

Package ‘gage’

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Type Package

Title Generally Applicable Gene-set Enrichment for Pathway Analysis

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Description GAGE is a published method for gene set (enrichment or GSEA) or pathway analysis. GAGE is generally applicable independent of microarray or RNA-Seq data attributes including sample sizes, experimental designs, assay platforms, and other types of heterogeneity, and consistently achieves superior performance over other frequently used methods. In gage package, we provide functions for basic GAGE analysis, result processing and presentation. We have also built pipeline routines for of multiple GAGE analyses in a batch, comparison between parallel analyses, and combined analysis of heterogeneous data from different sources/studies. In addition, we provide demo microarray data and commonly used gene set data based on KEGG pathways and GO terms. These functions and data are also useful for gene set analysis using other methods.

biocViews Pathways, GO, DifferentialExpression, Microarray, OneChannel, TwoChannel, RNASeq, Genetics, MultipleComparison, GeneSetEnrichment, GeneExpression, SystemsBiology, Sequencing

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Imports graph, KEGGREST, AnnotationDbi, GO.db

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License GPL (>=2.0)

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<http://www.biomedcentral.com/1471-2105/10/161>

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gageComp.R gage-internal.R gagePipe.R gagePrep.R gage.R
gageSum.R geneData.R go.gsets.R greenred.R gs.heatmap.R
gs.KSTest.R gs.tTest.R gs.zTest.R heatmap2.R heter.gage.R

invalid.R kegg.gsets.R kegg.species.code.R odd.R pairData.R
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eg2sym	<i>Conversion between Entrez Gene IDs and official gene symbols for human genes.</i>
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Description

These functions convert Entrez Gene IDs to official gene symbols for human genes, or vise versa.

Usage

eg2sym(eg)
sym2eg(sym)

Arguments

eg character vector for Entrez Gene IDs (human genes only).
sym character vector for official gene symbols (human genes only).

Details

Currently, only conversion for human genes are supported. Notice that some gene symbols are not official, hence not recognized and NA will be returned in such cases.

Value

A character vector giving the converted official gene symbols or Entrez IDs.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[egSymb](#) mapping data between Entrez Gene IDs and official symbols; [readList](#) read in gene set list

Examples

```
#genes in gse16873 was label by Entrez IDs
data(gse16873)
head(rownames(gse16873))
#may convert the gene IDs to official symbols
gse16873.sym<-gse16873
data(egSymb)
rownames(gse16873.sym)<-eg2sym(rownames(gse16873.sym))
head(rownames(gse16873.sym))

#convert kegg.gs correspondingly
data(kegg.gs)
kegg.gs.sym<-lapply(kegg.gs, eg2sym)
lapply(kegg.gs.sym[1:3],head)
#GAGE analysis with the converted data
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
gse16873.kegg.p2 <- gage(gse16873.sym, gsets = kegg.gs.sym,
  ref = hn, samp = dcis)
```

egSymb

*Mapping between Entrez Gene IDs and official symbols***Description**

A two column matrix listing the Entrez IDs and official symbols for all currently known human genes.

Usage

```
data(egSymb)
```

Format

The format is: chr [1:40784, 1:2] "1" "10" "100" "1000" ... - attr(*, "dimnames")=List of 2 ..\$: NULL ..\$: chr [1:2] "eg" "sym"

Details

This mapping matrix is commonly used together with functions `eg2sym` and `sym2eg`. Check the examples below.

Source

This mapping data matrix were compiled using the gene data from NCBI Entrez Gene database.

Similar information can also be derived from Bioconductor package `org.Hs.eg.db`. Please check the package for more information.

References

Entrez Gene <URL: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>>

Examples

```
#genes in gse16873 was label by Entrez IDs
data(gse16873)
head(rownames(gse16873))
#may convert the gene IDs to official symbols
gse16873.sym<-gse16873
data(egSymb)
rownames(gse16873.sym)<-eg2sym(rownames(gse16873.sym))
head(rownames(gse16873.sym))

#convert kegg.gs correspondingly
data(kegg.gs)
kegg.gs.sym<-lapply(kegg.gs, eg2sym)
lapply(kegg.gs.sym[1:3],head)
#GAGE analysis with the converted data
cn=colnames(gse16873)
```

```
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
gse16873.kegg.p2 <- gage(gse16873.sym, gsets = kegg.gs.sym,
  ref = hn, samp = dcis)
```

eset.grp

The non-redundant significant gene set list

Description

This function extract a non-redundant significant gene set list, groups of redundant gene sets, and related data from gage results. Redundant gene sets are those overlap heavily in their effective member gene lists or core genes.

Usage

```
eset.grp(setp, exprs, gsets, ref = NULL, samp = NULL, test4up = TRUE,
  same.dir = TRUE, compare = "paired", use.fold = TRUE, cutoff = 0.01,
  use.q = FALSE, pc = 10^-10, output = TRUE, outname = "eset.grp",
  make.plot = FALSE, pdf.size = c(7, 7), core.counts = FALSE, get.esets =
  TRUE, bins = 10, bsize = 1, cex = 0.5, layoutType = "circo", name.str =
  c(10, 100), ...)
```

Arguments

setp	a numeric matrix, the result p-value matrix returned by gage function. Check gage help information for details.
exprs	an expression matrix or matrix-like data structure, with genes as rows and samples as columns.
gsets	a named list, each element contains a gene set that is a character vector of gene IDs or symbols. For example, type head(kegg.gs). A gene set can also be a "smc" object defined in PGSEA package. Make sure that the same gene ID system is used for both gsets and exprs.
ref	a numeric vector of column numbers for the reference condition or phenotype (i.e. the control group) in the exprs data matrix. Default ref = NULL, all columns are considered as target experiments.
samp	a numeric vector of column numbers for the target condition or phenotype (i.e. the experiment group) in the exprs data matrix. Default samp = NULL, all columns other than ref are considered as target experiments.
test4up	boolean, whether the input gage result or significant gene sets are test results for up-regulated gene sets or not. This information is needed for selecting core member genes which contribute to the overall significance of a gene sets.
same.dir	boolean, whether the input gage result test for changes in a gene set toward a single direction (all genes up or down regulated) or changes towards both directions simultaneously.

<code>compare</code>	character, which comparison scheme to be used: 'paired', 'unpaired', 'lon-group', 'as.group'. 'paired' is the default, ref and samp are of equal length and one-on-one paired by the original experimental design; 'as.group', group-on-group comparison between ref and samp; 'unpaired' (used to be 'lon1'), one-on-one comparison between all possible ref and samp combinations, although the original experimental design may not be one-on-one paired; 'lon-group', comparison between one samp column at a time vs the average of all ref columns.
<code>use.fold</code>	Boolean, whether the input gage results used fold changes or t-test statistics as per gene statistics. Default <code>use.fold= TRUE</code> .
<code>cutoff</code>	numeric, p- or q-value cutoff, between 0 and 1. Default 0.01 (for p-value). When q-value is used, recommended cutoff value is 0.1.
<code>use.q</code>	boolean, whether to use q-value or not as the pre-selection of a significant gene set list. Default to be FALSE, i.e. use the p-value instead.
<code>pc</code>	numeric, cutoff p-value for the overlap between gene sets to be called 'redundant', default to $10e-10$, may need trial-and-error to find the best value.
<code>output</code>	boolean, whether output the non-redundant gene set list as tab-delimited text file? Default to be TRUE.
<code>outname</code>	character, the prefix used to label the output file names when <code>output = TRUE</code> .
<code>make.plot</code>	boolean, whether to generate the network graph to visualize the redundancy (overlap in core genes) between significant gene sets. Currently the only feasible option is FALSE.
<code>pdf.size</code>	numeric vector of length 2, specifies the PDF file size for network graph output. Currently unsupported.
<code>core.counts</code>	Currently unsupported.
<code>get.esets</code>	Currently unsupported.
<code>bins</code>	Currently unsupported.
<code>bsize</code>	Currently unsupported.
<code>cex</code>	Currently unsupported.
<code>layoutType</code>	Currently unsupported.
<code>name.str</code>	numeric vector of length 2, specifies the substring range of the gene set name to show in the network graph. Currently unsupported.
<code>...</code>	extra arguments to be passed into internal function <code>make.graph</code> . Currently unsupported.

Details

Redundant gene sets are defined to be those overlap heavily in their effective member gene lists or core genes. Core genes are those member genes that really contribute to the significance of the gene set in GAGE analysis in the interesting direction(s). Argument `pc` set the cutoff for the overlap to be called "redundant". The redundancy between gene sets is then represented by a undirected graph/network. Groups of redundant gene sets are then derived as the connected component in the network graph.

The selection criterion for gene sets here is p-value, instead of the commonly used q-value. This is because for extracting a non-redundant list of significant gene sets, p-value is relative stable, but q-value changes when the total number of gene sets being considered changes. Of course, q-value is also a sensible selection criterion, when one take this step as a further refinement on the list of significant gene sets.

Value

The value returned by pairData is a list of 7 elements:

essentialSets	character vector, the non-redundant significant gene set list.
setGroups	list, each element is a character vector of a group of redundant gene sets.
allSets	character vector, the full list of significant gene sets.
setGroups	list, each element is a character vector of a connected component in the redundancy graph representation of the gene set.
overlapCounts	numeric matrix, the overlap core gene counts between the significant gene sets.
overlapPvals	numeric matrix, the significance (in p-values) of the overlap core gene counts between the significant gene sets.
coreGeneSets	list, each element is a character vector of the core genes in a significant gene set.

Author(s)

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References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[gage](#) the main function for GAGE analysis; [sigGeneSet](#) significant gene set from GAGE analysis; [essGene](#) essential member genes in a gene set;

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
data(kegg.gs)

#kegg test for 1-directional changes
gse16873.kegg.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis)
#kegg test for 2-directional changes
gse16873.kegg.2d.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis, same.dir = FALSE)
gse16873.kegg.esg.up <- eset.grp(gse16873.kegg.p$greater,
```

```

gse16873, gsets = kegg.gs, ref = hn, samp = dcis,
test4up = TRUE, output = TRUE, outname = "gse16873.kegg.up", make.plot = FALSE)
gse16873.kegg.esg.dn <- esset.grp(gse16873.kegg.p$less,
gse16873, gsets = kegg.gs, ref = hn, samp = dcis,
test4up = FALSE, output = TRUE, outname = "gse16873.kegg.dn", make.plot = FALSE)
gse16873.kegg.esg.2d <- esset.grp(gse16873.kegg.2d.p$greater,
gse16873, gsets = kegg.gs, ref = hn, samp = dcis,
test4up = TRUE, output = TRUE, outname = "gse16873.kegg.2d", make.plot = FALSE)
names(gse16873.kegg.esg.up)
head(gse16873.kegg.esg.up$essentialSets, 4)
head(gse16873.kegg.esg.up$setGroups, 4)
head(gse16873.kegg.esg.up$coreGeneSets, 4)

```

essGene

Essential member genes in a gene set

Description

This function extracts data for essential member genes in a gene set. Essential genes are genes that have changes over noise level.

Usage

```

essGene(gs, exprs, ref = NULL, samp = NULL, gsets = NULL, compare
= "paired", use.fold = TRUE, rank.abs = FALSE, use.chi = FALSE, chi.p =
0.05, ...)

```

Arguments

gs	character, either the name of an interesting gene set in a gene set collection passed by gsets argument, or a vector of gene IDs. Make sure that the same gene ID system is used for both gs and exprs.
exprs	an expression matrix or matrix-like data structure, with genes as rows and samples as columns.
ref	a numeric vector of column numbers for the reference condition or phenotype (i.e. the control group) in the exprs data matrix. Default ref = NULL, all columns are considered as target experiments.
samp	a numeric vector of column numbers for the target condition or phenotype (i.e. the experiment group) in the exprs data matrix. Default samp = NULL, all columns other than ref are considered as target experiments.
gsets	a named list, each element contains a gene set that is a character vector of gene IDs or symbols. For example, type head(kegg.gs). A gene set can also be a "smc" object defined in PGSEA package. Make sure that the same gene ID system is used for both gsets and exprs. Default to be NULL, then argument gs needs to be a vector of gene IDs.

<code>compare</code>	character, which comparison scheme to be used: 'paired', 'unpaired', 'lon-group', 'as.group'. 'paired' is the default, ref and samp are of equal length and one-on-one paired by the original experimental design; 'as.group', group-on-group comparison between ref and samp; 'unpaired' (used to be 'lon1'), one-on-one comparison between all possible ref and samp combinations, although the original experimental design may not be one-on-one paired; 'lon-group', comparison between one samp column at a time vs the average of all ref columns.
<code>use.fold</code>	Boolean, whether the input gage results used fold changes or t-test statistics as per gene statistics. Default use.fold= TRUE.
<code>rank.abs</code>	boolean, whether to sort the essential gene data based on absolute changes. Default to be FALSE.
<code>use.chi</code>	boolean, whether to use chi-square test to select the essential genes. Default to be FALSE, use the mean plus standard deviation of all gene changes instead. Check details for more information.
<code>chi.p</code>	numeric value between 0 and 1, cutoff p-value for the chi-square test to select the essential genes. Default to 0.05.
<code>...</code>	other arguments to be passed into the inside gagePrep function.

Details

There are two different criteria for essential gene selection. One uses a chi-square test to determine whether the change of a gene is more than noise. A second considers any changes beyond 1 standard deviation from mean of all genes as real.

Note that essential genes are different from core genes considered in `eset.grp` function. Essential genes may change in a different direction than the overall change of a gene set. But core genes need to change in the interesting direction(s) of the gene set test.

Value

A expression data matrix extracted for the essential genes, with similar structure as `exprs`.

Author(s)

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References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[gage](#) the main function for GAGE analysis; [geneData](#) output and visualization of expression data for selected genes; [eset.grp](#) non-redundant significant gene set list;

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)

#kegg test for 1-directional changes
data(kegg.gs)
gse16873.kegg.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis)
rownames(gse16873.kegg.p$greater)[1:3]
gs=unique(unlist(kegg.gs[rownames(gse16873.kegg.p$greater)[1:3]]))
essData=essGene(gs, gse16873, ref =hn, samp =dcis)
head(essData)
ref1=1:6
samp1=7:12
#generated text file for data table, pdf files for heatmap and scatterplot
for (gs in rownames(gse16873.kegg.p$greater)[1:3]) {
  outname = gsub(" |:|/", "_", substr(gs, 10, 100))
  geneData(genes = kegg.gs[[gs]], exprs = essData, ref = ref1,
    samp = samp1, outname = outname, txt = TRUE, heatmap = TRUE,
    Colv = FALSE, Rowv = FALSE, dendrogram = "none", limit = 3, scatterplot = TRUE)
}
```

gage

GAGE (Generally Applicable Gene-set Enrichment) analysis

Description

Run GAGE analysis to infer gene sets (or pathways, functional groups etc) that are significantly perturbed relative to all genes considered. GAGE is generally applicable to essentially all microarray data independent of data attributes including sample size, experimental layout, study design, and all types of heterogeneity in data generation.

gage is the main function; gagePrep is the functions for the initial data preparation, especially sample pairing; gageSum carries out the final meta-test summarization.

Usage

```
gage(exprs, gsets, ref = NULL, samp = NULL, set.size = c(10, 500),
  same.dir = TRUE, compare = "paired", rank.test = FALSE, use.fold = TRUE,
  FDR.adj = TRUE, weights = NULL, full.table = FALSE, saaPrep = gagePrep,
  saaTest = gs.tTest, saaSum = gageSum, use.stouffer=TRUE, ...)

gagePrep(exprs, ref = NULL, samp = NULL, same.dir = TRUE, compare =
  "paired", rank.test = FALSE, use.fold = TRUE, weights = NULL, full.table =
  FALSE, ...)

gageSum(rawRes, ref = NULL, test4up = TRUE, same.dir =
```

```
TRUE, compare = "paired", use.fold = TRUE, weights = NULL, full.table =
FALSE, use.stouffer=TRUE, ...)
```

Arguments

<code>exprs</code>	an expression matrix or matrix-like data structure, with genes as rows and samples as columns.
<code>gsets</code>	a named list, each element contains a gene set that is a character vector of gene IDs or symbols. For example, <code>type head(kegg.gs)</code> . A gene set can also be a "smc" object defined in PGSEA package. Please make sure that the same gene ID system is used for both <code>gsets</code> and <code>exprs</code> .
<code>ref</code>	a numeric vector of column numbers for the reference condition or phenotype (i.e. the control group) in the <code>exprs</code> data matrix. Default <code>ref = NULL</code> , all columns are considered as target experiments.
<code>samp</code>	a numeric vector of column numbers for the target condition or phenotype (i.e. the experiment group) in the <code>exprs</code> data matrix. Default <code>samp = NULL</code> , all columns other than <code>ref</code> are considered as target experiments.
<code>set.size</code>	gene set size (number of genes) range to be considered for enrichment test. Tests for too small or too big gene sets are not robust statistically or informative biologically. Default to be <code>set.size = c(10, 500)</code> .
<code>same.dir</code>	boolean, whether to test for changes in a gene set toward a single direction (all genes up or down regulated) or changes towards both directions simultaneously. For experimentally derived gene sets, GO term groups, etc, coregulation is commonly the case, hence <code>same.dir = TRUE</code> (default); In KEGG, BioCarta pathways, genes frequently are not coregulated, hence it could be informative to let <code>same.dir = FALSE</code> . Although <code>same.dir = TRUE</code> could also be interesting for pathways.
<code>compare</code>	character, which comparison scheme to be used: 'paired', 'unpaired', 'lon-group', 'as.group'. 'paired' is the default, <code>ref</code> and <code>samp</code> are of equal length and one-on-one paired by the original experimental design; 'as.group', group-on-group comparison between <code>ref</code> and <code>samp</code> ; 'unpaired' (used to be 'lon1'), one-on-one comparison between all possible <code>ref</code> and <code>samp</code> combinations, although the original experimental design may not be one-on-one paired; 'lon-group', comparison between one <code>samp</code> column at a time vs the average of all <code>ref</code> columns. For PAGE-like analysis, the default is <code>compare='as.group'</code> , which is the only option provided in the original PAGE method. All other comparison schemas are set here for direct comparison to <code>gage</code> .
<code>rank.test</code>	<code>rank.test</code> : Boolean, whether do the optional rank based two-sample t-test (equivalent to the non-parametric Wilcoxon Mann-Whitney test) instead of parametric two-sample t-test. Default <code>rank.test = FALSE</code> . This argument should be used with respect to argument <code>saaTest</code> .
<code>use.fold</code>	Boolean, whether to use fold changes or t-test statistics as per gene statistics. Default <code>use.fold= TRUE</code> .
<code>FDR.adj</code>	Boolean, whether to do adjust for multiple testing as to control FDR (False discovery rate). Default to be <code>TRUE</code> .

<code>weights</code>	a numeric vector to specify the weights assigned to pairs of ref-samp. This is needed for data with both technical replicates and biological replicates as to count for the different contributions from the two types of replicates. This argument is also useful in manually paring ref-samp for unpaired data, as in <code>pairData</code> function. Default to be NULL.
<code>full.table</code>	This option is obsolete. Boolean, whether to output the full table of all individual p-values from the pairwise comparisons of ref and samp. Default to be FALSE.
<code>saaPrep</code>	function used for data preparation for single array based analysis, including sanity check, sample pairing, per gene statistics calculation etc. Default to be <code>gagePrep</code> , i.e. the default data preparation routine for gage analysis.
<code>saaTest</code>	function used for gene set tests for single array based analysis. Default to be <code>gs.tTest</code> , which features a two-sample t-test for differential expression of gene sets. Other options includes: <code>gs.zTest</code> , using one-sample z-test as in PAGE, or <code>gs.KSTest</code> , using the non-parametric Kolmogorov-Smirnov tests as in GSEA. The two non-default options should only be used when <code>rank.test = FALSE</code> .
<code>saaSum</code>	function used for summarization of the results from single array analysis (i.e. pairwise comparison between ref and samp). This function should include a meta-test for a global p-value or summary statistis and a FDR adjustment for multi-testing issue. Default to be <code>gageSum</code> , i.e. the default data summarization routine for gage analysis.
<code>rawRes</code>	a named list, the raw results of gene set tests. Check the help information of gene set test functions like <code>gs.tTest</code> for details.
<code>test4up</code>	boolean, whether to summarize the p-value results for up-regulation test (p.results) or not (ps.results for down-regulation). This argument is only needed when the argument <code>same.dir=TRUE</code> in the main gage function, i.e. when test for one-directional changes.
<code>use.stouffer</code>	Boolean, whether to use Stouffer's method when summarizing individual p-values into a global p-value. Default to be TRUE. This default setting is recommended as to avoid the "dual significance", i.e. a gene set is significant for both up-regulation and down-regulation tests simultaneously. Dual significance occurs sometimes for data with large sample size, due to extremely small p-values in a few pair-wise comparison. More robust p-value summarization using Stouffer's method is a important new feature added to GAGE since version 2.2.0 (Bioconductor 2.8). This new argument is set as to provide a option to the original summarization based on Gamma distribution (FALSE).
<code>...</code>	other arguments to be passed into the optional functions for <code>saaPrep</code> , <code>saaTest</code> and <code>saaSum</code> .

Details

We proposed a single array analysis (i.e. the one-on-one comparison) approach with GAGE. Here we made single array analysis a general workflow for gene set analysis. Single array analysis has 4 major steps: Step 1 sample pairing, Step 2 per gene tests, Step 3 gene set tests and Step 4 meta-test summarization. Correspondingly, this new main function, `gage`, is divided into 3 relatively independent modules. Module 1 input preparation covers step 1-2 of single array analysis. Module 2 corresponds to step 3 gene set test, and module 3 to step 4 meta-test summarization. These 3

modules become 3 argument functions to `gage`, `saaPrep`, `saaTest` and `saaSum`. The modularization made `gage` open to customization or plug-in routines at each steps and fully realize the general applicability of single array analysis. More examples will be included in a second vignette to demonstrate the customization with these modules.

some important updates has been made to `gage` package since version 2.2.0 (Bioconductor 2.8): First, more robust p-value summarization using Stouffer's method through argument `use.stouffer=TRUE`. The original p-value summarization, i.e. negative log sum following a Gamma distribution as the Null hypothesis, may produce less stable global p-values for large or heterogenous datasets. In other words, the global p-value could be heavily affected by a small subset of extremely small individual p-values from pair-wise comparisons. Such sensitive global p-value leads to the "dual significance" phenomenon. Dual-significant means a gene set is called significant simultaneously in both 1-direction tests (up- and down-regulated). "Dual significance" could be informative in revealing the sub-types or sub-classes in big clinical or disease studies, but may not be desirable in other cases. Second, output of `gage` function now includes the gene set test statistics from pair-wise comparisons for all proper gene sets. The output is always a named list now, with either 3 elements ("greater", "less", "stats") for one-directional test or 2 elements ("greater", "stats") for two-directional test. Third, the individual p-value (and test statistics) from dependent pair-wise comparisons, i.e. comparisons between the same experiment vs different controls, are now summarized into a single value. In other words, the column number of individual p-values or statistics is always the same as the sample number in the experiment (or disease) group. This change made the argument value `compare="longroup"` and argument `full.table` less useful. It also became easier to check the perturbations at gene-set level for individual samples. Fourth, whole gene-set level changes (either p-values or statistics) can now be visualized using heatmaps due to the third change above. Correspondingly, functions `sigGeneSet` and `gagePipe` have been revised to plot heatmaps for whole gene sets.

Value

The result returned by `gage` function is a named list, with either 3 elements ("greater", "less", "stats") for one-directional test (`same.dir = TRUE`) or 2 elements ("greater", "stats") for two-directional test (`same.dir = FALSE`). Elements "greater" and "less" are two data matrices of the same structure, mainly the p-values, element "stats" contains the test statistics. Each data matrix here has gene sets as rows sorted by global p- or q-values. Test significance or statistics columns include:

<code>p.geomean</code>	geometric mean of the individual p-values from multiple single array based gene set tests
<code>stat.mean</code>	mean of the individual statistics from multiple single array based gene set tests. Normally, its absolute value measures the magnitude of gene-set level changes, and its sign indicates direction of the changes. When <code>saaTest=gs.KSTest</code> , <code>stat.mean</code> is always positive.
<code>p.val</code>	global p-value or summary of the individual p-values from multiple single array based gene set tests. This is the default p-value being used.
<code>q.val</code>	FDR q-value adjustment of the global p-value using the Benjamini & Hochberg procedure implemented in <code>multtest</code> package. This is the default q-value being used.
<code>set.size</code>	the effective gene set size, i.e. the number of genes included in the gene set test

other columns columns of the individual p-values or statistics, each measures the gene set perturbation in a single experiment (vs its control or all controls, depends on the "compare argument value)

The result returned by `gagePrep` is a data matrix derived from `exprs`, but ready for column-wise gene set tests. In the matrix, genes are rows, and columns are the per gene test statistics from the ref-samp pairwise comparison.

The result returned by `gageSum` is almost identical to the results of `gage` function, it is also a named list but has only 2 elements, "p.glob" and "results", with one round of test results.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

`gs.tTest`, `gs.zTest`, and `gs.KSTest` functions used for gene set test; `gagePipe` and `heter.gage` function used for multiple GAGE analysis in a batch or combined GAGE analysis on heterogeneous data

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
data(kegg.gs)
data(go.gs)

#kegg test for 1-directional changes
gse16873.kegg.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis)
#go.gs with the first 1000 entries as a fast example.
gse16873.go.p <- gage(gse16873, gsets = go.gs,
  ref = hn, samp = dcis)
str(gse16873.kegg.p)
head(gse16873.kegg.p$greater)
head(gse16873.kegg.p$less)
head(gse16873.kegg.p$stats)
#kegg test for 2-directional changes
gse16873.kegg.2d.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis, same.dir = FALSE)
head(gse16873.kegg.2d.p$greater)
head(gse16873.kegg.2d.p$stats)

###alternative ways to do GAGE analysis###
```

```
#with unpaired samples
gse16873.kegg.unpaired.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis, compare = "unpaired")

#other options to tweak includes:
#saaTest, use.fold, rank.test, etc. Check arguments section above for
#details and the vignette for more examples.
```

gage-internal

Internal functions

Description

Not intended to be called by the users.

Details

These functions are not to be called by the user directly.

Function heatmap2 is revised based on the heatmap.2 function in package gplot. Secondary functions colorpanel, greenred, invalid and odd are identical to the version in gplots. gs.heatmap is a wrapper function of heatmap2 to visualize whole gene set perturbations.

Function vennDiagram2 is a revised form of function vennDiagram from package limma. Associated function vennCounts is identical to the version in limma.

Function kegg.species.code is identical to the version in pathview.

Functions deComp and rownorm were written by Weijun Luo, the author of gage package.

gageComp

Compare multiple GAGE analyses results

Description

This function is used to compare the results after running multiple rounds of GAGE analysis. It is frequently used after batch analysis using gagePipe, but may also be used after multiple runs of gage manually.

Usage

```
gageComp(samprnames, dataname, gsname = c("kegg.gs", "go.gs"), use.cols =
  c("stat.mean", "q.val"), q.cutoff = 0.1, do.plot = TRUE)
```

Arguments

sampnames	character vector, the names of the sample groups, on which the GAGE analysis has been done and to be compared. This same argument is used in gagePipe function. These sampnames have been used to label gage result objects.
dataname	character, the name of the data on which the GAGE analysis has been done. This same argument is used in gagePipe function. This name has been included as the prefix of the GAGE analysis output file names, and will be used in the comparison output file names.
gsname	character, the name(s) of the gene set collection(s) to be considered in the comparison. In other words, this argument specifies GAGE analysis results with what type(s) of gene sets are to be compared on. Default to be <code>c("kegg.gs", "go.gs")</code> .
use.cols	character, what columns in the gage analysis result objects will be used in the comparison. Default to be "stat.mean" (mean of gene set test statistics) and "q.val" (q-value using BH procedure). Check help information for gage function for more details on the result columns.
q.cutoff	numeric, q-value cutoff between 0 and 1 for significant gene sets selection. Default to be 0.1. The same argument is used in gagePipe function.
do.plot	boolean, whether to plot the venn diagram for the comparison results. Default to be TRUE.

Details

gageComp works with the results of gagePipe run by default. Try to load the .RData file named after dataname first. If there is no such file, it assumes that the gage result objects have been loaded and exist in the global environment.

For the GAGE analysis results with each gene set collection specified in gsname, gagePipe compares the significant gene set lists between the sample groups specified in sampnames. For each gene set collection, three comparisons will be done, on the 2-direction perturbed, up-regulated, and down-regulated gene sets.

The comparison results are output as tab-delimited text files. Venn diagrams are only plotted for comparison between 2-3 parties. But the text file outputs are not limited by the number of parties under comparison. The venn diagram is generated by calling a revised function based on the VennDiagram function from the limma package.

Value

The function returns invisible 1 when successfully executed.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[gagePipe](#) pipeline for multiple GAGE analysis in a batch; [gage](#) the main function for GAGE analysis

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
data(kegg.gs)

library(gageData)
data(gse16873.2)
cn2=colnames(gse16873.2)
hn2=grep('HN',cn2, ignore.case =TRUE)
dcis2=grep('DCIS',cn2, ignore.case =TRUE)

#multiple GAGE analysis in a batch with the combined data
gse16873=cbind(gse16873, gse16873.2)
dataname='gse16873' #output data prefix
samppnames=c('dcis.1', 'dcis.2')
refList=list(hn, hn2+12)
samplList=list(dcis, dcis2+12)
gagePipe(gse16873, gsname = "kegg.gs", dataname = "gse16873",
         samppnames = samppnames, ref.list = refList, sampl.list = samplList,
         comp.list = "paired")

#follow up comparison between the analyses
load('gse16873.gage.RData')
#list gage result objects
objects(pat = "[.]p$")
gageComp(samppnames, dataname, gsname = "kegg.gs",
         do.plot = TRUE)
```

gagePipe

GAGE analysis pipeline

Description

Function gagePipe runs mutliple rounds of GAGE in a batch without interference, and outputs signcant gene set lists in text format, heatmaps in pdf format, and save the results in RData format.

Usage

```
gagePipe(arraydata, dataname = "arraydata", trim.at=TRUE, samppnames, gsdata = NULL,
         gsname = c("kegg.gs", "go.gs"), ref.list, sampl.list, weight.list = NULL,
         comp.list = "paired", q.cutoff = 0.1, heatmap=TRUE, pdf.size = c(7,
         7), p.limit=c(0.5, 5.5), stat.limit=5, ... )
```

Arguments

<code>arraydata</code>	corresponds to <code>exprs</code> argument for <code>gage</code> function. But can either be a matrix-like data structure when the data has been loaded into R or the full path to the data file in <code>.RData</code> format if the data has not been loaded.
<code>dataname</code>	character, the name of the data to be analyzed. This name will be included as the prefix of the output file names. Default to be "arraydata".
<code>trim.at</code>	boolean, whether to trim the suffix "_at" from the probe set IDs or row names of the microarray data. Default to be TRUE.
<code>sampnames</code>	character vector, the names of the sample groups, on which the GAGE analysis is done. Each sample groups corresponds to one element of <code>samp.list</code> and the matching element of <code>ref.list</code> . These names will be included in the output file names or object names.
<code>gsdata</code>	character, the full path to the gene set data file in <code>.RData</code> format if the data has not been loaded. Default to be NULL, i.e. the gene set data has been loaded. Make sure that the same gene ID system is used for both <code>gsdata</code> and <code>arraydata</code> .
<code>gsname</code>	character, the name(s) of the gene set collections to be used. Default to be <code>c("kegg.gs", "go.gs")</code> .
<code>ref.list</code>	a list of <code>ref</code> inputs for <code>gage</code> function. In other words, each element of the list is a column number vector for the reference condition or phenotype (i.e. the control group) in the <code>exprs</code> data matrix.
<code>samp.list</code>	a list of <code>samp</code> inputs for <code>gage</code> function. In other words, each element of the list is a column number vector for the target condition or phenotype (i.e. the experiment group) in the <code>exprs</code> data matrix.
<code>weight.list</code>	a list or a vector of weights input(s) for <code>gage</code> function. As a list, the length of <code>weight.list</code> should equal to the length of <code>ref.list</code> and <code>samp.list</code> or 1. The <code>weight.list</code> vector or its element vectors of should match the elements of <code>ref.list</code> and <code>samp.list</code> in length or being NULL. When <code>weight.list</code> is a vector or length 1 list, all analyses will use the same weights setting.
<code>comp.list</code>	a list or a vector of compare input(s) for <code>gage</code> function. The length of the list or vector should equal to the length of <code>ref.list</code> and <code>samp.list</code> or 1. In the latter case, all analyses will use the same comparison scheme. The same as <code>compare</code> , the element value(s) in <code>comp.list</code> can be 'paired', 'unpaired', 'longroup' or 'as.group'. Default to be 'paired'.
<code>q.cutoff</code>	numeric, q-value cutoff between 0 and 1 for significant gene sets selection. Default to be 0.1.
<code>heatmap</code>	boolean, whether to plot heatmap for the selected gene data as a PDF file. Default to be FALSE.
<code>pdf.size</code>	a numeric vector to specify the the width and height of PDF graphics region in inches. Default to be <code>c(7, 7)</code> .
<code>stat.limit</code>	numeric vector of length 1 or 2 to specify the value range of gene set statistics to visualize using the heatmap. Statistics beyong will be reset to equal the proximal limit. Default to 5, i.e. plot all gene set statistics within (-5, 5) range. May also be NULL, i.e. plot all statistics without limit. This argument allows optimal differentiation between most gene set statistic values when extremely positive/negative values exsit and squeeze the normal-value region.

p.limit	numeric vector of length 1 or 2 to specify the value range of gene set $-\log_{10}(\text{p-values})$ to visualize using the heatmap. Values beyond will be reset to equal the proximal limit. Default to <code>c(0.5,5.5)</code> , i.e. plot all $-\log_{10}(\text{p-values})$ within this range. This argument is similar to argument <code>stat.limit</code> .
...	other arguments to be passed into <code>gage</code> or <code>gs.heatmap</code> function, which is a wrapper of the <code>heatmap2</code> function.

Details

`gagePipe` carries two rounds of GAGE analysis on each sample groups for each gene set collection specified in `gsnames`: one test for 1-direction changes (up- or down-regulated gene sets), one test for 2-direction changes (two-way perturbed gene sets). Correspondingly, the `gage` result p-value matrices for the significant gene sets are written into two tab-delimited text files, named after the `dataname` and `samprnames`. Note that the text file for 1-direction changes tests combines results for both up- and down-regulated gene sets. By default, heatmaps in pdf format are also produced to show the gene set perturbations using either $-\log_{10}(\text{p-value})$ or statistics. Meanwhile, the full `gage` analysis result objects (named lists of p-value or statistics matrices) are saved into a .RData file. The result objects are named after the `samprnames` and `gsnames`.

Value

The function returns invisible 1 when successfully executed.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[gage](#) the main function for GAGE analysis; [heter.gage](#) GAGE analysis for heterogeneous data

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
data(kegg.gs)

library(gageData)
data(gse16873.2)
cn2=colnames(gse16873.2)
hn2=grep('HN',cn2, ignore.case =TRUE)
dcis2=grep('DCIS',cn2, ignore.case =TRUE)

#multiple GAGE analysis in a batch with the combined data
```

```

gse16873=cbind(gse16873, gse16873.2)
dataname='gse16873' #output data prefix
samprnames=c('dcis.1', 'dcis.2')
refList=list(hn, hn2+12)
samplList=list(dcis, dcis2+12)
gagePipe(gse16873, gsname = "kegg.gs", dataname = "gse16873",
         samprnames = samprnames, ref.list = refList, sampl.list = samplList,
         comp.list = "paired")

#follow up comparison between the analyses
load('gse16873.gage.RData')
#list gage result objects
objects(pat = "[.]p$")
gageComp(samprnames, dataname, gsname = "kegg.gs",
         do.plot = TRUE)

```

geneData

View the expression data for selected genes

Description

This function outputs and visualizes the expression data for selected genes. Potential output files include: a tab-delimited text file, a heatmap in PDF format, and a scatter plot in PDF format.

Usage

```

geneData(genes, exprs, ref = NULL, samp = NULL, outname = "array",
txt = TRUE, heatmap = FALSE, scatterplot = FALSE, samp.mean = FALSE,
pdf.size = c(7, 7), cols = NULL, scale = "row", limit = NULL,
label.groups = TRUE, ...)

```

Arguments

genes	character, either a vector of interesting genes IDs or a 2-column matrix, where the first column specifies gene IDs used in expData while the second column gives another type of IDs to use for the output data files.
exprs	an expression matrix or matrix-like data structure, with genes as rows and samples as columns.
ref	a numeric vector of column numbers for the reference condition or phenotype (i.e. the control group) in the exprs data matrix. Default ref = NULL, all columns are considered as target experiments.
samp	a numeric vector of column numbers for the target condition or phenotype (i.e. the experiment group) in the exprs data matrix. Default samp = NULL, all columns other than ref are considered as target experiments.
outname	a character string, to be used as the prefix of the output data files. Default to be "array".

txt	boolean, whether to output the selected gene data as a tab-delimited text file. Default to be TRUE.
heatmap	boolean, whether to plot heatmap for the selected gene data as a PDF file. Default to be FALSE.
scatterplot	boolean, whether to make scatter plot for the selected gene data as a PDF file. Default to be FALSE.
samp.mean	boolean, whether to take the mean of gene data over the ref and samp group when making the scatter plot. Default to be FALSE, i.e. make scatter plots for the first two ref-samp pairs and label them differently on the same graph panel.
pdf.size	a numeric vector to specify the the width and height of PDF graphics region in inches. Default to be c(7, 7).
cols	a character vector to specify colors used for the heatmap image blocks. Default to be NULL, i.e. to generate a green-red spectrum based on the gene data automatically.
scale	character indicating if the values should be centered and scaled in either the row direction or the column direction, or none for the heatmap. The default is "row", other options include "column" and "none".
limit	numeric value to specify the maximal absolute value of gene data to visualize using the heatmap. Gene data beyond will be reset to equal this value. Default to NULL, i.e. plot all gene data values. This argument allows optimal differentiation between most gene data values when extremely positive/negative values exist and squeeze the normal-value region. Recommend limit = 3 when the gene data is scaled by row.
label.groups	boolean, whether to label the two sample groups, i.e. ref and samp, differently using side color bars along the heatmap area. Default to be TRUE.
...	other arguments to be passed into the inside heatmap2 function.

Details

This function integrated three most common presentation methods for gene expression data: tab-delimited text file, heatmap and scatter plot. Heatmap is ideal for visualizing relative changes with gene-wise standardized (or row-scaled) data. The heatmap is generated by calling a improved version of the heatmap.2 function from gplots package. Scatter plot is ideal for visualizing the modest or small but consistent changes over a gene set between two states under comparison.

Although geneData is designed to be a standard-alone function, it is frequently used in tandem with essGene function to present the changes of the essential genes in significant gene sets.

Value

The function returns invisible 1 when successfully executed.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[essGene](#) extract the essential member genes in a gene set; [gage](#) the main function for GAGE analysis;

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)

#kegg test for 1-directional changes
data(kegg.gs)
gse16873.kegg.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis)
rownames(gse16873.kegg.p$greater)[1:3]
gs=unique(unlist(kegg.gs[rownames(gse16873.kegg.p$greater)[1:3]]))
essData=essGene(gs, gse16873, ref =hn, samp =dcis)
head(essData)
ref1=1:6
samp1=7:12
#generated text file for data table, pdf files for heatmap and scatterplot
for (gs in rownames(gse16873.kegg.p$greater)[1:3]) {
  outname = gsub(" |:|/", "_", substr(gs, 10, 100))
  geneData(genes = kegg.gs[[gs]], exprs = essData, ref = ref1,
    samp = samp1, outname = outname, txt = TRUE, heatmap = TRUE,
    Colv = FALSE, Rowv = FALSE, dendrogram = "none", limit = 3, scatterplot = TRUE)
}
```

go.gsets

Generate up-to-date GO (Gene Ontology) gene sets

Description

Generate up-to-date GO gene sets for specified species, which has either Bioconductor or user supplied gene annotation package.

Usage

```
go.gsets(species = "human", pkg.name=NULL, id.type = "eg", keep.evidence=FALSE)
```

Arguments

<code>species</code>	character, common name of the target species. Note that scientific name is not used here. Default <code>species="human"</code> . For a list of other species names, type in: <code>data(bods); bods</code>
<code>pkg.name</code>	character, the gene annotation package name for the target species. It is either one of the Bioconductor OrgDb packages or a user supplied package with similar data structures created using AnnotationForge package. Default <code>species=NULL</code> , the right annotation package will be located for the specified species in Bioconductor automatically. Otherwise, the user need to prepare and supply a usable annotation package in the same format.
<code>id.type</code>	character, target ID type for the get sets, case insensitive. Default <code>gene.idtype="eg"</code> , i.e. Entrez Gene. Entrez Gene is the primary GO gene ID for many common model organisms, like human, mouse, rat etc. For other species may use "orf" (open reading frame) and other specific gene IDs, please type in: <code>data(bods); bods</code> to check the details.
<code>keep.evidence</code>	boolean, whether to keep the GO evidence codes for genes assigned to each GO term. The evidence codes describe what type of evidence was present in that reference to make the annotation. Default <code>keep.evidence = FALSE</code> , which removes all the evidence codes and redundant gene entries due to multiple evidence codes in a gene set.

Details

The updated GO gene sets are derived using Bioconductor or user supplied gene annotation packages. This way, we can create gene set data for GO analysis for 19 common species annotated in Bioconductor and more others by the users. Note that we have generated GO gene set for 4 species, human, mouse, rat and yeast, and provided the data in package `gageData`.

Value

A named list with the following elements:

<code>go.sets</code>	GO gene sets, a named list. Each element is a character vector of member gene IDs for a single GO. The number of elements of this list is the total number of GO terms defined for the specified species.
<code>go.subs</code>	<code>go.subs</code> is a named list of three elements: "BP", "CC" and "MF", corresponding to biological process, cellular component and molecular function subtrees. It may be more desirable to conduct separated GO enrichment test on each of these 3 subtrees as shown in the example code.
<code>go.mains</code>	<code>go.mains</code> is a named vector of three elements: "BP", "CC" and "MF", corresponding to the root node of biological process, cellular component and molecular function subtree, i.e. their corresponding indices in <code>go.sets</code> .

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[go.gs](#) for precompiled GO and other common gene set data collection

Examples

```
#GAGE analysis use the updated GO definitions, instead of
#go.gs.
#the following lines are not run due to long excution time.
#The data generation steop may take 10-30 seconds. You may want
#save the gene set data, i.e. go.hs, for future uses.
#go.hs=go.gsets(species="human")
#data(gse16873)
#hn=(1:6)*2-1
#dcis=(1:6)*2
#go.bp=go.hs$go.sets[go.hs$go.subs$BP]
#gse16873.bp.p <- gage(gse16873, gsets = go.bp,
#                      ref = hn, samp = dcis)

#Yeast and few othr species gene Id is different from Entre Gene
data(bods)
bods
#not run
#go.sc=go.gsets("Yeast")
#lapply(go.sc$go.sets[1:3], head, 3)
```

gs.tTest

Gene set differential expression test

Description

These functions test for perturbation of gene sets relative to all genes in the microarray data. They are the testing module for gage and single array analysis workflow.

They use different statistical tests: gs.tTest uses two-sample t-test, gs.zTest uses one-sample z-test, gs.KSTest uses Kolmogorov-Smirnov test.

Usage

```
gs.tTest(exprs, gsets, set.size = c(10, 500), same.dir = TRUE, ...)
gs.zTest(exprs, gsets, set.size = c(10, 500), same.dir = TRUE, ...)
gs.KSTest(exprs, gsets, set.size = c(10, 500), same.dir = TRUE, ...)
```


Arguments

<code>exprs</code>	an expression matrix or matrix-like data structure, with genes as rows and samples as columns.
<code>gsets</code>	a named list, each element contains a gene set that is a character vector of gene IDs or symbols. For example, type <code>head(kegg.gs)</code> . A gene set can also be a "smc" object defined in PGSEA package. Make sure that the same gene ID system is used for both <code>gsets</code> and <code>exprs</code> .
<code>set.size</code>	gene set size (number of genes) range to be considered for enrichment test. Tests for too small or too big gene sets are not robust statistically or informative biologically. Default to be <code>set.size = c(10, 500)</code> .
<code>same.dir</code>	whether to test for changes in a gene set toward a single direction (all genes up or down regulated) or changes towards both directions simultaneously. For experimentally derived gene sets, GO term groups, etc, coregulation is commonly the case, hence <code>same.dir = TRUE</code> (default); In KEGG, BioCarta pathways, genes frequently are not coregulated, hence it could be informative to let <code>same.dir = FALSE</code> . Although <code>same.dir = TRUE</code> could also be interesting for pathways.
<code>...</code>	other arguments to be passed into the secondary functions, not used currently.

Details

These functions are the gene set test module for gage and single array analysis workflow. When used in gage function, the function names are optional values for `saaTest` argument. Check help information for gage for details.

These functions may also used independently without calling gage function.

Value

As the raw results of gene set tests, a list of 5 components is returned:

<code>results</code>	matrix of test statistics, gene sets are rows, samp-ref pairs are columns
<code>p.results</code>	matrix of p-values for up-regulation (greater than) tests, gene sets are rows, samp-ref pairs are columns
<code>ps.results</code>	matrix of p-values for down-regulation (less than) tests, gene sets are rows, samp-ref pairs are columns
<code>mstat</code>	vector of test statistics mean for individual gene sets. Normally, its absolute value measures the magnitude of gene-set level changes, and its sign indicates direction of the changes. For <code>gs.KSTest</code> , <code>mstat</code> is always positive.
<code>setsizes</code>	vector of effective set size (number of genes) individual gene sets

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[gage](#) the main function for GAGE analysis

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
data(kegg.gs)

#kegg test
exprs.gage = gagePrep(gse16873, ref = hn, samp = dcis)
str(exprs.gage)
rawRes = gs.tTest(exprs.gage, gsets = kegg.gs)
str(rawRes)
head(rawRes$results)
head(rawRes$p.results)
```

gse16873

GSE16873: a breast cancer microarray dataset

Description

GSE16873 is a breast cancer study (Emery et al, 2009) downloaded from Gene Expression Omnibus (GEO). GSE16873 covers twelve patient cases, each with HN (histologically normal), ADH (ductal hyperplasia), and DCIS (ductal carcinoma in situ) RMA samples. Due to the size limit of this package, we split this GSE16873 into two halves, each including 6 patients with their HN and DCIS but not ADH tissue types. This gage package only includes the first half dataset for 6 patients as this example dataset gse16873. Most of our demo analyses are done on the first half dataset, except for the advanced analysis where we use both halves datasets with all 12 patients. Details section below gives more information.

Usage

```
data(gse16873)
```

Details

Raw data for these two half datasets were processed separately using two different methods, FARMS and RMA, respectively to generate the non-biological data heterogeneity. The first half dataset is named as gse16873, the second half dataset named gse16873.2. We also have the full dataset, gse16873.full, which includes all HN, ADH and DCIS samples of all 12 patients, processed together using FARMS. The second half dataset plus the full dataset and the original BMP6 dataset used in GAGE paper and earlier versions of gage is supplied with another package, gageData.

Source

GEO Dataset GSE16873: <URL: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE16873>>

References

Emery LA, Tripathi A, King C, Kavanah M, Mendez J, Stone MD, de las Morenas A, Sebastiani P, Rosenberg CL: Early dysregulation of cell adhesion and extracellular matrix pathways in breast cancer progression. *Am J Pathol* 2009, 175:1292-302.

Examples

```
data(gse16873)
#check the heterogeneity of the two half datasets
boxplot(data.frame(gse16873))

#column/sample names
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
adh=grep('ADH',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
print(hn)
print(dcis)

data(kegg.gs)
lapply(kegg.gs[1:3],head)
head(rownames(gse16873))
gse16873.kegg.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis)
```

heter.gage

GAGE analysis for heterogeneous data

Description

heter.gage is a wrapper function of gage for heterogeneous data. pairData prepares the heterogeneous data and related arguments for GAGE analysis.

Usage

```
heter.gage(exprs, gsets, ref.list, samp.list, comp.list = "paired",
  use.fold = TRUE, ...)

pairData(exprs, ref.list, samp.list, comp.list = "paired", use.fold =
  TRUE, ...)
```

Arguments

exprs	an expression matrix or matrix-like data structure, with genes as rows and samples as columns.
gsets	a named list, each element contains a gene set that is a character vector of gene IDs or symbols. For example, type head(kegg.gs). A gene set can also be a "smc" object defined in PGSEA package. Make sure that the same gene ID system is used for both gsets and exprs.

<code>ref.list</code>	a list of ref inputs for gage function. In other words, each element of the list is a column number vector for the reference condition or phenotype (i.e. the control group) in the <code>exprs</code> data matrix.
<code>samp.list</code>	a list of samp inputs for gage function. In other words, each element of the list is a column number vector for the target condition or phenotype (i.e. the experiment group) in the <code>exprs</code> data matrix.
<code>comp.list</code>	a list or a vector of compare input(s) for gage function. The length of the list or vector should equal to the length of <code>ref.list</code> and <code>samp.list</code> or 1. In the latter case, all analyses will use the same comparison scheme. The same as <code>compare</code> , the element value(s) in <code>comp.list</code> can be 'paired', 'unpaired', 'longroup' or 'as.group'. Default to be 'paired'.
<code>use.fold</code>	Boolean, whether to use fold changes or t-test statistics as per gene statistics. Default <code>use.fold= TRUE</code> .
<code>...</code>	other arguments to be passed into gage.

Details

`comp.list` can be a list or vector of mixture values of 'paired' and 'unpaired' matching the experiment layouts of the heterogeneous data. In such cases, each ref-samp pairs and corresponding columns in the result data matrix after calling `pairData` are assigned different weights when calling `gage` in the next step. The inclusion of 'longroup' and 'as.group' in `comp.list` would make weight assignment very complicated especially when the sample sizes are different for the individual experiments of the heterogeneous data.

Value

The output of `pairData` is a list of 2 elements:

<code>exprs</code>	a data matrix derived from the input expression data matrix <code>exprs</code> , but ready for column-wise gene test. In the matrix, genes are rows, and columns are the per gene test statistics from the ref-samp pairwise comparison.
<code>weights</code>	weights assigned to columns of the output data matrix <code>exprs</code> when calling <code>gage</code> next. The value may be NULL if <code>comp.list</code> are all 'paired'.

The result returned by `heter.gage` function is the same as result of `gage`, i.e. either a single data matrix (`same.dir = FALSE`, test for two-directional changes) or a named list of two data matrix (`same.dir = TRUE`, test for single-direction changes) for the results of up- (`$greater`) and down- (`$less`) regulated gene sets. Check help information for `gage` for details.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[gage](#) the main function for GAGE analysis; [gagePipe](#) pipeline for multiple GAGE analysis in a batch

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
data(kegg.gs)

library(gageData)
data(gse16873.2)
cn2=colnames(gse16873.2)
hn2=grep('HN',cn2, ignore.case =TRUE)
dcis2=grep('DCIS',cn2, ignore.case =TRUE)

#combined the two half dataset
gse16873=cbind(gse16873, gse16873.2)
refList=list(hn, hn2+12)
samplList=list(dcis, dcis2+12)

#quick look at the heterogeneity of the combined data
summary(gse16873[,hn[c(1:2,7:8)]])
#if graphic devices open:
#boxplot(data.frame(gse16873))
gse16873.kegg.heter.p <- heter.gage(gse16873, gsets = kegg.gs,
  ref.list = refList, samp.list = samplList)
gse16873.kegg.heter.2d.p <- heter.gage(gse16873, gsets = kegg.gs,
  ref.list = refList, samp.list = samplList, same.dir = FALSE)
str(gse16873.kegg.heter.p)
head(gse16873.kegg.heter.p$greater[, 1:5])
```

kegg.gs

Common gene set data collections

Description

The gene set data collections derived from KEGG, GO and BioCarta databases.

Usage

```
data(kegg.gs)
data(kegg.gs.dise)
data(go.gs)
data(cartagene.gs)
```

Format

kegg.gs is a named list of 177 elements. Each element is a character vector of member gene Entrez IDs for a single KEGG pathway. Type `head(kegg.gs, 3)` for the first 3 gene sets or pathways. Note that kegg.gs has been updated since gage version 2.9.1. From then, kegg.gs only include the subset of canonical signaling and metabolic pathways from KEGG pathway database, and kegg.gs.dise is the subset of disease pathways. And it is recommended to do KEGG pathway analysis with either kegg.gs or kegg.gs.dise separately (rather than combined altogether) for better defined results. In the near future, we will also generate subsets of go.gs for refined analysis. Note that kegg.gs in gageData package still keeps all KEGG pathways, where kegg.gs.sigmet and kegg.gs.dise are two subsets of kegg.gs.

go.gs is a named list of 1000 elements in this package. It is a truncated list in this package. The full list of go.gs has ~10000 elements and is provided with an experimental data package gageData. Each element is a character vector of member gene Entrez IDs for a single Gene Ontology term. Type `head(go.gs, 3)` for the first 3 gene sets or GO terms.

carta.gs is a named list of 259 elements. Each element is a character vector of member gene Entrez IDs for a single BioCarta pathway. Type `head(carta.gs, 3)` for the first 3 gene sets or pathways.

khier is a matrix of 442 rows and 3 columns. This records the category and subcategory assignments of all KEGG pathways. The data comes from KEGG BRITE Database. This data is used by kegg.gsets function to subset the KEGG pathways for more specific pathway analysis.

korg is a character matrix of ~3000 rows and 6 columns. First 3 columns are KEGG species code, scientific name and common name, followed columns on gene ID types used for each species: entrez.gnodes ("1" or "0", whether EntrezGene is the default gene ID) and representative KEGG gene ID and EntrezGene ID. This data comes from pathview package, and is used by kegg.gsets function internally.

bods is a character matrix of 19 rows and 4 columns on the mapping between gene annotation package names in Bioconductor, common name and KEGG code of most common research species. This data comes from pathview package, and is used by go.gsets function internally.

Details

These gene set data were compiled using Entrez Gene IDs, gene set names and mapping information from multiple Bioconductor packages, including: org.Hs.eg.db, kegg.db, go.db and cMAP. Please check the corresponding packages for more information.

We only provide gene set data for human with this package. For other species, please check the experiment data package list of Bioconductor website or use the Bioconductor package GSEABase to build the users' own gene set collections.

Source

Data from multiple Bioconductor packages, including: org.Hs.eg.db, kegg.db, go.db and cMAP.

References

Entrez Gene <URL: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>> KEGG pathways <URL: <ftp://ftp.genome.ad.jp/pub/kegg/pathways>> Gene Ontology <URL: <http://www.geneontology.org/>> cMAP <URL: <http://cmap.nci.nih.gov/PW>>

Examples

```
#load expression and gene set data
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)

data(kegg.gs)
data(go.gs)

#make sure the gene IDs are the same for expression data and gene set
#data
lapply(kegg.gs[1:3],head)
lapply(go.gs[1:3],head)
head(rownames(gse16873))

#GAGE analysis
gse16873.kegg.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis)
gse16873.go.p <- gage(gse16873, gsets = go.gs,
  ref = hn, samp = dcis)
```

kegg.gsets

Generate up-to-date KEGG pathway gene sets

Description

Generate up-to-date KEGG pathway gene sets for any specified KEGG species.

Usage

```
kegg.gsets(species = "hsa", id.type = "kegg", check.new=FALSE)
```

Arguments

species	character, either the kegg code, scientific name or the common name of the target species. This applies to both pathway and gene.data or cpd.data. When KEGG ortholog pathway is considered, species="ko". Default species="hsa", it is equivalent to use either "Homo sapiens" (scientific name) or "human" (common name).
id.type	character, desired ID type for the get sets, case insensitive. Default idtype="kegg", i.e. the primary KEGG gene ID. The other valid option is "entrez", i.e. Entrez Gene. Entrez Gene is the primary KEGG gene ID for many common model organisms, like human, mouse, rat etc, hence these two options have the same effect. For other species, primary KEGG gene ID is not Entrez Gene.
check.new	logical, whether to check for any newly added reference pathways online. Default to FALSE. Because such online checking takes time and new reference pathways are rarely added, it is usually not suggested to set this argument TRUE, unless this is really needed.

Details

The latest KEGG pathway gene sets are derived by connecting to the database in real time. This way, we can create high quality gene set data for pathway analysis for over 2400 KEGG species.

Note that we have generated GO gene set for 4 species, human, mouse, rat, yeast as well as KEGG Ortholog, and provided the data in package gageData.

Value

A named list with the following elements:

kg.sets	KEGG gene sets, a named list. Each element is a character vector of member gene IDs for a single KEGG pathway. The number of elements of this list is the total number of KEGG pathways defined for the specified species.
sigmet.idx	integer indice, which elements in kg.sets are signaling or metabolism pathways.
sig.idx	integer indice, which elements in kg.sets are signaling pathways.
met.idx	integer indice, which elements in kg.sets are metabolism pathways.
dise.idx	integer indice, which elements in kg.sets are disease pathways.

The *.idx elements here are all used to subset kg.sets for more specific type pathway analysis.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[kegg.gs](#) for precompiled KEGG and other common gene set data collection

Examples

```
#GAGE analysis use the latest KEGG pathway definitions, instead of
#kegg.gs
kg.hsa=kegg.gsets()
data(gse16873)
hn=(1:6)*2-1
dcis=(1:6)*2
kegg.sigmet=kg.hsa$kg.sets[kg.hsa$sigmet.idx]
gse16873.kegg.p <- gage(gse16873, gsets = kegg.sigmet,
                      ref = hn, samp = dcis)

#E coli KEGG Id is different from Entre Gene
kg.eco=kegg.gsets("eco")
kg.eco.eg=kegg.gsets("eco", id.type="entrez")
head(kg.eco$kg.sets,2)
head(kg.eco.eg$kg.sets,2)
```

readExpData	<i>Read in expression data</i>
-------------	--------------------------------

Description

This is a wrapper function of `read.delim` for reading in expression data matrix in tab-delimited format.

Usage

```
readExpData(file = "arrayData.txt", ...)
```

Arguments

file	character string, the full path name to the expression data file in tab-delimited format. Rows are genes, columns are array samples.
...	other arguments to be passed into <code>read.delim</code> function.

Details

`readExpData` is a wrapper function of `read.delim`. Please check help information of `read.delim` for more details.

Value

A `data.frame` (matrix-like) of gene expression data. Rows are genes, columns are array samples.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[readList](#) read in gene set list

Examples

```
filename=system.file("extdata/gse16873.demo", package = "gage")
demo.data=readExpData(filename, row.names=1)
head(demo.data)
```

`readList`*Read in gene set data as a named list*

Description

This function reads in gene set data in GMT (.gmt) format as a named list. GMT is defined originally by GSEA program. The code may be slightly revised for reading in gene set data in other tab-delimited formats too.

Usage

```
readList(file)
```

Arguments

`file` character string, the full path name to the gene set data file in GMT format.

Value

A named list, each element is a character vector giving the gene IDs of a gene set.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[readExpData](#) read in gene expression data

Examples

```
#an example GMT gene set data derived from MSigDB data
filename=system.file("extdata/c2.demo.gmt", package = "gage")
demo.gs=readList(filename)
demo.gs[1:3]
#to use these gene sets with gse16873, need to convert the gene symbols
#to Entrez IDs first
data(egSymb)
demo.gs.sym<-lapply(demo.gs, sym2eg)
demo.gs.sym[1:3]
```

sigGeneSet

*Significant gene set from GAGE analysis***Description**

This function sorts and counts significant gene sets based on q- or p-value cutoff.

Usage

```
sigGeneSet(setp, cutoff = 0.1, dualSig = (0:2)[2], qpval = c("q.val",
  "p.val")[1], heatmap=TRUE, outname="array", pdf.size = c(7,7),
  p.limit=c(0.5, 5.5), stat.limit=5, ...)
```

Arguments

setp	the result object returned by gage function, either a numeric matrix or a list of two such matrices. Check gage help information for details.
cutoff	numeric, q- or p-value cutoff, between 0 and 1. Default 0.1 (for q-value). When p-value is used, recommended cutoff value is 0.001 for data with more than 2 replicates per condition or 0.01 for less sample sizes.
dualSig	integer, switch argument controlling how dual-significant gene sets should be treated. This argument is only useful when Stouffer method is not used in gage function (use.stouffer=FALSE), hence makes no difference normally. 0: discard such gene sets from the final significant gene set list; 1: keep such gene sets in the more significant direction and remove them from the less significant direction; 2: keep such gene sets in the lists for both directions. default to 1. Dual-significant means a gene set is called significant simultaneously in both 1-direction tests (up- and down-regulated). Check the details for more information.
qpval	character, specifies the column name used for gene set selection, i.e. what type of q- or p-value to use in gene set selection. Default to be "q.val" (q-value using BH procedure). "p.val" is the unadjusted global p-value and may be used as selection criterion sometimes.
heatmap	boolean, whether to plot heatmap for the selected gene data as a PDF file. Default to be FALSE.
outname	a character string, to be used as the prefix of the output data files. Default to be "array".
pdf.size	a numeric vector to specify the width and height of PDF graphics region in inches. Default to be c(7, 7).
stat.limit	numeric vector of length 1 or 2 to specify the value range of gene set statistics to visualize using the heatmap. Statistics beyond will be reset to equal the proximal limit. Default to 5, i.e. plot all gene set statistics within (-5, 5) range. May also be NULL, i.e. plot all statistics without limit. This argument allows optimal differentiation between most gene set statistic values when extremely positive/negative values exist and squeeze the normal-value region.

p.limit	numeric vector of length 1 or 2 to specify the value range of gene set $-\log_{10}(\text{p-values})$ to visualize using the heatmap. Values beyond will be reset to equal the proximal limit. Default to <code>c(0.5,5.5)</code> , i.e. plot all $-\log_{10}(\text{p-values})$ within this range. This argument is similar to argument <code>stat.limit</code> .
...	other arguments to be passed into the inside <code>gs.heatmap</code> function, which is a wrapper of the <code>heatmap2</code> function.

Details

By default, heatmaps are produced to show the gene set perturbations using either $-\log_{10}(\text{p-value})$ or statistics.

Since gage package version 2.2.0, Stouffer's method is used as the default procedure for more robust p-value summarization. With the original p-value summarization, i.e. negative log sum following a Gamma distribution as the Null hypothesis, the global p-value could be heavily affected by a small subset of extremely small individual p-values from pair-wise comparisons. Such sensitive global p-value leads to the "dual significance" phenomenon. In other words, Gene sets are significantly up-regulated in a subset of experiments, but down-regulated in another subset. Note that dual-significant gene sets are not the same as gene sets called significant in 2-directional tests, although they are related.

Value

`sigGeneSet` function returns a named list of the same structure as `gage` result. Check `gage` help information for details.

Author(s)

Weijun Luo <luo_weijun@yahoo.com>

References

Luo, W., Friedman, M., Shedden K., Hankenson, K. and Woolf, P GAGE: Generally Applicable Gene Set Enrichment for Pathways Analysis. BMC Bioinformatics 2009, 10:161

See Also

[gage](#) the main function for GAGE analysis; [eset.grp](#) non-redundant significant gene set list; [essGene](#) essential member genes in a gene set;

Examples

```
data(gse16873)
cn=colnames(gse16873)
hn=grep('HN',cn, ignore.case =TRUE)
dcis=grep('DCIS',cn, ignore.case =TRUE)
data(kegg.gs)

#kegg test for 1-directional changes
gse16873.kegg.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis)
```

```
#kegg test for 2-directional changes
gse16873.kegg.2d.p <- gage(gse16873, gsets = kegg.gs,
  ref = hn, samp = dcis, same.dir = FALSE)
gse16873.kegg.sig<-sigGeneSet(gse16873.kegg.p, outname="gse16873.kegg")
str(gse16873.kegg.sig)
gse16873.kegg.2d.sig<-sigGeneSet(gse16873.kegg.2d.p, outname="gse16873.kegg")
str(gse16873.kegg.2d.sig)
#also check the heatmaps in pdf files named "*.heatmap.pdf".
```

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