

Package ‘GSVA’

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Title Gene Set Variation Analysis for microarray and RNA-seq data

Depends R (>= 2.13.0)

Imports methods, BiocGenerics, Biobase, GSEABase (>= 1.17.4)

Suggests limma, RColorBrewer, genefilter, mclust, edgeR, snow,
parallel, GSVAdata

Description Gene Set Variation Analysis (GSVA) is a non-parametric, unsupervised method for estimating variation of gene set enrichment through the samples of a expression data set. GSVA performs a change in coordinate systems, transforming the data from a gene by sample matrix to a gene-set by sample matrix, thereby allowing the evaluation of pathway enrichment for each sample. This new matrix of GSVA enrichment scores facilitates applying standard analytical methods like functional enrichment, survival analysis, clustering, CNV-pathway analysis or cross-tissue pathway analysis, in a pathway-centric manner.

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LazyLoad yes

biocViews Microarray, Pathways, GeneSetEnrichment

URL <http://www.sagebase.org>

Encoding latin1

NeedsCompilation yes

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`computeGeneSetsOverlap`*Compute gene-sets overlap*

Description

Calculates the overlap among every pair of gene-sets given as input.

Usage

```
## S4 method for signature 'list,character'  
computeGeneSetsOverlap(gSets, uniqGenes, min.sz=1, max.sz=Inf)  
## S4 method for signature 'list,ExpressionSet'  
computeGeneSetsOverlap(gSets, uniqGenes, min.sz=1, max.sz=Inf)  
## S4 method for signature 'GeneSetCollection,character'  
computeGeneSetsOverlap(gSets, uniqGenes, min.sz=1, max.sz=Inf)  
## S4 method for signature 'GeneSetCollection,ExpressionSet'  
computeGeneSetsOverlap(gSets, uniqGenes, min.sz=1, max.sz=Inf)
```

Arguments

<code>gSets</code>	Gene sets given either as a list or a GeneSetCollection object.
<code>uniqGenes</code>	Vector of unique genes to be considered when calculating the overlaps.
<code>min.sz</code>	Minimum size.
<code>max.sz</code>	Maximum size.

Details

This function calculates the overlap between every pair of gene sets of the input argument `gSets`. Before this calculation takes place, the gene sets in `gSets` are firstly filtered to discard genes that do not match to the identifiers in `uniqGenes`. Secondly, they are further filtered to meet the minimum and/or maximum size specified with the arguments `min.sz` and `max.sz`. The overlap between two gene sets is calculated as the number of common genes between the two gene sets divided by the smallest size of the two gene sets.

Value

A gene-set by gene-set matrix of the overlap among every pair of gene sets.

Author(s)

J. Guinney

References

Hänzelmann, S., Castelo, R. and Guinney, J. GSVA: Gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*, 14:7, 2013.

See Also

[filterGeneSets](#)

Examples

```
geneSets <- list(set1=as.character(1:4), set2=as.character(4:10))  
computeGeneSetsOverlap(geneSets, unique(unlist(geneSets)))
```

filterGeneSets	<i>Filter gene sets</i>
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Description

Filters gene sets through a given minimum and maximum set size.

Usage

```
## S4 method for signature 'list'  
filterGeneSets(gSets, min.sz=1, max.sz=Inf)  
## S4 method for signature 'GeneSetCollection'  
filterGeneSets(gSets, min.sz=1, max.sz=Inf)
```

Arguments

gSets	Gene sets given either as a list or a GeneSetCollection object.
min.sz	Minimum size.
max.sz	Maximum size.

Details

This function filters the input gene sets according to a given minimum and maximum set size.

Value

A collection of gene sets that meet the given minimum and maximum set size.

Author(s)

J. Guinney

References

Hänzelmann, S., Castelo, R. and Guinney, J. GSVA: Gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*, 14:7, 2013.

See Also

[computeGeneSetsOverlap](#)

Examples

```
geneSets <- list(set1=as.character(1:4), set2=as.character(4:10))

filterGeneSets(geneSets, min.sz=5)
```

gsva

Gene Set Variation Analysis

Description

Estimates GSVA enrichment scores.

Usage

```
## S4 method for signature 'ExpressionSet,list'
gsva(expr, gset.idx.list, annotation,
      method=c("gsva", "ssgsea", "zscore", "plage"),
      rnaseq=FALSE,
      abs.ranking=FALSE,
      min.sz=1,
      max.sz=Inf,
      no.bootstraps=0,
      bootstrap.percent = .632,
      parallel.sz=0,
      parallel.type="SOCK",
      mx.diff=TRUE,
      tau=switch(method, gsva=1, ssgsea=0.25, NA),
      kernel=TRUE,
      ssgsea.norm=TRUE,
      verbose=TRUE)

## S4 method for signature 'ExpressionSet,GeneSetCollection'
gsva(expr, gset.idx.list, annotation,
      method=c("gsva", "ssgsea", "zscore", "plage"),
      rnaseq=FALSE,
      abs.ranking=FALSE,
      min.sz=1,
      max.sz=Inf,
      no.bootstraps=0,
      bootstrap.percent = .632,
      parallel.sz=0,
      parallel.type="SOCK",
      mx.diff=TRUE,
      tau=switch(method, gsva=1, ssgsea=0.25, NA),
      kernel=TRUE,
      ssgsea.norm=TRUE,
      verbose=TRUE)

## S4 method for signature 'matrix,GeneSetCollection'
gsva(expr, gset.idx.list, annotation,
      method=c("gsva", "ssgsea", "zscore", "plage"),
```

```

rnaseq=FALSE,
abs.ranking=FALSE,
min.sz=1,
max.sz=Inf,
no.bootstraps=0,
bootstrap.percent = .632,
parallel.sz=0,
parallel.type="SOCK",
mx.diff=TRUE,
tau=switch(method, gsva=1, ssgsea=0.25, NA),
kernel=TRUE,
ssgsea.norm=TRUE,
verbose=TRUE)
## S4 method for signature 'matrix,list'
gsva(expr, gset.idx.list, annotation,
      method=c("gsva", "ssgsea", "zscore", "plage"),
      rnaseq=FALSE,
      abs.ranking=FALSE,
      min.sz=1,
      max.sz=Inf,
      no.bootstraps=0,
      bootstrap.percent = .632,
      parallel.sz=0,
      parallel.type="SOCK",
      mx.diff=TRUE,
      tau=switch(method, gsva=1, ssgsea=0.25, NA),
      kernel=TRUE,
      ssgsea.norm=TRUE,
      verbose=TRUE)

```

Arguments

<code>expr</code>	Gene expression data which can be given either as an <code>ExpressionSet</code> object or as a matrix of expression values where rows correspond to genes and columns correspond to samples.
<code>gset.idx.list</code>	Gene sets provided either as a <code>list</code> object or as a <code>GeneSetCollection</code> object.
<code>annotation</code>	In the case of calling <code>gsva()</code> with expression data in a matrix and gene sets as a <code>GeneSetCollection</code> object, the <code>annotation</code> argument can be used to supply the name of the Bioconductor package that contains annotations for the class of gene identifiers occurring in the row names of the expression data matrix. By default <code>gsva()</code> will try to match the identifiers in <code>expr</code> to the identifiers in <code>gset.idx.list</code> just as they are, unless the <code>annotation</code> argument is set.
<code>method</code>	Method to employ in the estimation of gene-set enrichment scores per sample. By default this is set to <code>gsva</code> (Hänzelmann et al, 2013) and other options are <code>ssgsea</code> (Barbie et al, 2009), <code>zscore</code> (Lee et al, 2008) or <code>plage</code> (Tomfohr et al, 2005). The latter two standardize first expression profiles into z-scores over the samples and, in the case of <code>zscore</code> , it combines them together as their sum divided by the square-root of the size of the gene set, while in the case of <code>plage</code> they are used to calculate the singular value decomposition (SVD) over the genes in the gene set and use the coefficients of the first right-singular vector as pathway activity profile.

<code>rnaseq</code>	Logical flag set by default to <code>rnaseq=FALSE</code> to inform whether the input gene expression data are continuous values, such as fluorescent units in logarithmic scale from microarray experiments or some other kind of continuous value derived from RNA-seq counts such as log-CPMs, log-RPKMs or log-TPMs. This flag should be set to <code>rnaseq=TRUE</code> only when the values of the input gene expression data are integer counts.
<code>abs.ranking</code>	Flag to determine whether genes should be ranked according to their sign (<code>abs.ranking=FALSE</code>) or by absolute value (<code>abs.ranking=TRUE</code>). In the latter, pathways with genes enriched on either extreme (high or low) will be regarded as 'highly' activated.
<code>min.sz</code>	Minimum size of the resulting gene sets.
<code>max.sz</code>	Maximum size of the resulting gene sets.
<code>no.bootstraps</code>	Number of bootstrap iterations to perform.
<code>bootstrap.percent</code>	.632 is the ideal percent samples bootstrapped.
<code>parallel.sz</code>	Number of processors to use when doing the calculations in parallel. This requires to previously load either the <code>parallel</code> or the <code>snow</code> library. If <code>parallel</code> is loaded and this argument is left with its default value (<code>parallel.sz=0</code>) then it will use all available core processors unless we set this argument with a smaller number. If <code>snow</code> is loaded then we must set this argument to a positive integer number that specifies the number of processors to employ in the parallel calculation.
<code>parallel.type</code>	Type of cluster architecture when using <code>snow</code> .
<code>mx.diff</code>	Offers two approaches to calculate the enrichment statistic (ES) from the KS random walk statistic. <code>mx.diff=FALSE</code> : ES is calculated as the maximum distance of the random walk from 0. <code>mx.diff=TRUE</code> (default): ES is calculated as the magnitude difference between the largest positive and negative random walk deviations.
<code>tau</code>	Exponent defining the weight of the tail in the random walk performed by both the <code>gsva</code> (Hänzelmann et al., 2013) and the <code>ssgsea</code> (Barbie et al., 2009) methods. By default, this <code>tau=1</code> when <code>method="gsva"</code> and <code>tau=0.25</code> when <code>method="ssgsea"</code> just as specified by Barbie et al. (2009) where this parameter is called alpha.
<code>kernel</code>	Logical, set to <code>TRUE</code> when the GSVa method employs a kernel non-parametric estimation of the empirical cumulative distribution function (default) and <code>FALSE</code> when this function is directly estimated from the observed data. This last option is justified in the limit of the size of the sample by the so-called Glivenko-Cantelli theorem.
<code>ssgsea.norm</code>	Logical, set to <code>TRUE</code> (default) with <code>method="ssgsea"</code> runs the SSGSEA method from Barbie et al. (2009) normalizing the scores by the absolute difference between the minimum and the maximum, as described in their paper. When <code>ssgsea.norm=FALSE</code> this last normalization step is skipped.
<code>verbose</code>	Gives information about each calculation step. Default: <code>FALSE</code> .

Details

GSVA assesses the relative enrichment of gene sets across samples using a non-parametric approach. Conceptually, GSVa transforms a p -gene by n -sample gene expression matrix into a g -geneset by n -sample pathway enrichment matrix. This facilitates many forms of statistical analysis in the 'space' of pathways rather than genes, providing a higher level of interpretability.

The `gsva()` function first maps the identifiers in the gene sets to the identifiers in the input expression data leading to a filtered collection of gene sets. This collection can be further filtered to require a minimum and/or maximum size of the gene sets for which we want to calculate GSVAs enrichment scores, by using the arguments `min.sz` and `max.sz`.

The name of the argument `rnaseq` can be misleading. When set to `rnaseq=FALSE`, the nonparametric estimation of the cumulative density function of the expression profile of each gene across samples is done using Gaussian kernels suited for continuous values. These were initially thought to be only microarray fluorescent units in logarithmic scale but nowadays these may also correspond to continuous values derived from RNA-seq experiments such as log-CPMs, log-RPKMs or log-TPMs. When `rnaseq=TRUE`, Poisson kernels are used instead and therefore, this option is only suitable when the input gene expression matrix is formed by integer counts. This implies that `rnaseq=FALSE` may also be used even when the expression data comes from a RNA-seq experiment. The name of this argument may change in the future to avoid this confusion.

Value

A gene-set by sample matrix of GSVAs enrichment scores.

Author(s)

J. Guinney and R. Castelo

References

- Barbie, D.A. et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature*, 462(5):108-112, 2009.
- Hänzelmann, S., Castelo, R. and Guinney, J. GSVAs: Gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*, 14:7, 2013.
- Lee, E. et al. Inferring pathway activity toward precise disease classification. *PLoS Comp Biol*, 4(11):e1000217, 2008.
- Tomfohr, J. et al. Pathway level analysis of gene expression using singular value decomposition. *BMC Bioinformatics*, 6:225, 2005.

See Also

[filterGeneSets](#) [computeGeneSetsOverlap](#)

Examples

```
library(limma)

p <- 10 ## number of genes
n <- 30 ## number of samples
nGrp1 <- 15 ## number of samples in group 1
nGrp2 <- n - nGrp1 ## number of samples in group 2

## consider three disjoint gene sets
geneSets <- list(set1=paste("g", 1:3, sep=""),
                 set2=paste("g", 4:6, sep=""),
                 set3=paste("g", 7:10, sep=""))

## sample data from a normal distribution with mean 0 and st.dev. 1
y <- matrix(rnorm(n*p), nrow=p, ncol=n,
```

```
dimnames=list(paste("g", 1:p, sep="") , paste("s", 1:n, sep=""))

## genes in set1 are expressed at higher levels in the last 10 samples
y[geneSets$set1, (nGrp1+1):n] <- y[geneSets$set1, (nGrp1+1):n] + 2

## build design matrix
design <- cbind(sampleGroup1=1, sampleGroup2vs1=c(rep(0, nGrp1), rep(1, nGrp2)))

## fit linear model
fit <- lmFit(y, design)

## estimate moderated t-statistics
fit <- eBayes(fit)

## genes in set1 are differentially expressed
topTable(fit, coef="sampleGroup2vs1")

## estimate GSEA enrichment scores for the three sets
gsva_es <- gsva(y, geneSets, mx.diff=1)$es.obs

## fit the same linear model now to the GSEA enrichment scores
fit <- lmFit(gsva_es, design)

## estimate moderated t-statistics
fit <- eBayes(fit)

## set1 is differentially expressed
topTable(fit, coef="sampleGroup2vs1")
```

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