# Package 'GSVA'

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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.

**License** GPL (>= 2) **VignetteBuilder** knitr

 $\boldsymbol{URL} \hspace{0.1cm} \textbf{https://github.com/rcastelo/GSVA}$ 

BugReports https://github.com/rcastelo/GSVA/issues

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computeGeneSetsOverlap

Compute gene-sets overlap

# Description

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

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 minSize and maxSize. 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.

#### Usage

```
## S4 method for signature 'list,character'
computeGeneSetsOverlap(gSets, uniqGenes, minSize = 1, maxSize = Inf)
## S4 method for signature 'list,ExpressionSet'
computeGeneSetsOverlap(gSets, uniqGenes, minSize = 1, maxSize = Inf)
## S4 method for signature 'GeneSetCollection,character'
computeGeneSetsOverlap(gSets, uniqGenes, minSize = 1, maxSize = Inf)
## S4 method for signature 'GeneSetCollection,ExpressionSet'
computeGeneSetsOverlap(gSets, uniqGenes, minSize = 1, maxSize = Inf)
```

## **Arguments**

gSets Gene sets given either as a list or a GeneSetCollection object.

uniqGenes Vector of unique genes to be considered when calculating the overlaps.

minSize Minimum size.

maxSize Maximum size.

#### 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)))</pre>
```

deduplicateGeneSets

Handling of Duplicated Gene Set Names

## **Description**

Offers a choice of ways for handling duplicated gene set names that may not be suitable as input to other gene set analysis functions.

## Usage

```
deduplicateGeneSets(
  geneSets,
  deduplUse = c("first", "drop", "union", "smallest", "largest")
)
```

## **Arguments**

geneSets

A named list of gene sets represented as character vectors of gene IDs as e.g. returned by readGMT.

dedup1Use

A character vector of length 1 specifying one of several methods to handle duplicated gene set names. Duplicated gene set names are explicitly forbidden by the GMT file format specification but can nevertheless be encountered in the wild. The available choices are:

- first (the default): drops all gene sets whose names are duplicated according to the base R function and retains only the first occurence of a gene set name.
- drop: removes *all* gene sets that have a duplicated name, including its first occurrence.
- union: replaces gene sets with duplicated names by a single gene set containing the union of all their gene IDs.
- smallest: drops gene sets with duplicated names and retains only the smallest of them, i.e. the one with the fewest gene IDs. If there are several smallest gene sets, the first will be selected.
- largest: drops gene sets with duplicated names and retains only the largest of them, i.e. the one with the most gene IDs. If there are several largest gene sets, the first will be selected.

# Value

A named list of gene sets represented as character vectors of gene IDs.

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	filterGeneSets	Filter gene sets	
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## **Description**

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

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

## Usage

```
## S4 method for signature 'list'
filterGeneSets(gSets, minSize = 1, maxSize = Inf)
## S4 method for signature 'GeneSetCollection'
filterGeneSets(gSets, minSize = 1, maxSize = Inf)
```

## **Arguments**

gSets Gene sets given either as a list or a GeneSetCollection object.

minSize Minimum size.
maxSize Maximum 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, minSize=5)</pre>
```

geneIdsToGeneSetCollection

Construct a GeneSetCollection object from a list of character vectors

## **Description**

This function is essentially the reverse of GSEABase::geneIds(), i.e., it takes as input a named list of character vectors representing gene sets and returns the corresponding GeneSetCollection object.

## Usage

```
geneIdsToGeneSetCollection(
  geneIdsList,
  geneIdType = "auto",
  collectionType = NullCollection()
)
```

## **Arguments**

geneIdsList

A named list of character vectors like the ones returned by geneIds(). Names must be unique; otherwise see deduplicateGeneSets() for a number of strategies to resolve this issue.

geneIdType

By default a character vector of length 1 with the special value "auto" or an object of a subclass of GeneIdentifierType. If set to "auto", the function will try to derive the gene ID type from argument geneIdsList using guessGeneIdType. Other values, including NULL, will be ignored with a warning and geneIdType=NullIdentifier() will be used instead. The gene ID type of all GeneSet objects in the resulting GeneSetCollection will be set to this value.

collectionType An object of class CollectionType. The collection type of all GeneSet objects in the resulting GeneSetCollection will be set to this value but can afterwards be modified for individual GeneSets if necessary.

#### Value

An object of class GeneSetCollection with all its GeneSet objects using the gene ID and collection types specified by the corresponding arguments. Applying function geneIds() to this object should return a list identical to the geneIdsList argument.

## See Also

 ${\tt GeneSetCollection, geneIds, deduplicateGeneSets, guessGeneIdType, GeneSetate} \\$ 

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geneSets

Retrieve or Determine Gene Sets

## **Description**

Retrieves or determines the gene sets that have been used or would be used in a gsva() gene set analysis. These are not necessarily the same as the input gene sets. See Details.

## Usage

```
## S4 method for signature 'GsvaMethodParam'
geneSets(obj)

## S4 method for signature 'SummarizedExperiment'
geneSets(obj)

## S4 method for signature 'SingleCellExperiment'
geneSets(obj)

## S4 method for signature 'SpatialExperiment'
geneSets(obj)

## S4 method for signature 'GsvaExprData'
geneSets(obj)

## S4 method for signature 'GsvaMethodParam'
geneSetSizes(obj)

## S4 method for signature 'GsvaExprData'
geneSetSizes(obj)
```

## **Arguments**

obj

An object of one of the following classes:

- An expression data object of one of the classes described in GsvaExprData that is the return value of a call to gsva().
- A parameter object of one of the classes described in GsvaMethodParam that could be used in a call to gsva().

## **Details**

The gene sets used in a gsva() gene set analysis, or just their sizes, may be a valuable input to subsequent analyses. However, they are not necessarily the same as the original input gene sets, or their sizes: based on user choices, the gene annotation used, or presence/absence of genes in gene sets and expression data set, gsva() may have to modify them during the preparation of an analysis run. In order to make use of these gene sets or their sizes, you can either

 retrieve them from the object returned by gsva() by passing this object to geneSets() or geneSetSizes(), or 8 gsva

• predict them by calling geneSets() or geneSetSizes() on the parameter object that would also be passed to gsva(). This is much slower and should only be done if you do not intend to run an actual gene set analysis.

geneSetSizes() is a convenience wrapper running lengths() on the list of gene sets returned by geneSets().

#### Value

The geneSets() methods return a named list of character vectors where each character vector contains the gene IDs of a gene set. The geneSetSizes() methods return a named integer vector of gene set sizes.

gsva

Gene Set Variation Analysis

## **Description**

Estimates GSVA enrichment scores. The API of this function has changed in the Bioconductor release 3.18 and this help page describes the new API. The old API is defunct and will be removed in the next Bioconductor release. If you are looking for the documentation of the old API to the gsva() function, please consult GSVA-pkg-defunct.

## Usage

```
## S4 method for signature 'plageParam'
gsva(param, verbose = TRUE, BPPARAM = SerialParam(progressbar = verbose))
## S4 method for signature 'zscoreParam'
gsva(param, verbose = TRUE, BPPARAM = SerialParam(progressbar = verbose))
## S4 method for signature 'ssgseaParam'
gsva(param, verbose = TRUE, BPPARAM = SerialParam(progressbar = verbose))
## S4 method for signature 'gsvaParam'
gsva(param, verbose = TRUE, BPPARAM = SerialParam(progressbar = verbose))
```

#### **Arguments**

param

A parameter object of one of the following classes:

- A gsvaParam object built using the constructor function gsvaParam. This
  object will trigger gsva() to use the GSVA algorithm by Hänzelmann et al.
  (2013).
- A plageParam object built using the constructor function plageParam. This object will trigger gsva() to use the PLAGE algorithm by Tomfohr et al. (2005).
- A zscoreParam object built using the constructor function zscoreParam. This object will trigger gsva() to use the combined z-score algorithm by Lee et al. (2008).
- A ssgseaParam object built using the constructor function ssgseaParam.
   This object will trigger gsva() to use the ssGSEA algorithm by Barbie et al. (2009).

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verbose Gives information about each calculation step. Default: TRUE.

BPPARAM An object of class BiocParallelParam specifying parameters related to the parallel execution of some of the tasks and calculations within this function.

#### Value

A gene-set by sample matrix of GSVA enrichment scores stored in a container object of the same type as the input expression data container. If the input was a base matrix or a dgCMatrix object, then the output will be a base matrix object with the gene sets employed in the calculations stored in an attribute called geneSets. If the input was an ExpressionSet object, then the output will be also an ExpressionSet object with the gene sets employed in the calculations stored in an attributed called geneSets. If the input was an object of one of the classes described in GsvaExprData, such as a SingleCellExperiment, then the output will be of the same class, where enrichment scores will be stored in an assay called es and the gene sets employed in the calculations will be stored in the rowData slot of the object under the column name gs.

#### References

Barbie, D.A. et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature*, 462(5):108-112, 2009. DOI

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

Lee, E. et al. Inferring pathway activity toward precise disease classification. *PLoS Comp Biol*, 4(11):e1000217, 2008. DOI

Tomfohr, J. et al. Pathway level analysis of gene expression using singular value decomposition. *BMC Bioinformatics*, 6:225, 2005. DOI

# See Also

plageParam, zscoreParam, ssgseaParam, gsvaParam

# **Examples**

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```
design <- cbind(sampleGroup1=1, sampleGroup2vs1=c(rep(0, nGrp1), rep(1, nGrp2)))</pre>
## fit linear model
fit <- lmFit(y, design)</pre>
## estimate moderated t-statistics
fit <- eBayes(fit)</pre>
## genes in set1 are differentially expressed
topTable(fit, coef="sampleGroup2vs1")
## build GSVA parameter object
gsvapar <- gsvaParam(y, geneSets)</pre>
## estimate GSVA enrichment scores for the three sets
gsva_es <- gsva(gsvapar)</pre>
## fit the same linear model now to the GSVA enrichment scores
fit <- lmFit(gsva_es, design)</pre>
## estimate moderated t-statistics
fit <- eBayes(fit)</pre>
## set1 is differentially expressed
topTable(fit, coef="sampleGroup2vs1")
```

gsva-defunct

Gene Set Variation Analysis

## **Description**

This is the old manual page of the defunct version of the function gsva().

## See Also

GSVA-pkg-defunct

GSVA-pkg-defunct

Defunct functions in package GSVA.

## **Description**

The functions listed below are defunct and will be removed in the next release.

#### **Details**

Instead of gsva(expr=., gset.idx.list=., method=., ...), use a method-specific parameter object, see plageParam zscoreParam ssgseaParam gsvaParam, followed by a call to the new gsva() function, see gsva.

GSVA-pkg-deprecated

Deprecated functions in package GSVA.

## **Description**

The functions listed below are deprecated and will be defunct in the near future. When possible, alternative functions with similar functionality are also mentioned.

gsvaAnnotation

Store and Retrieve Annotation Metadata

#### **Description**

Methods for storing and retrieving annotation metadata in expression data objects that support it. If gene sets and expression data are using different but known gene identifier types and an appropriate annotation database is available, gene set identifiers can be mapped to expression data identifiers without manual user intervention, e.g. from an MSigDb gene set using ENTREZ IDs or gene symbols to an expression data set using ENSEMBL IDs.

## Usage

```
## S4 method for signature 'GsvaExprData'
gsvaAnnotation(object)
## S4 replacement method for signature 'GsvaExprData,GeneIdentifierType'
gsvaAnnotation(object) <- value</pre>
## S4 method for signature 'ExpressionSet'
gsvaAnnotation(object)
## S4 replacement method for signature 'ExpressionSet, character'
gsvaAnnotation(object) <- value</pre>
## S4 replacement method for signature 'ExpressionSet,GeneIdentifierType'
gsvaAnnotation(object) <- value</pre>
## S4 method for signature 'SummarizedExperiment'
gsvaAnnotation(object)
## S4 replacement method for signature 'SummarizedExperiment,GeneIdentifierType'
gsvaAnnotation(object) <- value</pre>
## S4 method for signature 'SingleCellExperiment'
gsvaAnnotation(object)
## S4 replacement method for signature 'SingleCellExperiment,GeneIdentifierType'
gsvaAnnotation(object) <- value</pre>
## S4 method for signature 'SpatialExperiment'
```

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```
gsvaAnnotation(object)

## S4 replacement method for signature 'SpatialExperiment,GeneIdentifierType'
gsvaAnnotation(object) <- value

## S4 method for signature 'list'
gsvaAnnotation(object)

## S4 replacement method for signature 'list,GeneIdentifierType'
gsvaAnnotation(object) <- value

## S4 method for signature 'GeneSetCollection'
gsvaAnnotation(object)</pre>
```

## **Arguments**

object

An expression data object of one of the classes described in GsvaExprData. Simple matrix and dgCMatrix objects are not capable of storing annotation metadata and will return NULL.

value

For the replacement methods, the annotation metadata to be stored in the object. For ExpressionSet objects, this must be a character of length 1 specifying the name of the annotation database to be used. For SummarizedExperiment and its subclasses, this must be a GeneIdentifierType created by one of the constructors from package GSEABase where the annotation argument is typically the name of an organism or annotation database, e.g. org.Hs.eg.db. Simple matrix and dgCMatrix objects are not capable of storing annotation metadata and the attempt to do so will result in an error.

## Value

For the retrieval methods, the annotation metadata stored in the object of NULL. For the replacement methods, the updated object.

gsvaEnrichment

GSVA enrichment data and visualization

## **Description**

Extract and plot enrichment data from GSVA scores.

## Usage

```
## S4 method for signature 'gsvaRanksParam'
gsvaEnrichment(
  param,
  column = 1,
  geneSet = 1,
  plot = c("auto", "base", "ggplot", "no"),
  ...
)
```

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#### **Arguments**

A gsvaRanksParam object obtained with the method gsvaRanks. param column The column for which we want to retrieve the enrichment data. This parameter is only available in the gsvaEnrichment() method. geneSet Either a positive integer number between 1 and the number of available gene sets in param, or a character string with the name of one of the gene sets available in param. plot A character string indicating whether an enrichment plot should be produced using either base R graphics (plot="base") or the ggplot2 package (plot="ggplot"), or not (plot="no"). In the latter case, the enrichment data will be returned. By default plot="auto", which implies that if this method is called from an interactive session, a plot using base R graphics will be produced and, otherwise, the enrichment data is returned. Further arguments passed to the plot() function when the previous parameter

. . .

plot="base".

#### Value

When plot="no", this method returns the enrichment data. When plot="ggplot", this method returns a ggplot object. When plot="base" no value is returned.

## References

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

## **Examples**

```
library(GSVA)
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(gset1=paste0("g", 1:3),</pre>
                 gset2=paste0("g", 4:6),
                  gset3=paste0("g", 7:10))
## sample data from a normal distribution with mean 0 and st.dev. 1
y <- matrix(rnorm(n*p), nrow=p, ncol=n,</pre>
            dimnames=list(paste("g", 1:p, sep="") , paste("s", 1:n, sep="")))
## genes in set1 are expressed at higher levels in the last 'nGrp1+1' to 'n' samples
y[geneSets\$set1, (nGrp1+1):n] \leftarrow y[geneSets\$set1, (nGrp1+1):n] + 2
## build GSVA parameter object
gsvapar <- gsvaParam(y, geneSets)</pre>
## calculate GSVA ranks
gsvarankspar <- gsvaRanks(gsvapar)</pre>
gsvarankspar
```

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```
## by default the enrichment data for the first column and the first
## gene set are retrieved
gsvaEnrichment(gsvarankspar)
```

GsvaExprData-class

GsvaExprData class

## **Description**

Virtual superclass of expression data classes supported by GSVA.

#### **Details**

GSVA supports expression data matrices in a growing number of containers and representations. This class union allows to store any of these in a slot of another class as well as defining common methods for all of them.

## See Also

 $\verb|matrix|, \verb|dgCMatrix|, \verb|ExpressionSet|, \verb|SummarizedExperiment|, \verb|SingleCellExperiment|, \verb|SpatialExperiment|, \verb|SingleCellExperiment|, \verb|SpatialExperiment|, \verb|SingleCellExperiment|, \verb|Singl$ 

GsvaGeneSets-class

GsvaGeneSets class

# Description

Virtual superclass of gene set classes supported by GSVA.

## **Details**

GSVA supports gene sets in either a list of character vectors or an object of class GSEABase::GeneSetCollection. This class union allows to store any of these in a slot of another class as well as defining common methods for them.

## See Also

list, GeneSetCollection

GsvaMethodParam-class 15

GsvaMethodParam-class GsvaMethodParam class

#### **Description**

Virtual superclass of method parameter classes supported by GSVA.

A virtual superclass of the GSVA packages' method-specific parameter classes.

#### **Details**

GSVA implements four single-sample gene set analysis methods: PLAGE, combined z-scores, ss-GSEA, and GSVA. All of them take at least an expression data matrix and one or more gene sets as input. Further common parameters include an assay name for use with multi-assay expression data containers, the gene ID type used by the expression data set, and a minimum and maximum size for gene sets to limit the range of gene set sizes used in an analysis. This virtual class provides the necessary slots for this shared parameter set and serves as the parent class for all GSVA method parameter classes.

The GSVA package implements four single-sample gene set analysis methods (PLAGE, combined z-scores, ssGSEA, and GSVA) and a respective method-specific parameter class that is used to invoke each of them with a matching set of parameters.

#### **Slots**

- exprData The expression data set. Must be one of the classes supported by GsvaExprData. For a list of these classes, see its help page using help(GsvaExprData).
- geneSets The gene sets. Must be one of the classes supported by GsvaGeneSets. For a list of these classes, see its help page using help(GsvaGeneSets).
- assay Character vector of length 1. The name of the assay to use in case exprData is a multi-assay container, otherwise ignored. By default, the first assay is used.
- annotation An object of class GeneIdentifierType from package GSEABase describing the gene identifiers used as the row names of the expression data set. See GeneIdentifierType for help on available gene identifier types and how to construct them. This information can be used to map gene identifiers occurring in the gene sets. By default, this slot has value NullIdentifier and gene identifiers used in expression data set and gene sets are matched directly.
- minSize Numeric vector of length 1. Minimum size of the resulting gene sets after gene identifier mapping. By default, the minimum size is 1.
- maxSize Numeric vector of length 1. Maximum size of the resulting gene sets after gene identifier mapping. By default, the maximum size is Inf.

## See Also

 ${\tt GsvaExprData, GsvaGeneSets, zscoreParam, plageParam, ssgseaParam, gsvaParam, GeneIdentifierType} \\ {\tt GeneIdentifierType}$ 

plageParam, zscoreParam, ssgseaParam, gsvaParam

gsvaParam-class

gsvaParam class

## **Description**

S4 class for GSVA method parameter objects.

Objects of class gsvaParam contain the parameters for running the GSVA method.

## Usage

```
gsvaParam(
  exprData,
 geneSets,
  assay = NA_character_,
  annotation = NULL,
 minSize = 1,
 maxSize = Inf,
 kcdf = c("auto", "Gaussian", "Poisson", "none"),
 kcdfNoneMinSampleSize = 200,
  tau = 1,
 maxDiff = TRUE,
 absRanking = FALSE,
  sparse = TRUE,
 checkNA = c("auto", "yes", "no"),
  use = c("everything", "all.obs", "na.rm")
## S4 replacement method for signature 'gsvaRanksParam,GsvaGeneSets'
geneSets(object) <- value</pre>
```

## **Arguments**

assay

annotation

exprData The expression data set. Must be one of the classes supported by GsvaExprData.

For a list of these classes, see its help page using help(GsvaExprData).

geneSets The gene sets. Must be one of the classes supported by GsvaGeneSets. For a list of these classes, see its help page using help(GsvaGeneSets).

Character vector of length 1. The name of the assay to use in case exprData is

a multi-assay container, otherwise ignored. By default, the first assay is used.

An object of class GeneIdentifierType from package GSEABase describing the gene identifiers used as the row names of the expression data set. See GeneIdentifierType for help on available gene identifier types and how to construct them. This information can be used to map gene identifiers occurring in the gene sets.

If the default value NULL is provided, an attempt will be made to extract the gene identifier type from the expression data set provided as exprData (by calling gsvaAnnotation on it). If still not successful, the NullIdentifier() will be used as the gene identifier type, gene identifier mapping will be disabled and gene identifiers used in expression data set and gene sets can only be matched directly.

minSize

Numeric vector of length 1. Minimum size of the resulting gene sets after gene identifier mapping. By default, the minimum size is 1.

maxSize

Numeric vector of length 1. Maximum size of the resulting gene sets after gene identifier mapping. By default, the maximum size is Inf.

kcdf

Character vector of length 1 denoting the kernel to use during the non-parametric estimation of the empirical cumulative distribution function (ECDF) of expression levels across samples. The value kcdf="auto" will allow GSVA to automatically choose one of the possible values. The value kcdf="Gaussian" is suitable when input expression values are continuous, such as microarray fluorescent units in logarithmic scale, RNA-seq log-CPMs, log-RPKMs, or log-TPMs. When input expression values are integer counts, such as those derived from RNA-seq experiments, then this argument should be set to kcdf="Poisson". When we do not want to use a kernel approach for the estimation of the ECDF, then we should set kcdf="none".

#### kcdfNoneMinSampleSize

Integer vector of length 1. When kcdf="auto", this parameter decides at what minimum sample size kcdf="none", i.e., the estimation of the empirical cumulative distribution function (ECDF) of expression levels across samples is performed directly without using a kernel. By default, this value is set to 200; see the kcdf slot.

tau

Numeric vector of length 1. The exponent defining the weight of the tail in the random walk performed by the GSVA (Hänzelmann et al., 2013) method. The default value is 1 as described in the paper.

maxDiff

Logical vector of length 1 which offers two approaches to calculate the enrichment statistic (ES) from the KS random walk statistic.

- FALSE: ES is calculated as the maximum distance of the random walk from

   This approach produces a distribution of enrichment scores that is bimodal, but it can give large enrichment scores to gene sets whose genes are not concordantly activated in one direction only.
- TRUE (the default): ES is calculated as the magnitude difference between the largest positive and negative random walk deviations. This default value gives larger enrichment scores to gene sets whose genes are concordantly activated in one direction only.

absRanking

Logical vector of length 1 used only when maxDiff=TRUE. When absRanking=FALSE (default) a modified Kuiper statistic is used to calculate enrichment scores, taking the magnitude difference between the largest positive and negative random walk deviations. When absRanking=TRUE the original Kuiper statistic that sums the largest positive and negative random walk deviations is used.

sparse

Logical vector of length 1 used only when the input expression data in exprData is stored in a sparse matrix (e.g., a dgCMatrix or a SingleCellExperiment object storing the expression data in a dgCMatrix). In such a case, when sparse=TRUE (default), a sparse version of the GSVA algorithm will be applied. Otherwise, when sparse=FALSE, the classical version of the GSVA algorithm will be used.

checkNA

Character vector of length 1 specifying whether the input expression data should be checked for the presence of missing (NA) values. This must be one of the strings "auto" (default), "yes", or "no". The default value "auto" means that the software will perform that check only when the input expression data is provided as a base matrix, an ExpressionSet or a SummarizedExperiment object, while every other type of input expression data container (e.g., SingleCellExperiment, etc.) will not be checked. If checkNA="yes", then the input expression data will

be checked for missing values irrespective of the object class of the data container, and if checkNA="no", then that check will not be performed.

use

Character vector of length 1 specifying a policy for dealing with missing values (NAs) in the input expression data argument exprData. It only applies when either checkNA="yes", or checkNA="auto" (see the checkNA parameter. The argument value must be one of the strings "everything" (default), "all.obs", or "na.rm". The policy of the default value "everything" consists of propagating NAs so that the resulting enrichment score will be NA, whenever one or more of its contributing values is NA, giving a warning when that happens. When use="all.obs", the presence of NAs in the input expression data will produce an error. Finally, when use="na.rm", NA values in the input expression data will be removed from calculations, giving a warning when that happens, and giving an error if no values are left after removing the NA values.

object For the replacement method, an object of class gsvaRanksParam.

value For the replacement method, an object of the classes supported by GsvaGeneSets.

#### **Details**

In addition to the common parameter slots inherited from [GsvaMethodParam], this class has slots for the six method-specific parameters of the GSVA method described below.

In addition to a number of parameters shared with all methods implemented by package GSVA, GSVA takes six method-specific parameters. All of these parameters are described in detail below.

#### Value

A new gsvaParam object.

## **Slots**

kcdf Character vector of length 1 denoting the kernel to use during the non-parametric estimation of the empirical cumulative distribution function (ECDF) of expression levels across samples. The value kcdf="auto" will allow GSVA to automatically choose one of the possible values. The value kcdf="Gaussian" is suitable when input expression values are continuous, such as microarray fluorescent units in logarithmic scale, RNA-seq log-CPMs, log-RPKMs, or log-TPMs. When input expression values are integer counts, such as those derived from RNA-seq experiments, then this argument should be set to kcdf="Poisson". When we do not want to use a kernel approach for the estimation of the ECDF, then we should set kcdf="none".

kcdfNoneMinSampleSize Integer vector of length 1. When kcdf="auto", this parameter decides at what minimum sample size kcdf="none", i.e., the estimation of the empirical cumulative distribution function (ECDF) of expression levels across samples is performed directly without using a kernel; see the kcdf slot.

tau Numeric vector of length 1. The exponent defining the weight of the tail in the random walk performed by the GSVA (Hänzelmann et al., 2013) method.

maxDiff Logical vector of length 1 which offers two approaches to calculate the enrichment statistic (ES) from the KS random walk statistic.

- FALSE: ES is calculated as the maximum distance of the random walk from 0.
- TRUE: ES is calculated as the magnitude difference between the largest positive and negative random walk deviations.

absRanking Logical vector of length 1 used only when maxDiff=TRUE. When absRanking=FALSE a modified Kuiper statistic is used to calculate enrichment scores, taking the magnitude difference between the largest positive and negative random walk deviations. When absRanking=TRUE the original Kuiper statistic that sums the largest positive and negative random walk deviations, is used. In this latter case, gene sets with genes enriched on either extreme (high or low) will be regarded as 'highly' activated.

- sparse Logical vector of length 1 used only when the input expression data in exprData is stored in a sparse matrix (e.g., a dgCMatrix or a container object, such as a SingleCellExperiment, storing the expression data in a dgCMatrix). In such a case, when sparse=TRUE, a sparse version of the GSVA algorithm will be applied. Otherwise, when sparse=FALSE, the classical version of the GSVA algorithm will be used.
- checkNA Character vector of length 1. One of the strings "auto" (default), "yes", or "no", which refer to whether the input expression data should be checked for the presence of missing (NA) values.
- didCheckNA Logical vector of length 1, indicating whether the input expression data was checked for the presence of missing (NA) values.
- anyNA Logical vector of length 1, indicating whether the input expression data contains missing (NA) values.
- use Character vector of length 1. One of the strings "everything" (default), "all.obs", or "na.rm", which refer to three different policies to apply in the presence of missing values in the input expression data; see ssgseaParam.

#### References

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

## See Also

GsvaExprData, GsvaGeneSets, GsvaMethodParam, plageParam, zscoreParam, ssgseaParam

## **Examples**

```
library(GSVA)
library(GSVAdata)

data(leukemia)
data(c2BroadSets)

## for simplicity, use only a subset of the sample data
ses <- leukemia_eset[1:1000, ]
gsc <- c2BroadSets[1:100]
gp1 <- gsvaParam(ses, gsc)
gp1</pre>
```

20 gsvaRanks

gsvaRanks	GSVA ranks and scores	
-----------	-----------------------	--

# Description

Calculate GSVA scores in two steps: (1) calculate GSVA ranks; and (2) calculate GSVA scores using the previously calculated ranks.

## Usage

```
## S4 method for signature 'gsvaParam'
gsvaRanks(param, verbose = TRUE, BPPARAM = SerialParam(progressbar = verbose))
## S4 method for signature 'gsvaRanksParam'
gsvaScores(param, verbose = TRUE, BPPARAM = SerialParam(progressbar = verbose))
```

## **Arguments**

param A parameter object of the gsvaRanksParam class.

verbose Gives information about each calculation step. Default: TRUE.

BPPARAM An object of class BiocParallelParam specifying parameters related to the

parallel execution of some of the tasks and calculations within this function.

## Value

In the case of the gsvaRanks() method, an object of class gsvaRanksParam.

In the case of the <code>gsvaScores()</code> method, a gene-set by sample matrix of GSVA enrichment scores stored in a container object of the same type as the input ranks data container. If the input was a base matrix or a <code>dgCMatrix</code> object, then the output will be a base matrix object with the gene sets employed in the calculations stored in an attribute called <code>geneSets</code>. If the input was an <code>ExpressionSet</code> object, then the output will be also an <code>ExpressionSet</code> object with the gene sets employed in the calculations stored in an attributed called <code>geneSets</code>. If the input was an object of one of the classes described in <code>GsvaExprData</code>, such as a <code>SingleCellExperiment</code>, then the output will be of the same class, where enrichment scores will be stored in an assay called es and the gene sets employed in the calculations will be stored in the rowData slot of the object under the column name <code>gs</code>.

# References

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

#### See Also

```
gsvaParam, gsvaRanksParam, gsva
```

# Examples

```
library(GSVA)

p <- 10 ## number of genes
n <- 30 ## number of samples</pre>
```

guessGeneIdType 21

```
nGrp1 <- 15 ## number of samples in group 1
nGrp2 <- n - nGrp1 ## number of samples in group 2
## consider three disjoint gene sets
geneSets <- list(gset1=paste0("g", 1:3),</pre>
                  gset2=paste0("g", 4:6),
                 gset3=paste0("g", 7:10))
## sample data from a normal distribution with mean 0 and st.dev. 1
y <- matrix(rnorm(n*p), nrow=p, ncol=n,</pre>
            dimnames=list(paste("g", 1:p, sep="") , paste("s", 1:n, sep="")))
## genes in set1 are expressed at higher levels in the last 'nGrp1+1' to 'n' samples
y[geneSets\$set1, (nGrp1+1):n] \leftarrow y[geneSets\$set1, (nGrp1+1):n] + 2
## build GSVA parameter object
gsvapar <- gsvaParam(y, geneSets)</pre>
## calculate GSVA ranks
gsvarankspar <- gsvaRanks(gsvapar)</pre>
gsvarankspar
## calculate GSVA scores
gsva_es <- gsvaScores(gsvarankspar)</pre>
gsva_es
## calculate now GSVA scores in a single step
gsva_es1 <- gsva(gsvapar)</pre>
## both approaches give the same result with the same input gene sets
all.equal(gsva_es1, gsva_es)
## however, results will be (obviously) different with different gene sets
geneSets2 <- list(gset1=paste0("g", 3:6),</pre>
                   gset2=paste0("g", c(1, 2, 7, 8)))
## note that there is no need to calculate the GSVA ranks again
geneSets(gsvarankspar) <- geneSets2</pre>
gsvaScores(gsvarankspar)
```

 ${\tt guessGeneIdType}$ 

Guess the gene identifier type from a list of character vectors

## **Description**

This function tries to derive the type of gene IDs used in a named list of character vectors provided as input.

# Usage

```
guessGeneIdType(geneIdsList)
```

## **Arguments**

geneIdsList A named list of character vectors like the ones returned by geneIds().

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#### **Details**

In order to make this function useful and keep it as simple as possible, we limit ourselves to the most common types of gene identifiers: "Gene IDs" consisting of digits only are considered ENTREZ IDs, anything starting with 'ENS' an ENSEMBL identifier and anything else a HuGO gene symbol.

#### Value

An object of a subclass of GeneIdentifierType derived from the input.

#### See Also

GeneIdentifierType

igsva

Gene Set Variation Analysis

# Description

Starts an interactive GSVA shiny web app.

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 igsva() function starts an interactive shiny web app that allows the user to configure the arguments of the gsva() function and runs it on the computer. Please see the manual page of the gsva() function for a description of the arguments and their default and alternative values.

The input data may be loaded from the users workspace or by selecting a CSV file for the expression data, and a GMT file for the gene sets data.

# Usage

igsva()

## Value

A gene-set by sample matrix of GSVA enrichment scores after pressing the button 'Save & Close'. This result can be also downloaded as a CSV file with the 'Download' button.

## Author(s)

J. Fernández and R. Castelo

#### 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

gsva()

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#### **Examples**

```
## Not run:
res <- igsva() ## this will open your browser with the GSVA shiny web app
## End(Not run)</pre>
```

plageParam-class

plageParam class

## **Description**

S4 class for PLAGE method parameter objects.

Objects of class plageParam contain the parameters for running the PLAGE method.

## Usage

```
plageParam(
  exprData,
  geneSets,
  assay = NA_character_,
  annotation = NULL,
  minSize = 1,
  maxSize = Inf
)
```

# **Arguments**

exprData The expression data set. Must be one of the classes supported by GsvaExprData.

For a list of these classes, see its help page using help(GsvaExprData).

list of these classes, see its help page using help(GsvaGeneSets).

assay Character vector of length 1. The name of the assay to use in case exprData is a multi-assay container, otherwise ignored. By default, the first assay is used.

annotation An object of class GeneIdentifierType from package GSEABase describing

the gene identifiers used as the row names of the expression data set. See GeneIdentifierType for help on available gene identifier types and how to construct them. This information can be used to map gene identifiers occurring

in the gene sets.

If the default value NULL is provided, an attempt will be made to extract the gene identifier type from the expression data set provided as exprData (by calling gsvaAnnotation on it). If still not successful, the NullIdentifier() will be used as the gene identifier type, gene identifier mapping will be disabled and gene identifiers used in expression data set and gene sets can only be matched

directly.

minSize Numeric vector of length 1. Minimum size of the resulting gene sets after gene

identifier mapping. By default, the minimum size is 1.

maxSize Numeric vector of length 1. Maximum size of the resulting gene sets after gene

identifier mapping. By default, the maximum size is Inf.

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#### **Details**

Since method PLAGE does not take any method-specific parameters, this class does not add any slots to the common slots inherited from GsvaMethodParam.

PLAGE takes a number of parameters shared with all methods implemented by package GSVA but does not take any method-specific parameters. These parameters are described in detail below.

## Value

A new plageParam object.

## References

Tomfohr, J. et al. Pathway level analysis of gene expression using singular value decomposition. *BMC Bioinformatics*, 6:225, 2005. DOI

#### See Also

GsvaExprData, GsvaGeneSets, GsvaMethodParam, zscoreParam, ssgseaParam, gsvaParam

## **Examples**

```
library(GSVA)
library(GSVAdata)

data(leukemia)
data(c2BroadSets)

## for simplicity, use only a subset of the sample data
ses <- leukemia_eset[1:1000, ]
gsc <- c2BroadSets[1:100]
pp1 <- plageParam(ses, gsc)
pp1</pre>
```

readGMT

Import Gene Sets from a GMT File

## **Description**

Imports a list of gene sets from a GMT (Gene Matrix Transposed) format file, offering a choice of ways to handle duplicated gene set names.

# Usage

```
readGMT(
   con,
   sep = "\t",
   geneIdType = "auto",
   collectionType = NullCollection(),
   valueType = c("GeneSetCollection", "list"),
   deduplUse = c("first", "drop", "union", "smallest", "largest"),
   ...
)
```

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## **Arguments**

con

A connection object or a non-empty character string of length 1 containing e.g. the filename or URL of a (possibly compressed) GMT file.

sep

The character string separating members of each gene set in the GMT file.

geneIdType

By default a character vector of length 1 with the special value "auto" or an object of a subclass of GeneIdentifierType. If set to "auto", the function will try to derive the gene ID type from argument geneIdsList using guessGeneIdType. Other values, including NULL, will be ignored with a warning and geneIdType=NullIdentifier() will be used instead. Depending on the value of argument valueType, the gene ID type of the resulting list or of all GeneSet objects in the resulting GeneSetCollection will be set to this value.

collectionType

Only used when valueType == "GeneSetCollection". See getGmt for more information.

valueType

A character vector of length 1 specifying the desired type of return value. It must be one of:

- GeneSetCollection (the default): a GeneSetCollection object as defined and described by package GSEABase.
- list: a named list of gene sets represented as character vectors of gene IDs. This format is much simpler and cannot store the metadata required for automatic mapping of gene IDs.

deduplUse

A character vector of length 1 specifying one of several methods to handle duplicated gene set names. Duplicated gene set names are explicitly forbidden by the GMT file format specification but can nevertheless be encountered in the wild. The available choices are:

- first (the default): drops all gene sets whose names are duplicated according to the base R function and retains only the first occurence of a gene set name.
- drop: removes *all* gene sets that have a duplicated name, including its first occurrence.
- union: replaces gene sets with duplicated names by a single gene set containing the union of all their gene IDs.
- smallest: drops gene sets with duplicated names and retains only the smallest of them, i.e. the one with the fewest gene IDs. If there are several smallest gene sets, the first will be selected.
- largest: drops gene sets with duplicated names and retains only the largest of them, i.e. the one with the most gene IDs. If there are several largest gene sets, the first will be selected.

Further arguments passed on to readLines()

#### Value

The gene sets imported from the GMT file, with duplicate gene sets resolved according to argument deduplUse and in the format determined by argument valueType.

## See Also

readLines, GeneSetCollection, getGmt

26 spatCor

#### **Examples**

spatCor

Compute Spatial Autocorrelation for SpatialExperiment objects

## **Description**

Computes spatial autocorrelation using Moran's I statistic for a SpatialExperiment object, using an inverse squared distance weight matrix as default, or an inverse distance weight matrix as an alternative. It also tests for spatial autocorrelation assuming normality.

# Usage

```
## S4 method for signature 'SpatialExperiment'
spatCor(spe, na.rm = FALSE, alternative = "two.sided", squared = TRUE)
```

# **Arguments**

spe An object of SpatialExperiment class.

na.rm A logical indicating whether missing values should be removed.

alternative A character string specifying the alternative hypothesis tested against the null

hypothesis of no spatial autocorrelation; must be one of "two.sided", "less", or

"greater", or any unambiguous abbreviation of these.

squared A logical indicating whether the inverse distance weight matrix should be squared

or not.

#### Value

A data frame with the same row names as the original SpatialExperiment object. Columns include the observed Moran's I statistic, the expected Moran's I statistic under no spatial autocorrelation, the expected standard deviation under no spatial autocorrelation, and the p-value of the test.

ssgseaParam-class 27

ssgseaParam-class ssgseaParam class

## **Description**

S4 class for ssGSEA method parameter objects.

Objects of class ssgseaParam contain the parameters for running the ssGSEA method.

## Usage

```
## S4 method for signature 'gsvaParam'
anyNA(x, recursive = FALSE)
ssgseaParam(
  exprData,
 geneSets,
 assay = NA_character_,
  annotation = NULL,
 minSize = 1,
 maxSize = Inf,
 alpha = 0.25,
 normalize = TRUE,
 checkNA = c("auto", "yes", "no"),
  use = c("everything", "all.obs", "na.rm")
)
## S4 method for signature 'ssgseaParam'
anyNA(x, recursive = FALSE)
```

## Arguments

x An object of class ssgseaParam.

recursive Not used with x being an object of class ssgseaParam.

exprData The expression data set. Must be one of the classes supported by GsvaExprData.

For a list of these classes, see its help page using help(GsvaExprData).

list of these classes, see its help page using help(GsvaGeneSets).

assay Character vector of length 1. The name of the assay to use in case exprData is

a multi-assay container, otherwise ignored. By default, the first assay is used.

annotation An object of class GeneIdentifierType from package GSEABase describing the gene identifiers used as the row names of the expression data set. See

GeneIdentifierType for help on available gene identifier types and how to construct them. This information can be used to map gene identifiers occurring

in the gene sets.

If the default value NULL is provided, an attempt will be made to extract the gene identifier type from the expression data set provided as exprData (by calling gsvaAnnotation on it). If still not successful, the NullIdentifier() will be used as the gene identifier type, gene identifier mapping will be disabled and gene identifiers used in expression data set and gene sets can only be matched directly.

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minSize Numeric vector of length 1. Minimum size of the resulting gene sets after gene

identifier mapping. By default, the minimum size is 1.

Numeric vector of length 1. Maximum size of the resulting gene sets after gene maxSize

identifier mapping. By default, the maximum size is Inf.

Numeric vector of length 1. The exponent defining the weight of the tail in the alpha

random walk performed by the ssGSEA (Barbie et al., 2009) method. The default

value is 0.25 as described in the paper.

normalize Logical vector of length 1; if TRUE 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. Otherwise this final normalization

step is skipped.

checkNA Character vector of length 1 specifying whether the input expression data should

be checked for the presence of missing (NA) values. This must be one of the strings "auto" (default), "yes", or "no". The default value "auto" means that the software will perform that check only when the input expression data is provided as a base matrix, an ExpressionSet or a SummarizedExperiment object, while every other type of input expression data container (e.g., SingleCellExperiment,

etc.) will not be checked. If checkNA="yes", then the input expression data will be checked for missing values irrespective of the object class of the data con-

tainer, and if checkNA="no", then that check will not be performed.

Character vector of length 1 specifying a policy for dealing with missing values (NAs) in the input expression data argument exprData. It only applies when

either checkNA="yes", or checkNA="auto" (see the checkNA parameter. The argument value must be one of the strings "everything" (default), "all.obs", or "na.rm". The policy of the default value "everything" consists of propagating NAs so that the resulting enrichment score will be NA, whenever one or more of its contributing values is NA, giving a warning when that happens. When use="all.obs", the presence of NAs in the input expression data will produce an error. Finally, when use="na.rm", NA values in the input expression data will be removed from calculations, giving a warning when that happens, and giving

an error if no values are left after removing the NA values.

#### **Details**

In addition to the common parameter slots inherited from [GsvaMethodParam], this class has slots for the two method-specific parameters of the ssGSEA method described below as well as four more slots for implementing a missing value policy.

In addition to a number of parameters shared with all methods implemented by package GSVA, ssGSEA takes two method-specific parameters as well as two more parameters for implementing a missing value policy. All of these parameters are described in detail below.

## Value

A new ssgseaParam object.

## **Slots**

alpha Numeric vector of length 1. The exponent defining the weight of the tail in the random walk performed by the ssGSEA (Barbie et al., 2009) method.

normalize Logical vector of length 1. If TRUE 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. Otherwise this final normalization step is skipped.

use

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checkNA Character vector of length 1. One of the strings "auto" (default), "yes", or "no", which refer to whether the input expression data should be checked for the presence of missing (NA) values.

didCheckNA Logical vector of length 1, indicating whether the input expression data was checked for the presence of missing (NA) values.

anyNA Logical vector of length 1, indicating whether the input expression data contains missing (NA) values.

use Character vector of length 1. One of the strings "everything" (default), "all.obs", or "na.rm", which refer to three different policies to apply in the presence of missing values in the input expression data; see ssgseaParam.

#### References

Barbie, D.A. et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature*, 462(5):108-112, 2009. DOI

## See Also

GsvaExprData, GsvaGeneSets, GsvaMethodParam, plageParam, zscoreParam, gsvaParam

## **Examples**

```
library(GSVA)
library(GSVAdata)

data(leukemia)
data(c2BroadSets)

## for simplicity, use only a subset of the sample data
ses <- leukemia_eset[1:1000, ]
gsc <- c2BroadSets[1:100]
sp1 <- ssgseaParam(ses, gsc)
sp1</pre>
```

zscoreParam-class

zscoreParam class

## **Description**

S4 class for combined z-scores method parameter objects.

Objects of class zscoreParam contain the parameters for running the combined z-scores method.

## Usage

```
zscoreParam(
  exprData,
  geneSets,
  assay = NA_character_,
  annotation = NULL,
  minSize = 1,
  maxSize = Inf
)
```

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## **Arguments**

exprData The expression data set. Must be one of the classes supported by GsvaExprData. For a list of these classes, see its help page using help(GsvaExprData). The gene sets. Must be one of the classes supported by GsvaGeneSets. For a geneSets list of these classes, see its help page using help(GsvaGeneSets). Character vector of length 1. The name of the assay to use in case exprData is assay a multi-assay container, otherwise ignored. By default, the first assay is used. annotation An object of class GeneIdentifierType from package GSEABase describing the gene identifiers used as the row names of the expression data set. See GeneIdentifierType for help on available gene identifier types and how to construct them. This information can be used to map gene identifiers occurring in the gene sets. If the default value NULL is provided, an attempt will be made to extract the gene identifier type from the expression data set provided as exprData (by calling gsvaAnnotation on it). If still not successful, the NullIdentifier() will be used as the gene identifier type, gene identifier mapping will be disabled and gene identifiers used in expression data set and gene sets can only be matched directly. minSize Numeric vector of length 1. Minimum size of the resulting gene sets after gene identifier mapping. By default, the minimum size is 1.

# Details

maxSize

Since the combined z-scores method does not take any method-specific parameters, this class does not add any slots to the common slots inherited from GsvaMethodParam.

identifier mapping. By default, the maximum size is Inf.

Numeric vector of length 1. Maximum size of the resulting gene sets after gene

The combined z-scores method takes a number of parameters shared with all methods implemented by package GSVA but does not take any method-specific parameters. These parameters are described in detail below.

## Value

A new zscoreParam object.

#### References

Lee, E. et al. Inferring pathway activity toward precise disease classification. *PLoS Comp Biol*, 4(11):e1000217, 2008. DOI

## See Also

GsvaExprData, GsvaGeneSets, GsvaMethodParam, plageParam, ssgseaParam, gsvaParam

## **Examples**

library(GSVA)
library(GSVAdata)

data(leukemia)
data(c2BroadSets)

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```
## for simplicity, use only a subset of the sample data
ses <- leukemia_eset[1:1000, ]
gsc <- c2BroadSets[1:100]
zp1 <- zscoreParam(ses, gsc)
zp1</pre>
```

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