sets with both activator and inhibitor genes. The mean is a democratic score that gives priority to detecting gene sets in which a large fraction of their genes present similar effect sizes going at the same direction. The maxmean (default) falls in between the mean and the absmean scores, being capable to recover both type of gene sets consistently.

Some of the defined sets are composed by genes that interact together in any particular biological condition, leading to intra-gene set correlation structures with high levels of correlation. We encourage the usage of effective signatures size, that can be a proxy for the number of uncorrelated genes in the gene set used for GSA ([varrotrand](#) and [ploteffsignaturesize](#)). Through the argument weights, we provide the possibility to redefining the gene set by weighting the importance of each gene in the list.

GSEA and GSVA scores are computationally much more intensive than the other scores.

Value

return an object of class `roastgsa` with attributes

| | |
|------------|--|
| "res" | data.frame with main results obtained in hypothesis testing. Total genes in the geneset, the number of genes also in the y, the test statistic, the normalized score and the significance of the tests |
| "stats" | Moderated t-statistics for all genes |
| "contrast" | contrast used in a vector form |
| "index" | list with gene set symbols |

Author(s)

Adria Caballe Mestres

References

- [1] A comparison of rotation-based scores for gene set analysis Adria Caballe Mestres, Antonio Berenguer Llergo, Camille Stephan-Otto Attolini bioRxiv 2021.03.23.436604; doi:
- [2] E. Lim, D. Wu, G. K. Smyth, M.-L. Asselin-Labat, F. Vaillant, and J. E. Visvader. ROAST: rotation gene set tests for complex microarray experiments. *Bioinformatics*, 26(17):2176-2182, 2010.

See Also

[roast](#)

Examples

```
y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)

roastgsa1 <- roastgsa(y, covar, form = form, self.contained = TRUE,
  set.statistic = "maxmean", index = index, nrot = 200,
  mcores = 1, execution.info = FALSE)
print(roastgsa1)
```

sorttable

sorttable for html writings

Description

from sorttable v2.0 of Stuart Langridge.

Usage

```
sorttable
```

Format

character vector

Value

Character vector with sorttable

Source

<http://www.kryogenix.org/code/browser/sorttable/>

References

<http://www.kryogenix.org/code/browser/sorttable/>

 ssGSA

Single sample Gene Set Analysis

Description

Single sample gene set analysis using z-score summarized data for linear model hypothesis testing

Usage

```
ssGSA(y, obj = NULL, design = NULL, contrast = NULL, index = NULL,
      method = c("GScor", "GSadj", "zscore"))
```

Arguments

| | |
|----------|--|
| y | expression matrix with columns indicating samples and rows indicating genes |
| obj | object of class 'roastgsa' used to extract the design, the contrast and the index arguments |
| design | the design matrix of the experiment. Considered only if obj is NULL |
| contrast | comparison to consider in the model. Considered only if obj is NULL |
| index | list with index vectors specifying which rows of y are in the testing sets. Either integer indexes with row positions or gene identifiers can be stated. Considered only if obj is NULL |
| method | If "GSadj", a correction variable with the average trend in the data enters in the model as confounding variable. If "GScor", gene signatures are adjusted a priori by subtracting the correction variable values. Check details for more information. |

Details

A correction by the overall tendency can be done a priori (GScor) or it can be incorporated as a covariate in the linear model (GSadj). The correction variable used here is what we have called the global signature (GS) of the experiment, that for each sample can be calculated as the average z-score of all genes measured in y. This GS corrects or centers global technical / sampling directions in the data.

Value

return an object of class ssGSA with attributes

| | |
|---------|---|
| "res" | data.frame with main results obtained in hypothesis testing. Total genes in the gene set, the average score, the test statistic, p-value and adjusted pvalue. |
| "stats" | adjusted z-scores matrix |

Author(s)

Adria Caballe Mestres

References

[1] Caballe Mestres A, Berenguer Llergo A and Stephan-Otto Attolini C. Adjusting for systematic technical biases in risk assessment of gene signatures in transcriptomic cancer cohorts. *bioRxiv* (2018).

See Also

[plot.ssGSA](#)

Examples

```
y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)
design <- model.matrix(form, covar)

ssgsa1 <- ssGSA(y, obj=NULL, design = design, contrast = 2, index = index,
  method = c("GScor"))
```

varrotrand

roastgsa variance rotations under gene randomization

Description

Computation of the sample variance of rotation scores under gene randomization

Usage

```
varrotrand(obj, y, testedsizes = c(3:30,seq(32,50, by=2),
  seq(55,200,by=5)), nrep = 200, nrot = NULL,
  mcores = NULL, psel = NULL)
```

Arguments

| | |
|-------------|---|
| obj | an object of class 'roastgsa' |
| y | data used in roastgsa call |
| testedsizes | effective sizes to be tested |
| nrep | number of randomly selected gene sets created for each tested effective size |
| nrot | number of rotations used for hypothesis testing |
| mcores | the number of cores to use for parallel executions |
| psel | character vector with probesets (one per gene) to be used for roastgsa statistic in a microarray experiment |

Details

When a specific gene that is highly correlated to the rest of the gene set finds an extreme value, even under H_0 , it is likely that many other genes in the gene set follow it with large values as well. We define the concept of effective signature size of a gene set by the number of randomly selected (not necessarily independent) genes that are needed to achieve comparable variability levels on rotation summary test statistics. This can be viewed as a realistic measure of the total number of independent variables that contribute to the power of the test. The function presented here computes the sample variance of the rotation scores in randomly generated signatures of several sizes. The comparison to the observed variances (using the testing gene sets in the `roastgsa` call) is done through the function `ploteffsignaturesize`.

Value

return an object of class `varrotrand` with attributes

"varrot" matrix `nrep` x `testedsizes` with the estimated variance of the rotation scores using `nrot` rotations
 "testedsizes" effective sizes being tested
 "nrep" number of gene sets created for each tested effective size

Author(s)

Adria Caballe Mestres

References

[1] A comparison of rotation-based scores for gene set analysis Adria Caballe Mestres, Antonio Berenguer Llergo, Camille Stephan-Otto Attolini bioRxiv 2021.03.23.436604;

See Also

`ploteffsignaturesize` to visualize results and `roastgsa` for `gsa` approach

Examples

```
y <- array(rnorm(10000),dim = c(1000,10))
covar <- data.frame(voi = factor(c(rep(0,5),rep(1,5))))
colnames(y) <- rownames(covar) <- paste0("sample",1:10)
rownames(y) <- paste0("gene",1:1000)
form <- as.formula("~ voi")
index <- lapply(1:10, function(o) sample(1:1000,50))
names(index) <- paste0("gset",1:10)

roastgsa1 <- roastgsa(y, covar, form = form, self.contained = TRUE,
  set.statistic = "maxmean", index = index, nrot = 100,
  mcores = 1, execution.info = FALSE)

varrot <- varrotrand(roastgsa1, y,
  testedsizes = c(seq(5,50, by=5), seq(55,200,by=10)),
  nrep = 50)
```

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