

# Package ‘roastgsa’

November 23, 2024

**Type** Package

**Version** 1.5.0

**Date** 2023-06-13

**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, mRNAArray, 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

**git\_url** <https://git.bioconductor.org/packages/roastgsa>

**git\_branch** devel

**git\_last\_commit** 5f94c99

**git\_last\_commit\_date** 2024-10-29

**Repository** Bioconductor 3.21

**Date/Publication** 2024-11-22

**Author** Adria Caballe [aut, cre] (ORCID:  
<<https://orcid.org/0000-0002-2388-4713>>)

**Maintainer** Adria Caballe <[adria.caballe@irbbarcelona.org](mailto:adria.caballe@irbbarcelona.org)>

## Contents

dragtable . . . . .	2
expr.tcga . . . . .	3
fd.tcga . . . . .	4
hallmarks.hs . . . . .	4
heatmaprgsa_hm . . . . .	5
htmlrgsa . . . . .	7
kegg.hs . . . . .	10
pd.tcga . . . . .	11
plot.roastgsa . . . . .	11
plot.ssGSA . . . . .	13
ploteffsignaturesize . . . . .	14
plotGSEA . . . . .	16
plotStats . . . . .	17
roastgsa . . . . .	18
sorttable . . . . .	21
ssGSA . . . . .	22
varrotrand . . . . .	23
<b>Index</b>	<b>26</b>

---

dragtable

*dragtable for html writings*

---

### 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/sortable](http://kryogenix.org/code/browser/sortable)

---

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*Hallmarks homo sapiens gene symbol*

---

**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, topplot = 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
topplot	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 <code>obj\$statistic = "maxmean"</code> . 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 <code>set.statistic</code> operations) samplewise (see details below).
psel	character vector with probesets (one per gene) to be used for roastgsa statistic in a microarray experiment
dendrogram	<a href="#">heatmap.2</a> parameter. Character string indicating whether to draw 'none', 'row', 'column' or 'both' dendrograms. Defaults to 'n'
col	<a href="#">heatmap.2</a> parameter. Colors used for the image
trace	<a href="#">heatmap.2</a> 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	<a href="#">heatmap.2</a> parameter. Color of note
notecex	<a href="#">heatmap.2</a> parameter. Size of note
keysize	<a href="#">heatmap.2</a> parameter. Numeric value indicating the size of the key
cexCol	<a href="#">heatmap.2</a> parameter. Cex.axis in for the column axis labeling
Rowv	<a href="#">heatmap.2</a> parameter. Determines if and how the row dendrogram should be reordered
Colv	<a href="#">heatmap.2</a> 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 <code>pathwaylevel = TRUE</code>
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 restandardization 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 <a href="#">plotStats</a> are created
plotgsea	plots using <a href="#">plotGSEA</a> are created
indheatmap	plots using <a href="#">heatmaprgsa_hm</a> at the gene level are created
ploteffsize	plots using <a href="#">ploteffsignaturesize</a> 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 <a href="#">roastgsa</a>
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 <a href="#">ggplot2</a> type or <a href="#">heatmap.2</a> type
intvar	for <a href="#">heatmaprgsa_hm</a> . Name of variable of interest in obj\$formula. If missing, last term of obj\$formula is used
adj.var	for <a href="#">heatmaprgsa_hm</a> . 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 <a href="#">varrotrand</a> ) with estimated rotation score variances for randomly selected genesets of several sizes. Cannot be missing if ploteffsize = TRUE



psel	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,
```

```
mccores = 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	<i>Tumor Bladder TCGA data</i>
---------	--------------------------------

---

**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	<i>roastgsa plot</i>
---------------	----------------------

---

**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 <a href="#">varrotrand</a> ) 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	minimum 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
mcores	the number of cores to use for parallel executions
execution.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 <a href="#">roastgsa</a> 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)),
```



*varrotrand*

25

nrep = 50)

# Index

## \* datasets

- dragtable, [2](#)
- expr.tcga, [3](#)
- fd.tcga, [4](#)
- hallmarks.hs, [4](#)
- kegg.hs, [10](#)
- pd.tcga, [11](#)
- sorttable, [21](#)

dragtable, [2](#)

expr.tcga, [3](#)

fd.tcga, [4](#)

hallmarks.hs, [4](#)

heatmap.2, [6](#)

heatmaprgsa\_hm, [5](#), [8](#), [9](#)

htmlrgsa, [7](#)

kegg.hs, [10](#)

pd.tcga, [11](#)

plot.roastgsa, [11](#)

plot.ssGSA, [13](#), [23](#)

ploteffsignaturesize, [8](#), [9](#), [14](#), [20](#), [24](#)

plotGSEA, [7-9](#), [12](#), [13](#), [16](#), [18](#)

plotStats, [7-9](#), [12](#), [16](#), [17](#)

print.roastgsa (roastgsa), [18](#)

print.ssGSA (ssGSA), [22](#)

roast, [20](#)

roastgsa, [7-9](#), [12](#), [15](#), [16](#), [18](#), [18](#), [23](#), [24](#)

sorttable, [21](#)

ssGSA, [14](#), [22](#)

varrotrand, [8](#), [9](#), [14](#), [15](#), [20](#), [23](#)