Package ‘EGSEA’

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Description This package implements the Ensemble of Gene Set Enrichment Analyses (EGSEA) method for gene set testing.

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EGSEA-package

Ensemble of Gene Enrichment Analysis (EGSEA)

Description
This package provides the implementation of the EGSEA algorithm and additional functions to help perform GSEA analysis.

Author(s)
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buildCustomIdx

Custom Gene Set Collection Index

Description
It creates gene set collections from a given list of gene sets to be used for the EGSEA analysis.

Usage
buildCustomIdx(entrezIDs, gsets, anno = NULL, label = "custom", name = "User-Defined Gene Sets", species = "Human", min.size = 1)

Arguments
- entrezIDs: character, a vector that stores the Entrez Gene IDs tagged in your dataset. The order of the Entrez Gene IDs should match those of the count/expression matrix row names.
- gsets: list, list of gene sets. Each gene set is a character vector of Entrez IDs. The names of the list should match the GeneSet column in the anno argument (if it is provided).
- anno: list, dataframe that stores a detailed annotation for each gene set. Some of its fields can be ID, GeneSet, PubMed, URLs, etc. The GeneSet field is mandatory and should have the same names as the gsets’ names.
- label: character, a unique id that identifies the collection of gene sets
- name: character, the collection name to be used in the EGSEA report
- species: character, determine the organism of selected gene sets: "human", "mouse" or "rat".
- min.size: integer, the minimum number of genes required in a testing gene set


**buildGeneSetDBIdx**

**Details**

It indexes newly created gene sets and attach gene set annotation if provided.

**Value**

indexed gene set annotation that can be used with other functions in the package. Each annotation is a list of seven elements: original stores the original gene sets, idx stores the indexed gene sets, anno that stores detailed annotation for each gene set, label a unique id that identifies the collection of gene sets, featureIDs stores the entrezIDs used in building the annotation, species stores that organism name of gene sets and name stores the collection name to be used in the EGSEA report.

**Examples**

library(EGSEAdata)
data(i113.data)
v = i113.data$voom
data(kegg.pathways)
gsets = kegg.pathways$human$kg.sets[1:50]
gs.annot = buildCustomIdx(entrezIDs=rownames(v$E), gsets= gsets, species="human")
class(gs.annot)

**Description**

It prepares the GeneSetDB gene set collections to be used for the EGSEA analysis.

**Usage**

buildGeneSetDBIdx(entrezIDs, species, geneSets = "all", min.size = 1)

**Arguments**

- **entrezIDs** character, a vector that stores the Entrez Gene IDs tagged in your dataset. The order of the Entrez Gene IDs should match those of the count/expression matrix row names.
- **species** character, determine the organism of selected gene sets: "human", "mouse" or "rat".
- **geneSets** character, a vector determines which gene set collections are loaded from the GeneSetDB. It takes "all", "gsdbdis", "gsdbgo", "gsdbdrug", "gsdbpath" or "gsdbreg". "all" includes all the GeneSetDB collections. "gsdbdis" is to load the disease collection, "gsdbgo" to load the GO terms collection, "gsdbdrug" to load the drug/chemical collection, "gsdbpath" to load the pathways collection and "gsdbreg" to load the gene regulation collection.
- **min.size** integer, the minimum number of genes required in a testing gene set.
Details

It indexes the GeneSetDB gene sets and loads gene set annotation.

Value

indexed gene set annotation that can be used with other functions in the package. Each annotation is a list of seven elements: original stores the original gene sets, idx stores the indexed gene sets, anno that stores detailed annotation for each gene set, label a unique id that identifies the collection of gene sets, featureIDs stores the entrezIDs used in building the annotation, species stores that organism name of gene sets and name stores the collection name to be used in the EGSEA report.

Examples

library(EGSEAdata)
data(lll3.data)
v = lll3.data$voom
gs.annots = buildGeneSetDBIdx(entrezIDs=rownames(v$E), species="human")
names(gs.annots)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>entrezIDs</td>
<td>character, a vector that stores the Entrez Gene IDs tagged in your dataset. The order of the Entrez Gene IDs should match those of the count/expression matrix row names.</td>
</tr>
<tr>
<td>species</td>
<td>character, determine the organism of selected gene sets: &quot;human&quot;, &quot;mouse&quot; or &quot;rat&quot;.</td>
</tr>
<tr>
<td>msigdb.gsets</td>
<td>character, a vector determines which gene set collections should be used from MSigDB. It can take values from this list: &quot;h&quot;, &quot;c1&quot;, &quot;c2&quot;, &quot;c3&quot;, &quot;c4&quot;, &quot;c5&quot;, &quot;c6&quot;, &quot;c7&quot;. &quot;h&quot; and &quot;c1&quot; are human specific. If &quot;all&quot;, all available gene set collections are loaded. If &quot;none&quot;, MSigDB collections are excluded.</td>
</tr>
<tr>
<td>gsdb.gsets</td>
<td>character, a vector determines which gene set collections are loaded from the GeneSetDB. It takes &quot;none&quot;, &quot;all&quot;, &quot;gsdbdis&quot;, &quot;gsdbgo&quot;, &quot;gsdbdrug&quot;, &quot;gsdbpath&quot; or &quot;gsdbreg&quot;. &quot;none&quot; excludes the GeneSetDB collections. &quot;all&quot; includes all the GeneSetDB collections. &quot;gsdbdis&quot; to load the disease collection, &quot;gsdbgo&quot; to load the GO terms collection, &quot;gsdbdrug&quot; to load the drug/chemical collection,</td>
</tr>
</tbody>
</table>

BuildIdx

Generate Gene Set Collection Indexes from the MSigDB and KEGG Databases

Description

It prepares the MSigDB and KEGG gene set collections to be used for the EGSEA analysis.

Usage

buildIdx(entrezIDs, species = "human", msigdb.gsets = "all",
          gsdb.gsets = "none", kegg.updated = FALSE, kegg.exclude = c(),
          min.size = 1)
buildKEGGIdx

"gsdbpath" to load the pathways collection and "gsdbreg" to load the gene regulation collection.

kegg.updated logical, set to TRUE if you want to download the most recent KEGG pathways.

kegg.exclude character, vector used to exclude KEGG pathways of specific type(s): Disease, Metabolism, Signaling. If "all", none of the KEGG collections is included.

min.size integer, the minimum number of genes required in a testing gene set

Details

It indexes the MSigDB and KEGG gene sets and loads gene set annotation.

Value

Indexed gene set annotation that can be used with other functions in the package. Each annotation is a list of seven elements: original stores the original gene sets, idx stores the indexed gene sets, anno that stores detailed annotation for each gene set, label a unique id that identifies the collection of gene sets, featureIDs stores the entrezIDs used in building the annotation, species stores that organism name of gene sets and name stores the collection name to be used in the EGSEA report.

Examples

library(EGSEAdata)
data(ll13.data)
v = ll13.data$voom
gs.annots = buildIdx(entrezIDs=rownames(v$E), species="human",
msgdb.gsets = c("h", "c2"),
kegg.exclude = c("Metabolism"))
names(gs.annots)

buildKEGGIdx

Gene Set Collection Index from the KEGG Database

Description

It prepares the KEGG pathway collection to be used for the EGSEA analysis.

Usage

buildKEGGIdx(entrezIDs, species = "human", min.size = 1, updated = FALSE,
exclude = c())

Arguments

entrezIDs character, a vector that stores the Entrez Gene IDs tagged in your dataset. The order of the Entrez Gene IDs should match those of the count/expression matrix row names.

species character, determine the organism of selected gene sets: "human", "mouse" or "rat".

min.size integer, the minimum number of genes required in a testing gene set

updated logical, set to TRUE if you want to download the most recent KEGG pathways.

exclude character, vector used to exclude KEGG pathways of specific category. Accepted values are "Disease", "Metabolism", or "Signaling".
Details

It indexes the KEGG pathway gene sets and loads gene set annotation.

Value

indexed gene set annotation that can be used with other functions in the package. Each annotation is a list of seven elements: original stores the original gene sets, idx stores the indexed gene sets, anno that stores detailed annotation for each gene set, label a unique id that identifies the collection of gene sets, featureIDs stores the entrezIDs used in building the annotation, species stores that organism name of gene sets and name stores the collection name to be used in the EGSEA report.

Examples

```r
library(EGSEAdata)
data(iil3.data)
v = iil3.data$voom
gs.annots = buildKEGGIdx(entrezIDs=rownames(v$E), species="human")
```

buildMSigDBIdx

Gene Set Collection Indexes from the MSigDB Database

Description

It prepares the MSigDB gene set collections to be used for the EGSEA analysis.

Usage

```r
buildMSigDBIdx(entrezIDs, geneSets = "all", species = "Homo sapiens",
               min.size = 1)
```

Arguments

- **entrezIDs** character, a vector that stores the Entrez Gene IDs tagged in your dataset. The order of the Entrez Gene IDs should match those of the count/expression matrix row names.
- **geneSets** character, a vector determines which gene set collections should be used from the MSigDB. It can take values from this list: "all", "h", "c1", "c2", "c3", "c4", "c5", "c6", "c7". "c1" is human specific. If "all", all available gene set collections are loaded.
- **species** character, determine the organism of selected gene sets: "human", "mouse" or "rat".
- **min.size** integer, the minimum number of genes required in a testing gene set

Details

It indexes the MSigDB gene sets and loads gene set annotation.
indexed gene set annotation that can be used with other functions in the package. Each annotation is a list of seven elements: original stores the original gene sets, idx stores the indexed gene sets, anno that stores detailed annotation for each gene set, label a unique id that identifies the collection of gene sets, featureIDs stores the entrezIDs used in building the annotation, species stores that organism name of gene sets and name stores the collection name to be used in the EGSEA report.

Examples

```r
library(EGSEAdata)
data(il13.data)
v = il13.data$voom
gs.annots = buildMSigDBIdx(entrezIDs=rownames(v$E), geneSets=c("h", "c2"), species="human")
names(gs.annots)
```

Description

This is the main function to carry out gene set enrichment analysis using the EGSEA algorithm. This function is aimed to extend the limma-voom pipeline of RNA-seq analysis.

Usage

```r
egsea(voom.results, contrasts, logFC = NULL, gs.annots, symbolsMap = NULL, 
baseGSEAs = egsea.base(), minSize = 2, display.top = 20, 
combineMethod = "fisher", combineWeights = NULL, sort.by = "p.adj", 
egsea.dir = NULL, kegg.dir = NULL, logFC.cutoff = 0, 
sum.plot.axis = "p.adj", sum.plot.cutoff = NULL, vote.bin.width = 5, 
um.threads = 4, report = TRUE, print.base = FALSE, verbose = FALSE, 
keep.limma = FALSE, keep.set.scores = FALSE)
```

Arguments

- **voom.results**
  - list, an EList object generated using the `voom` function. Entrez Gene IDs should be used as row names.

- **contrasts**
  - double, an N x L matrix indicates the contrast of the linear model coefficients for which the test is required. N is number of experimental conditions and L is number of contrasts.

- **logFC**
  - double, an K x L matrix indicates the log2 fold change of each gene for each contrast. K is the number of genes included in the analysis. If logFC=NULL, the logFC values are estimated using the `ebayes` for each contrast.

- **gs.annots**
  - list, list of objects of class GSCollectionIndex. It is generated using one of these functions: `buildIdx`, `buildMSigDBIdx`, `buildKEGGIdx`, `buildGeneSetDBIdx`, and `buildCustomIdx`.

- **symbolsMap**
  - dataframe, an K x 2 matrix stores the gene symbol of each Entrez Gene ID. It is used for the heatmap visualization. The order of rows should match that of the `voom.results`. Default symbolsMap=NULL.
egsea

baseGSEAs character, a vector of the gene set tests that should be included in the ensemble. Type `egsea.base` to see the supported GSE methods. By default, all supported methods are used.

minSize integer, the minimum size of a gene set to be included in the analysis. Default `minSize=2`.

display.top integer, the number of top gene sets to be displayed in the EGSEA report. You can always access the list of all tested gene sets using the returned gsa list. Default is 20.

combineMethod character, determines how to combine p-values from different GSEA method. Type `egsea.combine()` to see supported methods.

combineWeights double, a vector determines how different GSEA methods will be weighted. Its values should range between 0 and 1. This option is not supported currently.

sort.by character, determines how to order the analysis results in the stats table. Type `egsea.sort()` to see all available options.

egsea.dir character, directory into which the analysis results are written out.

kegg.dir character, the directory of KEGG pathway data file (.xml) and image file (.png). Default `kegg.dir=paste0(egsea.dir, "/kegg-dir/ ")`.

logFC.cutoff numeric, cut-off threshold of logFC and is used for Significance Score and Regulation Direction Calculations. Default `logFC.cutoff=0`.

sum.plot.axis character, the x-axis of the summary plot. All the values accepted by the `sort.by` parameter can be used. Default `sum.plot.axis="p.value"`.

sum.plot.cutoff numeric, cut-off threshold to filter the gene sets of the summary plots based on the values of the `sum.plot.axis`. Default `sum.plot.cutoff=NULL`.

vote.bin.width numeric, the bin width of the vote ranking. Default `vote.bin.width=5`.

num.threads numeric, number of CPU threads to be used. Default `num.threads=2`.

report logical, whether to generate the EGSEA interactive report. It takes longer time to run. Default is `TRUE`.

print.base logical, whether to write out the results of the individual GSE methods. Default `FALSE`.

verbose logical, whether to print out progress messages and warnings.

keep.limma logical, whether to return the results of the limma analysis.

keep.set.scores logical, whether to calculate the gene set enrichment scores per sample for the methods that support this option, i.e., "sgsea".

Details

EGSEA, an acronym for Ensemble of Gene Set Enrichment Analyses, utilizes the analysis results of eleven prominent GSE algorithms from the literature to calculate collective significance scores for gene sets. These methods include: `ora`, `globaltest`, `plage`, `safe`, `zscore`, `gage`, `ssgsea`, `roast`, `fry`, `padog`, `camera` and `gsva`. The `ora`, `gage`, `camera` and `gsva` methods depend on a competitive null hypothesis while the remaining seven methods are based on a self-contained hypothesis. Conveniently, the algorithm proposed here is not limited to these twelve GSE methods and new GSE tests can be easily integrated into the framework. This function takes the voom object and the contrast matrix as parameters. The results of EGSEA can be seen using the `topSets` function.

EGSEA report is an interactive HTML report that is generated if `report=TRUE` to enable a swift
navigation through the results of an EGSEA analysis. The following pages are generated for each gene set collection and contrast/comparison:

1. Stats Table page shows the detailed statistics of the EGSEA analysis for the display.top gene sets. It shows the EGSEA scores, individual rankings and additional annotation for each gene set. Hyperlinks to the source of each gene set can be seen in this table when they are available. The "Direction" column shows the regulation direction of a gene set which is calculated based on the logFC, which is either calculated from the limma differential expression analysis or provided by the user. The logFC.cutoff is applied for this calculation. The calculations of the EGSEA scores can be seen in the references section. The method topSets can be used to generate custom Stats Table.

2. Heatmaps page shows the heatmaps of the gene fold changes for the gene sets that are presented in the Stats Table page. Red indicates up-regulation while blue indicates down-regulation. Only genes that appear in the input expression/count matrix are visualized in the heat map. Gene names are coloured based on their statistical significance in the limma differential expression analysis. The "Interpret Results" link below each heat map allows the user to download the original heat map values along with additional statistics from limma DE analysis (if available) so that they can be used to perform further analysis in R, e.g., customizing the heat map visualization. Additional heat maps can be generated and customized using the method plotHeatmap.

3. Summary Plots page shows the methods ranking plot along with the summary plots of EGSEA analysis. The method plot uses multidimensional scaling (MDS) to visualize the ranking of individual methods on a given gene set collection. The summary plots are bubble plots that visualize the distribution of gene sets based on the EGSEA Significance Score and another EGSEA score (default, p-value). Two summary plots are generated: ranking and directional plots. Each gene set is represented with a bubble which is coloured based on the EGSEA ranking (in ranking plots) or gene set regulation direction (in directional plots) and sized based on the gene set cardinality (in ranking plots) or EGSEA Significance score (in directional plots). Since the EGSEA "Significance Score" is proportional to the p-value and the absolute fold changes, it could be useful to highlight gene sets that have high Significance scores. The blue labels on the summary plot indicate gene sets that do not appear in the top 10 list of gene sets based on the "sort.by" argument (black labels) yet they appear in the top 5 list of gene sets based on the EGSEA "Significance Score". If two contrasts are provided, the rank is calculated based on the "comparison" analysis results and the "Significance Score" is calculated as the mean. If sort.by = NULL, the slot sort.by of the object is used to order gene sets. The method plotSummary can be used to customize the Summary plots by changing the x-axis score and filtering bubbles based on the values of the x-axis. The method plotMethods can be used to generate Methods plots.

4. Pathways page shows the KEGG pathways for the gene sets that are presented in the Stats Table of a KEGG gene set collection. The gene fold changes are overlaid on the pathway maps and coloured based on the gene regulation direction: blue for down-regulation and red for up-regulation. The method plotPathway can be used to generate additional pathway maps. Note that this page only appears if a KEGG gene set collection is used in the EGSEA analysis.

5. Go Graphs page shows the Gene Ontology graphs for top 5 GO terms in each of three GO categories: Biological Processes (BP), Molecular Functions (MF), and Cellular Components (CC). Nodes are coloured based on the default sort.by score where red indicates high significance and yellow indicates low significance. The method plotGOGraph can be used to customize GO graphs by changing the default sorting score and the number of significance nodes that can be visualized. It is recommended that a small number of nodes is selected. Note that this page only appears if a Gene Ontology gene set collection is used, i.e., for the c5 collection from MSigDB or the gsdbgo collection from GeneSetDB.

Finally, the "Interpret Results" hyperlink in the EGSEA report allows the user to download the fold changes and limma analysis results and thus improve the interpretation of the results. Note that the running time of this function significantly increases when report = TRUE. For example, the analysis in the example section below was conducted on the $203S$ signaling and disease
KEGG pathways using a MacBook Pro machine that had a 2.8 GHz Intel Core i7 CPU and 16 GB of RAM. The execution time varied between 23.1 seconds (single thread) to 7.9 seconds (16 threads) when the HTML report generation was disabled. The execution time took 145.5 seconds when the report generation was enabled using 16 threads.

Value

A list of elements, each with two/three elements that store the top gene sets and the detailed analysis results for each contrast and the comparative analysis results.

References

Monther Alhamdoosh, Milica Ng, Nicholas J. Wilson, Julie M. Sheridan, Huy Huynh, Michael J. Wilson and Matthew E. Ritchie. Combining multiple tools outperforms individual methods in gene set enrichment analyses.

See Also

topSets, egsea.base, egsea.sort, buildIdx, buildMSigDBIdx, buildKEGGIdx, buildGeneSetDBIdx, and buildCustomIdx

Examples

```r
# Example of egsea
library(EGSEAdata)
data(ll13.data)
v = ll13.data$voom
contrasts = ll13.data$contra
gs.annots = buildIdx(entrezIDs=rownames(v$E), species="human",
msigdb.gsets="none",
    kegg.updated=FALSE, kegg.exclude = c("Metabolism"))
# set report = TRUE to generate the EGSEA interactive report
gsa = egsea(voom.results=v, contrasts=contrasts, gs.annots=gs.annots,
symbolsMap=v$genes, baseGSEAs=egsea.base()[c(2,5,6,9,12)],
display.top = 5, sort.by="avg.rank",
    egsea.dir="/ll13-egsea-report",
    num.threads = 2, report = FALSE)
topSets(gsa)
```

egsea.base

**EGSEA Base GSE Methods**

Description

It lists the supported GSEA methods. Since EGSEA base methods are implemented in the Bioconductor project, the most recent version of each individual method is always used.

Usage

```r
egsea.base()
```
Details

These methods include: ora[1], globaltest[2], plage[3], safe[4], zscore[5], gage[6], ssgsea[7], roast[8], fry[8], padog[9], camera[10] and gsva[11]. The ora, gage, camera and gsva methods depend on a competitive null hypothesis while the remaining seven methods are based on a self-contained hypothesis. Conveniently, EGSEA is not limited to these twelve GSE methods and new GSE tests can be easily integrated into the framework.

Note: the execution time of base methods can vary depending on the size of gene set collections, number of samples, number of genes and number of contrasts. When a gene set collection of around 200 gene sets was tested on a dataset of 17,500 genes, 8 samples and 2 contrasts, the execution time of base methods in ascending order was as follows: globaltest; safe; gage; gsva; zscore; plage; fry; camera; ora; ssgsea; padog. When the same dataset was tested on a large gene set collection of 3,700 gene sets, the execution time of base methods in ascending order was as follows: globaltest; camera; fry; zscore; plage; safe; gsva; ora; gage; padog; ssgsea. Apparently, the size of gene set collection plays a key role in the execution time of most of the base methods. The reduction rate of execution time between the large and small gene set collections varied between 18% and 88%. camera, fry, plage, zscore and ora showed the least reduction rate of execution time. As a result, there is no guarantee that a single combination of base methods would run faster than other combinations. It is worth mentioning that our simulation results showed that the increasing number of base methods in the EGSEA analysis is desirable to achieve high performance.

Value

It returns a character vector of supported GSE methods.

References


Examples

egsea.base()
**egsea.cnt**

*Ensemble of Gene Set Enrichment Analyses Function*

**Description**

This is the main function to carry out gene set enrichment analysis using the EGSEA algorithm. This function is aimed to use the raw count matrix to perform the EGSEA analysis.

**Usage**

```r
egsea.cnt(counts, group, design = NULL, contrasts, logFC = NULL, gs.annots,
symbolsMap = NULL, baseGSEAs = egsea.base(), minSize = 2,
display.top = 20, combineMethod = "fisher", combineWeights = NULL,
sort.by = "p.adj", egsea.dir = NULL, kegg.dir = NULL,
logFC.cutoff = 0, sum.plot.axis = "p.adj", sum.plot.cutoff = NULL,
vote.bin.width = 5, num.threads = 4, report = TRUE,
print.base = FALSE, verbose = FALSE, keep.limma = FALSE,
keep.set.scores = FALSE)
```

**Arguments**

- `counts`: double, numeric matrix of read counts where genes are the rows and samples are the columns.
- `group`: character, vector or factor giving the experimental group/condition for each sample/library.
- `design`: double, numeric matrix giving the design matrix of the linear model fitting.
- `contrasts`: double, an N x L matrix indicates the contrast of the linear model coefficients for which the test is required. N is number of experimental conditions and L is number of contrasts.
- `logFC`: double, an K x L matrix indicates the log2 fold change of each gene for each contrast. K is the number of genes included in the analysis. If logFC=NULL, the logFC values are estimated using the `eBayes` for each contrast.
- `gs.annots`: list, list of objects of class GSCollectionIndex. It is generated using one of these functions: `buildIdx`, `buildMSigDBIdx`, `buildKEGGIdx`, `buildGeneSetDBIdx`, and `buildCustomIdx`.
- `symbolsMap`: dataframe, an K x 2 matrix stores the gene symbol of each Entrez Gene ID. It is used for the heatmap visualization. The order of rows should match that of the `counts`. Default symbolsMap=NULL.
- `baseGSEAs`: character, a vector of the gene set tests that should be included in the ensemble. Type egsea.base to see the supported GSE methods. By default, all supported methods are used.
- `minSize`: integer, the minimum size of a gene set to be included in the analysis. Default minSize=2.
- `display.top`: integer, the number of top gene sets to be displayed in the EGSEA report. You can always access the list of all tested gene sets using the returned gsa list. Default is 20.
Details

EGSEA, an acronym for Ensemble of Gene Set Enrichment Analyses, utilizes the analysis results of eleven prominent GSE algorithms from the literature to calculate collective significance scores for gene sets. These methods include: ora, globaltest, plage, safe, zscore, gage, ssgsea, roast, fry, padog, camera and gsva. The ora, gage, camera and gsva methods depend on a competitive null hypothesis while the remaining seven methods are based on a self-contained hypothesis. Conveniently, the algorithm proposed here is not limited to these eleven GSE methods and new GSE tests can be easily integrated into the framework. This function takes the raw count matrix, the experimental group of each sample, the design matrix and the contrast matrix as parameters. It performs TMM normalization and then applies voom to calculate the logCPM and weighting factors. The results of EGSEA can be seen using the topSets function.

EGSEA report is an interactive HTML report that is generated if report=TRUE to enable a swift navigation through the results of an EGSEA analysis. The following pages are generated for each gene set collection and contrast/comparison:
1. Stats Table page shows the detailed statistics of the EGSEA analysis for the display.top gene sets. It shows the EGSEA scores, individual rankings and additional annotation for each gene set. Hyperlinks to the source of each gene set can be seen in this table when they are available. The “Direction” column shows the regulation direction of a gene set which is calculated based on the logFC, which is either calculated from the limma differential expression analysis or provided by the
user. The logFC.cutoff is applied for this calculation. The calculations of the EGSEA scores can be seen in the references section. The method topSets can be used to generate custom Stats Table.

2. Heatmaps page shows the heatmaps of the gene fold changes for the gene sets that are presented in the Stats Table page. Red indicates up-regulation while blue indicates down-regulation. Only genes that appear in the input expression/count matrix are visualized in the heat map. Gene names are coloured based on their statistical significance in the limma differential expression analysis. The "Interpret Results" link below each heat map allows the user to download the original heat map values along with additional statistics from limma DE analysis (if available) so that they can be used to perform further analysis in R, e.g., customizing the heat map visualization. Additional heat maps can be generated and customized using the method plotHeatmap.

3. Summary Plots page shows the methods ranking plot along with the summary plots of EGSEA analysis. The method plot uses multidimensional scaling (MDS) to visualize the ranking of individual methods on a given gene set collection. The summary plots are bubble plots that visualize the distribution of gene sets based on the EGSEA Significance Score and another EGSEA score (default, p-value). Two summary plots are generated: ranking and directional plots. Each gene set is represented with a bubble which is coloured based on the EGSEA ranking (in ranking plots) or gene set regulation direction (in directional plots) and sized based on the gene set cardinality (in ranking plots) or EGSEA Significance score (in directional plots). Since the EGSEA "Significance Score" is proportional to the p-value and the absolute fold changes, it could be useful to highlight gene sets that have high Significance scores. The blue labels on the summary plot indicate gene sets that do not appear in the top 10 list of gene sets based on the "sort.by" argument (black labels) yet they appear in the top 5 list of gene sets based on the EGSEA "Significance Score". If two contrasts are provided, the rank is calculated based on the "comparison" analysis results and the "Significance Score" is calculated as the mean. If sort.by = NULL, the slot sort.by of the object is used to order gene sets. The method plotSummary can be used to customize the Summary plots by changing the x-axis score and filtering bubbles based on the values of the x-axis. The method plotMethods can be used to generate Methods plots.

4. Pathways page shows the KEGG pathways for the gene sets that are presented in the Stats Table of a KEGG gene set collection. The gene fold changes are overlaid on the pathway maps and coloured based on the gene regulation direction: blue for down-regulation and red for up-regulation. The method plotPathway can be used to generate additional pathway maps. Note that this page only appears if a KEGG gene set collection is used in the EGSEA analysis.

5. Go Graphs page shows the Gene Ontology graphs for top 5 GO terms in each of three GO categories: Biological Processes (BP), Molecular Functions (MF), and Cellular Components (CC). Nodes are coloured based on the default sort.by score where red indicates high significance and yellow indicates low significance. The method plotGOGraph can be used to customize GO graphs by changing the default sorting score and the number of significance nodes that can be visualized. It is recommended that a small number of nodes is selected. Note that this page only appears if a Gene Ontology gene set collection is used, i.e., for the c5 collection from MSigDB or the gsdbgo collection from GeneSetDB.

Finally, the "Interpret Results" hyperlink in the EGSEA report allows the user to download the fold changes and limma analysis results and thus improve the interpretation of the results. Note that the running time of this function significantly increases when report = TRUE. For example, the analysis in the example section below was conducted on the $203$ signaling and disease KEGG pathways using a MacBook Pro machine that had a 2.8 GHz Intel Core i7 CPU and 16 GB of RAM. The execution time varied between 23.1 seconds (single thread) to 7.9 seconds (16 threads) when the HTML report generation was disabled. The execution time took 145.5 seconds when the report generation was enabled using 16 threads.
Value
A list of elements, each with two/three elements that store the top gene sets and the detailed analysis results for each contrast and the comparative analysis results.

References
Monther Alhamdoosh, Milica Ng, Nicholas J. Wilson, Julie M. Sheridan, Huy Huynh, Michael J. Wilson and Matthew E. Ritchie. Combining multiple tools outperforms individual methods in gene set enrichment analyses.

See Also
topSets, egsea.base, egsea.sort, buildIdx, buildMSigDBIdx, buildKEGGIdx, buildGeneSetDBIdx, buildCustomIdx

Examples
# Example of egsea.cnt
library(EGSEAdata)
data(il13.data.cnt)
cnt = il13.data.cnt$count
group = il13.data.cnt$group
design = il13.data.cnt$design
contrasts = il13.data.cnt$contra
genes = il13.data.cnt$genes
gs.annots = buildIdx(entrezIDs=rownames(cnt), species="human",
msigdb.gsets="none",
kegg.updated=FALSE, kegg.exclude = c("Metabolism"))
# set report = TRUE to generate the EGSEA interactive report
gsa = egsea.cnt(counts=cnt, group=group, design=design, contrasts=contrasts,
gs.annots=gs.annots,
symbolsMap=genes, baseGSEAs=egsea.base()[-c(2,5,6,9,12)],
display.top = 5,
sort.by="avg.rank",
egsea.dir="/il13-egsea-cnt-report",
um.threads = 2, report = FALSE)
topSets(gsa)
Examples

egsea.combine()

egsea.ora

Over-representation Analysis with EGSEA Reporting Capabilities

Description

This is the main function to carry out gene set enrichment analysis using the over-representation analysis (ORA) only.

Usage

egsea.ora(entrezIDs, universe = NULL, logFC = NULL, title = NULL, gs.annots, symbolsMap = NULL, minSize = 2, display.top = 20, sort.by = "p.adj", egsea.dir = NULL, kegg.dir = NULL, logFC.cutoff = 0, sum.plot.axis = "p.adj", sum.plot.cutoff = NULL, vote.bin.width = 5, num.threads = 4, report = TRUE, print.base = FALSE, verbose = FALSE)

Arguments

entrezIDs character, a vector of Entrez Gene IDs to be tested for ORA.
universe character, a vector of Enterz IDs to be used as a background list. If universe=NULL, the background list is created from the AnnotationDbi package.
logFC double, is a matrix or vector of log fold changes of the same length of entrezIDs. If logFC=NULL, 1 is used as a default value. Then, the regulation direction in heatmaps and pathway maps is not indicative to the gene regulation direction.
title character, a short description of the experimental contrast.
gs.annots list, list of objects of class GSCollectionIndex. It is generated using one of these functions: buildIdx, buildMSigDBIdx, buildKEGGIdx, buildGeneSetDBIdx, and buildCustomIdx.
symbolsMap dataframe, an K x 2 matrix stores the gene symbol of each Entrez Gene ID. It is used for the heatmap visualization. The order of rows should match that of the entrezIDs. Default symbolsMap=NULL.
minSize integer, the minimum size of a gene set to be included in the analysis. Default minSize= 2.
display.top integer, the number of top gene sets to be displayed in the EGSEA report. You can always access the list of all tested gene sets using the returned gsa list. Default is 20.
sort.by character, determines how to order the analysis results in the stats table. It takes "p.value", "p.adj" or "Significance".
egsea.dir character, directory into which the analysis results are written out.
kegg.dir character, the directory of KEGG pathway data file (.xml) and image file (.png). Default kegg.dir=paste0(egsea.dir, "/kegg-dir/").
logFC.cutoff numeric, cut-off threshold of logFC and is used for Significance Score and Regulation Direction Calculations. Default logFC.cutoff=0.
sum.plot.axis character, the x-axis of the summary plot. All the values accepted by the `sort.by` parameter can be used. Default `sum.plot.axis="p.adj"`.

sum.plot.cutoff numeric, cut-off threshold to filter the gene sets of the summary plots based on the values of the `sum.plot.axis`. Default `sum.plot.cutoff=NULL`.

vote.bin.width numeric, the bin width of the vote ranking. Default `vote.bin.width=5`.

num.threads numeric, number of CPU threads to be used. Default `num.threads=2`.

report logical, whether to generate the EGSEA interactive report. It takes longer time to run. Default is `True`.

print.base logical, whether to write out the results of the individual GSE methods. Default `FALSE`.

verbose logical, whether to print out progress messages and warnings.

Details

This function takes a list of Entrez gene IDs and uses the gene set collections from `EGSEData` or a custom-built collection to find over-represented gene sets in this list. It takes the advantage of the existing EGSEA reporting capabilities and generate an interactive report for the ORA analysis. The results can be explored using the `topSets` function.

Value

A list of elements, each with two/three elements that store the top gene sets and the detailed analysis results for each contrast and the comparative analysis results.

References

Monther Alhamdoosh, Milica Ng, Nicholas J. Wilson, Julie M. Sheridan, Huy Huynh, Michael J. Wilson and Matthew E. Ritchie. Combining multiple tools outperforms individual methods in gene set enrichment analyses.

See Also

topSets, buildIdx, buildMSigDBIdx, buildKEGGIdx, buildGeneSetDBIdx, and buildCustomIdx

Examples

```r
# Example of egsea.ora
library(EGSEData)
data(ill13.data)
voom.results = ill13.data$voom
contrast = ill13.data$contra
library(limma)
vfit = lmFit(voom.results, voom.results$design)
vfit = contrasts.fit(vfit, contrast)
vfit = eBayes(vfit)
top.Table = topTable(vfit, coef=1, number=Inf, p.value=0.05, lfc=1)
deGenes = as.character(top.Table$FeatureID)
logFC = top.Table$logFC
names(logFC) = deGenes
gs.annots = buildIdx(entrezIDs=deGenes, species="human",
msigdb.gsets="none",
kegg.updated=FALSE, kegg.exclude = c("Metabolism"))
```
### egsea.sort

**EGSEA Result Sorting Options**

**Description**

It lists the accepted sorting methods for analysis results.

**Usage**

```r
egsea.sort()
```

**Value**

It returns a character vector of the accepted values for the `sort.by` argument in `egsea`.

**Examples**

```r
egsea.sort()
```

### EGSEAResults

**The EGSEAResults class**

**Description**

The EGSEAResults class stores the results of an EGSEA analysis.

- `op$` extracts a slot from an object of class EGSEAResults.
- `topSets` extracts a table of the top-ranked gene sets from an EGSEA analysis.
- `show` displays the parameters of an EGSEAResults object.
- `summary` displays a brief summary of the analysis results stored in an EGSEAResults object.
- `limmaTopTable` returns a dataframe of the top table of the limma analysis for a given contrast.
- `getlimmaResults` returns the linear model fit produced by `limma::eBayes`.
- `plotHeatmap` generates a heatmap of fold changes for a selected gene set.
- `plotSummaryHeatmap` generates a summary heatmap for the top n gene sets of the comparative analysis across multiple contrasts.
- `plotPathway` generates a visual map for a selected KEGG pathway with the gene fold changes overlaid on it.
plotMethods generates a multi-dimensional scaling (MDS) plot for the gene set rankings of different base GSE methods.

plotSummary generates a Summary plot for EGSEA analysis.

plotGOGraph generates a graph of the top significant GO terms in a GO term collection, which could be C5 from MSigDB or Gene Ontolog from the GeneSetDB.

showSetByName shows the details of a given gene set indicated by name.

showSetByID shows the details of a given gene set indicated by ID.

getSetScores returns a dataframe of the gene set enrichment scores per sample. This can be only calculated using specific base methods, namely, "ssgsea".

Usage

```r
## S4 method for signature 'EGSEAResults'
x$name

topSets(object, gs.label = 1, contrast = 1, sort.by = NULL, number = 10,
     names.only = TRUE, verbose = TRUE)

## S4 method for signature 'EGSEAResults'
show(object)

## S4 method for signature 'EGSEAResults'
summary(object)

limmaTopTable(object, contrast = 1)

getlimmaResults(object)

plotHeatmap(object, gene.set, gs.label = 1, contrast = 1,
     file.name = "heatmap", format = "pdf", fc.colors = c("#67A9CF",
     "#F7F7F7", "#EF8A62"), verbose = TRUE)

plotSummaryHeatmap(object, gs.label = 1, number = 20, sort.by = NULL,
     show.vals = NULL, file.name = "sum_heatmap", format = "pdf",
     verbose = TRUE)

plotPathway(object, gene.set, gs.label = 1, contrast = 1,
     file.name = "pathway", verbose = TRUE)

plotMethods(object, gs.label = 1, contrast = 1, file.name = "methods.mds",
     format = "pdf", verbose = TRUE)

plotSummary(object, gs.label = 1, contrast = 1, file.name = "summary",
     format = "pdf", x.axis = "p.adj", x.cutoff = NULL, sort.by = NULL,
     use.names = FALSE, verbose = TRUE)

plotGOGraph(object, gs.label = "c5", contrast = 1, sort.by = NULL,
    noSig = 5, file.name = "c5-top-", format = "pdf", verbose = TRUE)

showSetByName(object, gs.label = 1, set.name)
```
showSetByID(object, gs.label = 1, id)

getSetScores(object, gs.label = 1)

Arguments

- **x**: EGSEAResults object, the analysis result object from `egsea`, `egsea.cnt` or `egsea.ora`.
- **name**: character, the slot name.
- **object**: EGSEAResults object, the analysis result object from `egsea`, `egsea.cnt` or `egsea.ora`.
- **gs.label**: the number or label of the gene set collection of interest.
- **contrast**: contrast column number or column name specifying which contrast is of interest. If contrast = 0 or "comparison" and the number of contrasts greater than 1, the comparative gene sets are returned.
- **sort.by**: character, determines how to order the analysis results in the stats table. The accepted values depend on the function used to generate the EGSEA results.
- **number**: integer, maximum number of gene sets to list.
- **names.only**: logical, whether to display the EGSEA statistics or not.
- **verbose**: logical, whether to print out progress messages and warnings.
- **gene.set**: character, the name of the gene set. See the output of `topSets`.
- **file.name**: character, the prefix of the output file name.
- **format**: character, takes "pdf" or "png".
- **fc.colors**: vector, determines the fold change colors of the heatmap. Three colors of the negative, zero and positive log fold changes, respectively, should be assigned. Default is c("#67A9CF", "#F7F7F7", "#EF8A62"). These colors were generated using `rev(RColorBrewer::brewer.pal(3, "RdBu"))`.
- **show.vals**: character, determines which EGSEA score values are shown on the map. Default is NULL which does not show anything.
- **x.axis**: character, the x-axis of the summary plot. All the values accepted by the **sort.by** parameter can be used. Default x.axis="p.value".
- **x.cutoff**: numeric, cut-off threshold to filter the gene sets of the summary plots based on the values of the **x.axis**. Default x.cutoff=NULL.
- **use.names**: logical, determines whether to display the GeneSet IDs or GeneSet Names. Default is FALSE.
- **noSig**: numeric, number of significant GO terms to be displayed. A number larger than 5 might not work due to the size of the generated graph.
- **set.name**: character, a vector of gene set names as they appear in `topSets`.
- **id**: character, a vector of gene set IDs as they appear in the `plotSummary`.

Details

The EGSEAResults class is used by `egsea`, `egsea.cnt` and `egsea.ora` to store the results of an EGSEA analysis. This helps in mining the analysis results and generating customized tables and plots.

`limmaTopTable` output can be understood from `limma::topTable`. 
getlimmaResults's output can be manipulated using `limma::topTable` and `limma::topTreat`. `plotHeatmap` fold changes are colored based on the `fc.colors` and only genes that appear in the EGSEA analysis are visualized in the heatmap. Gene names are coloured based on the statistical significance level from limma DE analysis.

`plotSummaryHeatmap` creates a summary heatmap for the rankings of top number gene sets of the comparative analysis across all the contrasts. The `show.vals` score can be displayed on the heatmap for each gene set. This can help to identify gene sets that are highly ranked/significant across multiple contrasts.

`plotSummary` generates a Summary Plot for an EGSEA analysis. Since the EGSEA "Significance Score" is proportional to the p-value and the absolute fold changes, it could be useful to highlight gene sets that have high Significance scores. The blue labels on the summary plot indicate gene sets that do not appear in the top 10 list of gene sets based on the "sort.by" argument (black labels) yet they appear in the top 5 list of gene sets based on the EGSEA "Significance Score". If two contrasts are provided, the rank is calculated based on the "comparison" analysis results and the "Significance Score" is calculated as the mean. If `sort.by = NULL`, the slot `sort.by` of the object is used to order gene sets.

**Value**

$ returns the selected slot.

`topSets` returns a dataframe of top gene sets with the calculated statistics for each if `names_only = FALSE`.

`show` does not return data.

`summary` does not return data.

`limmaTopTable` returns a dataframe.

`getlimmaResults` returns an `MArrayLM` object.

`plotHeatmap` does not return data but creates image and CSV files.

`plotSummaryHeatmap` does not return data but creates image and CSV files.

`plotPathway` does not return data but creates a file.

`plotMethods` does not return data but creates an image file.

`plotSummary` does not return data but creates an image file.

`plotGOGraph` does not return data but creates an image file.

`showSetByName` does not return data.

`showSetByID` does not return data.

`getSetScores` returns a dataframe where rows are gene sets and columns are samples.

**Slots**

`results` list, EGSEA analysis results

`limmaResults` `MArrayLM`, is a limma linear fit model

`contrasts` character, the contrasts defined in the analysis

`sampleSize` numeric, number of samples

`gs.annots` list, the gene set collection annotation index

`baseMethods` character, vector of base GSE methods

`baseInfo` list, additional information on the base methods (e.g., version).
EGSEAResults

combineMethod character, the p-value combining method
sort.by character, the results ordering argument
symbolsMap data.frame, the mapping between Entrez IDs and Gene Symbols
logFC matrix, the logFC matrix of contrasts
report logical, whether the report was generated
report.dir character, the directory of the EGSEA HTML report

Examples

# Example of EGSEAResults
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
print(gsa$baseMethods)

# Example of topSets
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
topSets(gsa, gs.label="kegg", contrast=1, number = 10)
topSets(gsa, gs.label=1, contrast=1, sort.by="ora", number = 10, names.only=FALSE)
topSets(gsa, gs.label="kegg", contrast=0, number = 10)

# Example of show
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
show(gsa)

# Example of summary
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
summary(gsa)

# Example of limmaTopTable
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
colnames(limmaTopTable(gsa))
head(limmaTopTable(gsa))

# Example of getlimmaResults
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
fit = getlimmaResults(gsa)
class(fit)
# Example of plotHeatmap
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
plotHeatmap(gsa, "Asthma", gs.label="kegg")
plotHeatmap(gsa, "Asthma", gs.label="kegg", contrast = "comparison", file.name = "asthma.hm.cmp")

# Example of plotHeatmap
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
plotSummaryHeatmap(gsa, gs.label="kegg")

# Example of plotPathway
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
plotPathway(gsa, gs.label="kegg", "Asthma")
plotPathway(gsa, gs.label="kegg", "Asthma", contrast="comparison", file.name = "asthma.map.cmp")

# Example of plotMethods
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
plotMethods(gsa)

# Example of plotSummary
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
plotSummary(gsa)
plotSummary(gsa, contrast=c(1,2), file.name = "summary.cmp")

# Example of plotGOGraph
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
plotGOGraph(gsa, sort.by="avg.rank")

# Example of showSetByName
library(EGSEAdata)
data(il13.gsa)
gsa = il13.gsa
class(gsa)
showSetByName(gsa, "kegg", "Asthma")

# Example of showSetByID
The GSCollectionIndex class

Description

The GSCollectionIndex class stores an indexed gene set collection. The operator \$extracts a slot from an object of class GSCollectionIndex.

summary displays a brief summary of a gene set collection
show displays the details of a gene set collection
getSetByName retrieves the details of a given gene set indicated by name
getSetByID retrieves the details of a given gene set indicated by ID

Usage

## S4 method for signature 'GSCollectionIndex'
x$name

## S4 method for signature 'GSCollectionIndex'
summary(object)

## S4 method for signature 'GSCollectionIndex'
show(object)

getSetByName(object, set.name)

getSetByID(object, id)

Arguments

x GSCollectionIndex, the indexed gene set collection generated from buildIdx, buildMSigDBIdx, buildKEGGIdx, buildGeneSetDBIdx, and buildCustomIdx.

name character, the slot name

object GSCollectionIndex, the indexed gene set collection generated from buildIdx, buildMSigDBIdx, buildKEGGIdx, buildGeneSetDBIdx, and buildCustomIdx.

set.name character, a vector of gene set names as they appear in topSets.

id character, a vector of gene set IDs as they appear in the plotSummary.
Details

The GSCollectionIndex is used by buildIdx, buildCustomIdx, buildKEGGIdx, buildMSigDBIdx and buildGeneSetDBIdx.

Value

$ returns the selected slot data.
summary does not return data.
show does not return data.
getSetByName returns a list of annotation records
getSetByID returns a list of the annotation records.

Slots

original list, the original gene sets
idx list, the gene set indexes
anno data.frame, the annotations of the gene sets
featureIDs character, vector of the original Entrez IDs that are used in the indexing procedure
species character, the species name
name character, the name of the gene set collection
label character, a label to distinguish this collection
version character, the database version from which the collection was extracted
date character, the update/download date of the database from other collections

Examples

# Example of GSCollectionIndex
library(EGSEAdata)
data(il13.data)
v = il13.data$voom
gs.annots = buildIdx(entrezIDs=rownames(v$E), species="human",
msigdb.gsets="none",
   kegg.updated=FALSE, kegg.exclude = c("Metabolism"))
print(gs.annots[[1]]$name)

# Example of summary
library(EGSEAdata)
data(il13.data)
v = il13.data$voom
gs.annots = buildIdx(entrezIDs=rownames(v$E), species="human",
msigdb.gsets="none",
   kegg.updated=FALSE, kegg.exclude = c("Metabolism"))
summary(gs.annots[[1]])

# Example of show
library(EGSEAdata)
data(il13.data)
v = il13.data$voom
gs.annots = buildIdx(entrezIDs=rownames(v$E), species="human",
msigdb.gsets="none",
   kegg.updated=FALSE, kegg.exclude = c("Metabolism"))
library(EGSEAdata)
data(il13.data)
v = il13.data$voom
gs.annots = buildIdx(entrezIDs=rownames(v$E), species="human",
msigdb.gsets="none",
    kegg.updated=FALSE, kegg.exclude = c("Metabolism"))
getSetByName(gs.annots[[1]], "Asthma")

library(EGSEAdata)
data(il13.data)
v = il13.data$voom
gs.annots = buildIdx(entrezIDs=rownames(v$E), species="human",
msigdb.gsets="none",
    kegg.updated=FALSE, kegg.exclude = c("Metabolism"))
getSetByID(gs.annots[[1]], "hsa04060")
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