

# Package ‘GeneTonic’

April 12, 2022

**Title** Enjoy Analyzing And Integrating The Results From Differential Expression Analysis And Functional Enrichment Analysis

**Version** 1.6.4

**Date** 2022-03-24

**Description** This package provides a Shiny application that aims to combine at different levels the existing pieces of the transcriptome data and results, in a way that makes it easier to generate insightful observations and hypothesis - combining the benefits of interactivity and reproducibility, e.g. by capturing the features and gene sets of interest highlighted during the live session, and creating an HTML report as an artifact where text, code, and output coexist.

**Depends** R (>= 4.0.0)

**Imports** AnnotationDbi, backbone, bs4Dash (>= 2.0.0), circlize, colorspace, colourpicker, ComplexHeatmap, dendextend, DESeq2, dplyr, DT, dynamicTreeCut, expm, ggforce, ggplot2, ggrepel, GO.db, graphics, grDevices, grid, igraph, matrixStats, methods, plotly, RColorBrewer, rintrojs, rlang, rmarkdown, S4Vectors, scales, shiny, shinyAce, shinycssloaders, shinyWidgets, stats, SummarizedExperiment, tidyr, tippy, tools, utils, viridis, visNetwork

**Suggests** knitr, BiocStyle, htmltools, clusterProfiler, macrophage, org.Hs.eg.db, magrittr, testthat (>= 2.1.0)

**License** MIT + file LICENSE

**Encoding** UTF-8

**VignetteBuilder** knitr

**URL** <https://github.com/federicomarini/GeneTonic>

**BugReports** <https://github.com/federicomarini/GeneTonic/issues>

**RoxygenNote** 7.1.2

**Roxygen** list(markdown = TRUE)

**biocViews** GUI, GeneExpression, Software, Transcription, Transcriptomics, Visualization, DifferentialExpression, Pathways, ReportWriting, GeneSetEnrichment, Annotation, GO

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

**git\_branch** RELEASE\_3\_14

**git\_last\_commit** c9ee95b

**git\_last\_commit\_date** 2022-03-24

**Date/Publication** 2022-04-12

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.check_pandoc	<i>Check whether pandoc and pandoc-citeproc are available</i>
---------------	---

---

**Description**

Check whether pandoc and pandoc-citeproc are available

**Usage**

.check\_pandoc(ignore\_pandoc)

**Arguments**

ignore\_pandoc Logical. If TRUE, just give a warning if one of pandoc or pandoc-citeproc is not available. If FALSE, an error is thrown.

**Details**

Credits to the original implementation proposed by Charlotte Sonesson, upon which this function is **heavily** inspired.

**Value**

No value is returned. If pandoc or pandoc-citeproc are missing, either warning or error messages are triggered.

---

checkup_GeneTonic	<i>Checking the input objects for GeneTonic</i>
-------------------	---

---

**Description**

Checking the input objects for GeneTonic, whether these are all set for running the app

**Usage**

```
checkup_GeneTonic(dds, res_de, res_enrich, annotation_obj, verbose = FALSE)
```

**Arguments**

dds	A DESeqDataSet object, normally obtained after running your data through the DESeq2 framework.
res_de	A DESeqResults object. As for the dds parameter, this is also commonly used in the DESeq2 framework.
res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
annotation_obj	A data.frame object, containing two columns, gene_id with a set of unambiguous identifiers (e.g. ENSEMBL ids) and gene_name, containing e.g. HGNC-based gene symbols.
verbose	Logical, to control level of verbosity of the messages generated

**Details**

Some suggestions on the requirements for each parameter are returned in the error messages.

**Value**

Invisible NULL

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
```

```

rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

checkup_GeneTonic(
  dds = dds_macrophage,
  res_de = res_de,
  res_enrich = res_enrich,
  annotation_obj = anno_df
)
# if all is fine, it should return an invisible NULL and a simple message

```

---

checkup\_gtl

*Checking the gtl input object for GeneTonic*

---

### Description

Checking the gtl ("GeneTonic list") input object for GeneTonic, with the correct content and format expected

### Usage

```
checkup_gtl(gtl, verbose = FALSE)
```

### Arguments

**gtl** A DESeqDataSet object, normally obtained after running your data through the DESeq2 framework. This list should contain

- in the dds slot: A DESeqDataSet object

- in the `res_de`: A `DESeqResults` object
- in the `res_enrich`: A `data.frame` object, storing the result of the functional enrichment analysis
- in the `annotation_obj`: A `data.frame` object, containing two columns, `gene_id` with a set of unambiguous identifiers (e.g. ENSEMBL ids) and `gene_name`, containing e.g. HGNC-based gene symbols.

`verbose` Logical, to control level of verbosity of the messages generated

## Details

Some suggestions on the requirements for the `gtl` are returned in the error messages.

## Value

Invisible NULL

## Examples

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

gtl <- list(
```

```
    dds = dds_macrophage,  
    res_de = res_de,  
    res_enrich = res_enrich,  
    annotation_obj = anno_df  
  )  
  
  checkup_gtl(gtl)  
  # if all is fine, it should return an invisible NULL and a simple message
```

---

check\_colors

*Check colors*

---

## Description

Check correct specification of colors

## Usage

```
check_colors(x)
```

## Arguments

x                    A vector of strings specifying colors

## Details

This is a vectorized version of [grDevices::col2rgb\(\)](#)

## Value

A vector of logical values, one for each specified color - TRUE if the color is specified correctly

## Examples

```
# simple case  
mypal <- c("steelblue", "#FF1100")  
check_colors(mypal)  
mypal2 <- rev(  
  scales::alpha(  
    colorRampPalette(RColorBrewer::brewer.pal(name = "RdYlBu", 11))(50), 0.4  
  )  
)  
check_colors(mypal2)  
# useful with long vectors to check at once if all cols are fine  
all(check_colors(mypal2))
```

---

cluster\_markov

*Markov Clustering (MCL) for community detection*


---

### Description

This function implements the Markov Clustering (MCL) algorithm for finding community structure, in an analogous way to other existing algorithms in igraph.

### Usage

```
cluster_markov(
  g,
  add_self_loops = TRUE,
  loop_value = 1,
  mcl_expansion = 2,
  mcl_inflation = 2,
  allow_singletons = TRUE,
  max_iter = 100,
  return_node_names = TRUE,
  return_esm = FALSE
)
```

### Arguments

<code>g</code>	The input graph object
<code>add_self_loops</code>	Logical, whether to add self-loops to the matrix by setting the diagonal to <code>loop_value</code>
<code>loop_value</code>	Numeric, the value to use for self-loops
<code>mcl_expansion</code>	Numeric, cluster expansion factor for the Markov clustering iteration - defaults to 2
<code>mcl_inflation</code>	Numeric, cluster inflation factor for the Markov clustering iteration - defaults to 2
<code>allow_singletons</code>	Logical; if TRUE, single isolated vertices are allowed to form their own cluster. If set to FALSE, all clusters of size = 1 are grouped in one cluster (to be interpreted as background noise).
<code>max_iter</code>	Numeric value for the maximum number of iterations for the Markov clustering
<code>return_node_names</code>	Logical, if the graph is named and set to TRUE, returns the node names.
<code>return_esm</code>	Logical, controlling whether the equilibrium state matrix should be returned

### Details

This implementation has been driven by the nice explanations provided in

- [https://sites.cs.ucsb.edu/~xian/classes/CS595D-2009winter/MCL\\_Presentation2.pdf](https://sites.cs.ucsb.edu/~xian/classes/CS595D-2009winter/MCL_Presentation2.pdf)



- <https://medium.com/analytics-vidhya/demystifying-markov-clustering-aeb6cdabbfc7>
- [https://github.com/GuyAllard/markov\\_clustering](https://github.com/GuyAllard/markov_clustering) (python implementation)

More info on the MCL: <https://micans.org/mcl/index.html>, and [https://micans.org/mcl/sec\\_description1.html](https://micans.org/mcl/sec_description1.html)

## Value

This function returns a `communities` object, containing the numbers of the assigned membership (in the slot `membership`). Please see the `igraph::communities()` manual page for additional details

## References

van Dongen, S.M., Graph clustering by flow simulation (2000) PhD thesis, Utrecht University Repository - <https://dspace.library.uu.nl/handle/1874/848>

Enright AJ, van Dongen SM, Ouzounis CA, An efficient algorithm for large-scale detection of protein families (2002) *Nucleic Acids Research*, Volume 30, Issue 7, 1 April 2002, Pages 1575–1584, <https://doi.org/10.1093/nar/30.7.1575>

## Examples

```
library("igraph")
g <- make_full_graph(5) %du% make_full_graph(5) %du% make_full_graph(5)
g <- add_edges(g, c(1, 6, 1, 11, 6, 11))
cluster_markov(g)
V(g)$color <- cluster_markov(g)$membership
plot(g)
```

---

`create_jaccard_matrix` *Compute the overlap matrix for enrichment results*

---

## Description

Compute the overlap matrix for enrichment results, based on the Jaccard Index between each pair of sets

## Usage

```
create_jaccard_matrix(
  res_enrich,
  gtl = NULL,
  n_gs = nrow(res_enrich),
  gs_ids = NULL,
  return_sym = FALSE
)
```

**Arguments**

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to see the formatting requirements.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
n_gs	Integer value, corresponding to the maximal number of gene sets to be included (from the top ranked ones). Defaults to the number of rows of res_enrich
gs_ids	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be included, additionally to the ones specified via n_gs. Defaults to NULL.
return_sym	Logical, whether to return the symmetrical matrix or just the upper triangular - as needed by <a href="#">enrichment_map()</a> , for example.

**Value**

A matrix with the kappa scores between gene sets

**See Also**

[gs\\_mds\(\)](#), [enrichment\\_map\(\)](#)

**Examples**

```
# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOTableResult(topgoDE_macrophage_IFNg_vs_naive)

jmat <- create_jaccard_matrix(res_enrich[1:200, ])
dim(jmat)
```

---

create\_kappa\_matrix    *Compute the kappa matrix for enrichment results*

---

**Description**

Compute the kappa matrix for enrichment results, as a measure of overlap

**Usage**

```
create_kappa_matrix(
  res_enrich,
  gtl = NULL,
  n_gs = nrow(res_enrich),
  gs_ids = NULL
)
```

**Arguments**

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to see the formatting requirements.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
n_gs	Integer value, corresponding to the maximal number of gene sets to be included (from the top ranked ones). Defaults to the number of rows of res_enrich
gs_ids	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be included, additionally to the ones specified via n_gs. Defaults to NULL.

**Value**

A matrix with the kappa scores between gene sets

**See Also**

[gs\\_mds\(\)](#)

**Examples**

```
# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)

kmat <- create_kappa_matrix(res_enrich[1:200, ])
dim(kmat)
```

---

describe\_gtl

*Describe a GeneTonic list*

---

**Description**

Obtain a quick textual overview of the essential features of the components of the GeneTonic list object

**Usage**

```
describe_gtl(gtl)
```

**Arguments**

gtl	A GeneTonic-list object, containing in its named slots the required dds, res_de, res_enrich, and annotation_obj
-----	---

**Value**

Invisible NULL - the information is displayed as a message in the console

---

deseqresult2df	<i>Generate a table from the DESeq2 results</i>
----------------	---

---

**Description**

Generate a tidy table with the results of DESeq2

**Usage**

```
deseqresult2df(res_de, FDR = NULL)
```

**Arguments**

res_de	A DESeqResults object.
FDR	Numeric value, specifying the significance level for thresholding adjusted p-values. Defaults to NULL, which would return the full set of results without performing any subsetting based on FDR.

**Value**

A tidy data.frame with the results from differential expression, sorted by adjusted p-value. If FDR is specified, the table contains only genes with adjusted p-value smaller than the value.

**Examples**

```
data(res_de_macrophage, package = "GeneTonic")
head(res_macrophage_IFNg_vs_naive)
res_df <- deseqresult2df(res_macrophage_IFNg_vs_naive)
head(res_df)
```

---

distill_enrichment	<i>Distill enrichment results</i>
--------------------	-----------------------------------

---

**Description**

Distill the main topics from the enrichment results, based on the graph derived from constructing an enrichment map

## Usage

```
distill_enrichment(  
  res_enrich,  
  res_de,  
  annotation_obj,  
  gtl = NULL,  
  n_gs = nrow(res_enrich),  
  cluster_fun = "cluster_markov"  
)
```

## Arguments

<code>res_enrich</code>	A data.frame object, storing the result of the functional enrichment analysis.
<code>res_de</code>	A DESeqResults object. As for the <code>dds</code> parameter, this is also commonly used in the DESeq2 framework.
<code>annotation_obj</code>	A data.frame object, containing two columns, <code>gene_id</code> with a set of unambiguous identifiers (e.g. ENSEMBL ids) and <code>gene_name</code> , containing e.g. HGNC-based gene symbols.
<code>gtl</code>	A GeneTonic-list object, containing in its slots the arguments specified above: <code>dds</code> , <code>res_de</code> , <code>res_enrich</code> , and <code>annotation_obj</code> - the names of the list <i>must</i> be specified following the content they are expecting
<code>n_gs</code>	Integer value, corresponding to the maximal number of gene sets to be used.
<code>cluster_fun</code>	Character, referring to the name of the function used for the community detection in the enrichment map graph. Could be one of "cluster_markov", "cluster_louvain", or "cluster_walktrap", as they all return a communities object.

## Value

A list containing three objects:

- the distilled table of enrichment, `distilled_table`, where the new meta-genesets are identified and defined, specifying e.g. the names of each component, and the genes associated to these.
- the distilled graph for the enrichment map, `distilled_em`, with the information on the membership
- the original `res_enrich`, augmented with the information of the membership related to the meta-genesets

## Examples

```
library("macrophage")  
library("DESeq2")  
library("org.Hs.eg.db")  
library("AnnotationDbi")  
  
# dds object  
data("gse", package = "macrophage")
```

```

dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

distilled <- distill_enrichment(res_enrich,
  res_de,
  annotation_obj,
  n_gs = 100,
  cluster_fun = "cluster_markov"
)
colnames(distilled$distilled_table)
distilled$distilled_em

```

---

enhance\_table

*Visually enhances a functional enrichment result table*

---

## Description

Creates a visual summary for the results of a functional enrichment analysis, by displaying also the components of each gene set and their expression change in the contrast of interest

## Usage

```

enhance_table(
  res_enrich,
  res_de,
  annotation_obj,
  gtl = NULL,
  n_gs = 50,

```

```

    gs_ids = NULL,
    chars_limit = 70,
    plot_title = NULL
  )

```

### Arguments

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <code>GeneTonic()</code> , to check the formatting requirements (a minimal set of columns should be present).
res_de	A DESeqResults object.
annotation_obj	A data.frame object with the feature annotation. information, with at least two columns, <code>gene_id</code> and <code>gene_name</code> .
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: <code>dds</code> , <code>res_de</code> , <code>res_enrich</code> , and <code>annotation_obj</code> - the names of the list <i>must</i> be specified following the content they are expecting
n_gs	Integer value, corresponding to the maximal number of gene sets to be displayed.
gs_ids	Character vector, containing a subset of <code>gs_id</code> as they are available in <code>res_enrich</code> . Lists the gene sets to be displayed.
chars_limit	Integer, number of characters to be displayed for each geneset name.
plot_title	Character string, used as title for the plot. If left NULL, it defaults to a general description of the plot and of the DE contrast

### Value

A ggplot object

### Examples

```

library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),

```

```

stringsAsFactors = FALSE,
row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)
enhance_table(res_enrich,
  res_de,
  anno_df,
  n_gs = 10
)

```

---

enrichment\_map

*Creates an enrichment map for the results of functional enrichment*


---

### Description

Generates a graph for the enrichment map, combining information from `res_enrich` and `res_de`. This object can be further plotted, e.g. statically via `igraph::plot.igraph()`, or dynamically via `visNetwork::visIgraph()`

### Usage

```

enrichment_map(
  res_enrich,
  res_de,
  annotation_obj,
  gtl = NULL,
  n_gs = 50,
  gs_ids = NULL,
  overlap_threshold = 0.1,
  scale_edges_width = 200,
  scale_nodes_size = 5,
  color_by = "gs_pvalue"
)

```

### Arguments

<code>res_enrich</code>	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <code>GeneTonic()</code> , to check the formatting requirements (a minimal set of columns should be present).
<code>res_de</code>	A <code>DESeqResults</code> object.



annotation_obj	A data.frame object with the feature annotation information, with at least two columns, gene_id and gene_name.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
n_gs	Integer value, corresponding to the maximal number of gene sets to be displayed
gs_ids	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be displayed.
overlap_threshold	Numeric value, between 0 and 1. Defines the threshold to be used for removing edges in the enrichment map - edges below this value will be excluded from the final graph. Defaults to 0.1.
scale_edges_width	A numeric value, to define the scaling factor for the edges between nodes. Defaults to 200 (works well chained to visNetwork functions).
scale_nodes_size	A numeric value, to define the scaling factor for the node sizes. Defaults to 5 - works well chained to visNetwork functions.
color_by	Character, specifying the column of res_enrich to be used for coloring the plotted gene sets. Defaults to gs_pvalue.

**Value**

An igraph object to be further manipulated or processed/plotted

**See Also**

[GeneTonic\(\)](#) embeds an interactive visualization for the enrichment map

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL")
)
```

```

),
stringsAsFactors = FALSE,
row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

em <- enrichment_map(res_enrich,
  res_de,
  anno_df,
  n_gs = 20
)

em

# could be viewed interactively with
# library("visNetwork")
# library("magrittr")
# em %>%
#   visIgraph() %>%
#   visOptions(highlightNearest = list(enabled = TRUE,
#                                       degree = 1,
#                                       hover = TRUE),
#               nodesIdSelection = TRUE)

```

---

enrichr\_output\_macrophage

*A sample output from Enrichr*

---

## Description

A sample output object as created from a call to Enrichr, with the interface provided by enrichR - using the `enrichr()` function

## Details

This object has been created on the data from the macrophage package by analyzing downstream the differentially expressed genes when comparing IFNg treated samples vs naive samples, accounting for the different cell lines included.

Details on how this object has been created are included in the `create_gt_data.R` script, included in the `scripts` folder of the GeneTonic package.

## References

Alasoo, et al. "Shared genetic effects on chromatin and gene expression indicate a role for enhancer priming in immune response", Nature Genetics, January 2018 doi: 10.1038/s41588-018-0046-7.

## See Also

Other pathway-analysis-results: [gostres\\_macrophage](#), [topgoDE\\_macrophage\\_IFNg\\_vs\\_naive](#)

---

export_for_iSEE	<i>export_for_iSEE</i>
-----------------	------------------------

---

## Description

Combine data from a typical DESeq2 run

## Usage

```
export_for_iSEE(dds, res_de, gtl = NULL)
```

## Arguments

dds	A <a href="#">DESeqDataSet</a> object.
res_de	A <a href="#">DESeqResults</a> object.
gtl	A <a href="#">GeneTonic</a> -list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting

## Details

Combines the [DESeqDataSet](#) input and [DESeqResults](#) into a [SummarizedExperiment](#) object, which can be readily explored with [iSEE](#).

A typical usage would be after running the [DESeq2](#) pipeline and/or after exploring the functional enrichment results with [GeneTonic\(\)](#)

## Value

A [SummarizedExperiment](#) object, with raw counts, normalized counts, and variance-stabilizing transformed counts in the assay slots; and with `colData` and `rowData` extracted from the corresponding input parameters - mainly the results for differential expression analysis.

**Examples**

```

library("macrophage")
library("DESeq2")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# now everything is in place to launch the app
# dds_macrophage <- DESeq2::DESeq(dds_macrophage)
se_macrophage <- export_for_iSEE(dds_macrophage, res_de)
# iSEE(se_macrophage)

```

---

export\_to\_sif

*Export to sif*


---

**Description**

Export a graph to a Simple Interaction Format file

**Usage**

```
export_to_sif(g, sif_file = "", edge_label = "relates_to")
```

**Arguments**

<code>g</code>	An igraph object
<code>sif_file</code>	Character string, the path to the file where to save the exported graph as .sif file
<code>edge_label</code>	Character string, defining the name of the interaction type. Defaults here to "relates_to"

**Value**

Returns the path to the exported file, invisibly

**Examples**

```

library("igraph")
g <- make_full_graph(5) %du% make_full_graph(5) %du% make_full_graph(5)
g <- add_edges(g, c(1, 6, 1, 11, 6, 11))
export_to_sif(g, tempfile())

```

fgseaRes

*A sample output from fgsea***Description**

A sample output object as created from a call to the `fgsea()` function, in the `fgsea` package, as a practical framework for performing GSEA

**Details**

This object has been created on the data from the `macrophage` package by analyzing downstream the differentially expressed genes when comparing IFN $\gamma$  treated samples vs naive samples, accounting for the different cell lines included.

Details on how this object has been created are included in the `create_gt_data.R` script, included in the `scripts` folder of the `GeneTonic` package.

**References**

Alasoo, et al. "Shared genetic effects on chromatin and gene expression indicate a role for enhancer priming in immune response", *Nature Genetics*, January 2018 doi: 10.1038/s41588-018-0046-7.

geneinfo\_2\_html

*Information on a gene***Description**

Assembles information, in HTML format, regarding a gene symbol identifier

**Usage**

```
geneinfo_2_html(gene_id, res_de = NULL)
```

**Arguments**

<code>gene_id</code>	Character specifying the gene identifier for which to retrieve information
<code>res_de</code>	A <code>DESeqResults</code> object, storing the result of the differential expression analysis. If not provided, the experiment-related information is not shown, and only some generic info on the identifier is displayed. The information about the gene is retrieved by matching on the <code>SYMBOL</code> column, which should be provided in <code>res_de</code> .

**Details**

Creates links to the NCBI and the GeneCards databases

**Value**

HTML content related to a gene identifier, to be displayed in web applications (or inserted in Rmd documents)

**Examples**

```
geneinfo_2_html("ACTB")
geneinfo_2_html("Pf4")
```

---

GeneTonic

*GeneTonic*


---

**Description**

GeneTonic, main function for the Shiny app

**Usage**

```
GeneTonic(dds, res_de, res_enrich, annotation_obj, gtl = NULL, project_id = "")
```

**Arguments**

dds	A DESeqDataSet object, normally obtained after running your data through the DESeq2 framework.
res_de	A DESeqResults object. As for the dds parameter, this is also commonly used in the DESeq2 framework.
res_enrich	A data.frame object, storing the result of the functional enrichment analysis. Required columns for enjoying the full functionality of <code>GeneTonic()</code> include: <ul style="list-style-type: none"> <li>• a gene set identifier (e.g. GeneOntology id, <code>gs_id</code>) and its term description (<code>gs_description</code>)</li> <li>• a numeric value for the significance of the enrichment (<code>gs_pvalue</code>)</li> <li>• a column named <code>gs_genes</code> containing a comma separated vector of the gene names associated to the term, one for each term</li> <li>• the number of genes in the geneset of interest detected as differentially expressed (<code>gs_de_count</code>), or in the background set of genes (<code>gs_bg_count</code>)</li> </ul> See <code>shake_topG0tableResult()</code> or <code>shake_enrichResult()</code> for examples of such formatting helpers
annotation_obj	A data.frame object, containing two columns, <code>gene_id</code> with a set of unambiguous identifiers (e.g. ENSEMBL ids) and <code>gene_name</code> , containing e.g. HGNC-based gene symbols. This object can be constructed via the <code>org.eg.XX.db</code> packages, e.g. with convenience functions such as <code>pcaExplorer::get_annotation_orgdb()</code> .
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: <code>dds</code> , <code>res_de</code> , <code>res_enrich</code> , and <code>annotation_obj</code> - the names of the list <i>must</i> be specified following the content they are expecting
project_id	A character string, which can be considered as an identifier for the set/session, and will be e.g. used in the title of the report created via <code>happy_hour()</code>

**Value**

A Shiny app object is returned, for interactive data exploration

**Author(s)**

Federico Marini

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

# now everything is in place to launch the app
if (interactive()) {
  GeneTonic(
    dds = dds_macrophage,
    res_de = res_de,
    res_enrich = res_enrich,
    annotation_obj = anno_df,
    project_id = "myexample"
  )
}
```

```
# alternatively...
gtl_macrophage <- GeneTonic_list(
  dds = dds_macrophage,
  res_de = res_de,
  res_enrich = res_enrich,
  annotation_obj = anno_df
)
# GeneTonic(gtl = gtl_macrophage)
```

---

GeneTonic-pkg

*GeneTonic*

---

### Description

GeneTonic is a Bioconductor package that provides an interactive Shiny-based graphical user interface for streamlining the interpretation of RNA-seq data

### Details

GeneTonic simplifies and optimizes the integration of all components of Differential Expression analysis, with functional enrichment analysis and the original expression quantifications. It does so in a way that makes it easier to generate insightful observations and hypothesis - combining the benefits of interactivity and reproducibility, e.g. by capturing the features and gene sets of interest highlighted during the live session, and creating an HTML report as an artifact where text, code, and output coexist.

### Author(s)

Federico Marini <marinif@uni-mainz.de>

---

GeneTonic\_list

*Create a list for GeneTonic*

---

### Description

Create a list for GeneTonic from the single required components.

### Usage

```
GeneTonic_list(dds, res_de, res_enrich, annotation_obj)
```



**Arguments**

dds	A DESeqDataSet object, normally obtained after running your data through the DESeq2 framework.
res_de	A DESeqResults object. As for the dds parameter, this is also commonly used in the DESeq2 framework.
res_enrich	A data.frame object, storing the result of the functional enrichment analysis. Required columns for enjoying the full functionality of <code>GeneTonic()</code> include: <ul style="list-style-type: none"> <li>• a gene set identifier (e.g. GeneOntology id, <code>gs_id</code>) and its term description (<code>gs_description</code>)</li> <li>• a numeric value for the significance of the enrichment (<code>gs_pvalue</code>)</li> <li>• a column named <code>gs_genes</code> containing a comma separated vector of the gene names associated to the term, one for each term</li> <li>• the number of genes in the geneset of interest detected as differentially expressed (<code>gs_de_count</code>), or in the background set of genes (<code>gs_bg_count</code>) See <code>shake_topG0tableResult()</code> or <code>shake_enrichResult()</code> for examples of such formatting helpers</li> </ul>
annotation_obj	A data.frame object, containing two columns, <code>gene_id</code> with a set of unambiguous identifiers (e.g. ENSEMBL ids) and <code>gene_name</code> , containing e.g. HGNC-based gene symbols. This object can be constructed via the <code>org.eg.XX.db</code> packages, e.g. with convenience functions such as <code>pcaExplorer::get_annotation_orgdb()</code> .

**Details**

Having this dedicated function saves the pain of remembering which names the components of the list should have.

**Value**

A GeneTonic-list object, containing in its named slots the arguments specified above: `dds`, `res_de`, `res_enrich`, and `annotation_obj` - the names of the list are specified following the requirements for using it as single input to `GeneTonic()`

**Author(s)**

Federico Marini

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)
```

```

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

gtl_macrophage <- GeneTonic_list(
  dds = dds_macrophage,
  res_de = res_de,
  res_enrich = res_enrich,
  annotation_obj = anno_df
)

# now everything is in place to launch the app
if (interactive()) {
  GeneTonic(gtl = gtl_macrophage)
}

```

---

gene\_plot

*Plot expression values for a gene*

---

### Description

Plot expression values (e.g. normalized counts) for a gene of interest, grouped by experimental group(s) of interest

### Usage

```

gene_plot(
  dds,
  gene,
  intgroup = "condition",
  assay = "counts",

```

```

    annotation_obj = NULL,
    normalized = TRUE,
    transform = TRUE,
    labels_display = TRUE,
    labels_repel = TRUE,
    plot_type = "auto",
    return_data = FALSE,
    gtl = NULL
  )

```

### Arguments

dds	A DESeqDataSet object, normally obtained after running your data through the DESeq2 framework.
gene	Character, specifies the identifier of the feature (gene) to be plotted
intgroup	A character vector of names in colData(dds) to use for grouping. Note: the vector components should be categorical variables.
assay	Character, specifies with assay of the dds object to use for reading out the expression values. Defaults to "counts".
annotation_obj	A data.frame object with the feature annotation information, with at least two columns, gene_id and gene_name.
normalized	Logical value, whether the expression values should be normalized by their size factor. Defaults to TRUE, applies when assay is "counts"
transform	Logical value, corresponding whether to have log scale y-axis or not. Defaults to TRUE.
labels_display	Logical value. Whether to display the labels of samples, defaults to TRUE.
labels_repel	Logical value. Whether to use ggrepel's functions to place labels; defaults to TRUE
plot_type	Character, one of "auto", "jitteronly", "boxplot", "violin", or "sina". Defines the type of geom_ to be used for plotting. Defaults to auto, which in turn chooses one of the layers according to the number of samples in the smallest group defined via intgroup
return_data	Logical, whether the function should just return the data.frame of expression values and covariates for custom plotting. Defaults to FALSE.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting

### Details

The result of this function can be fed directly to `plotly::ggplotly()` for interactive visualization, instead of the static ggplot viz.

### Value

A ggplot object

**Examples**

```

library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

gene_plot(dds_macrophage,
  gene = "ENSG00000125347",
  intgroup = "condition",
  annotation_obj = anno_df
)

```

---

`get_aggrscores`*Compute aggregated scores for gene sets*

---

**Description**

Computes for each gene set in the `res_enrich` object a Z score and an aggregated score (using the `log2FoldChange` values, provided in the `res_de`)

**Usage**

```
get_aggrscores(res_enrich, res_de, annotation_obj, gtl = NULL, aggrfun = mean)
```

**Arguments**

`res_enrich` A `data.frame` object, storing the result of the functional enrichment analysis. See more in the main function, `GeneTonic()`, to check the formatting requirements (a minimal set of columns should be present).

`res_de` A `DESeqResults` object.

`annotation_obj` A `data.frame` object with the feature annotation information, with at least two columns, `gene_id` and `gene_name`.

gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
aggrfun	Specifies the function to use for aggregating the scores for each term. Common values could be mean or median.

### Value

A data.frame with the same columns as provided in the input, with additional information on the `z_score` and the `aggr_score` for each gene set. This information is used by other functions such as `gs_volcano()` or `enrichment_map()`

### See Also

`gs_volcano()` and `enrichment_map()` make efficient use of the computed aggregated scores

### Examples

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)

res_enrich <- get_aggrscores(
  res_enrich,
  res_de,
```

```

    anno_df
  )

```

---

get\_expression\_values *Get expression values*

---

## Description

Extract expression values, with the possibility to select other assay slots

## Usage

```

get_expression_values(
  dds,
  gene,
  intgroup,
  assay = "counts",
  normalized = TRUE,
  gtl = NULL
)

```

## Arguments

dds	A DESeqDataSet object, normally obtained after running your data through the DESeq2 framework.
gene	Character, specifies the identifier of the feature (gene) to be extracted
intgroup	A character vector of names in colData(dds) to use for grouping.
assay	Character, specifies with assay of the dds object to use for reading out the expression values. Defaults to "counts".
normalized	Logical value, whether the expression values should be normalized by their size factor. Defaults to TRUE, applies when assay is "counts"
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting

## Value

A tidy data.frame with the expression values and covariates for further processing

## Examples

```

library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object

```

```

data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

df_exp <- get_expression_values(dds_macrophage,
  gene = "ENSG00000125347",
  intgroup = "condition"
)
head(df_exp)

```

ggs\_backbone

*Extract the backbone for the gene-geneset graph***Description**

Extract the backbone for the gene-geneset graph, either for the genes or for the genesets

**Usage**

```

ggs_backbone(
  res_enrich,
  res_de,
  annotation_obj = NULL,
  gtl = NULL,
  n_gs = 15,
  gs_ids = NULL,
  bb_on = c("genesets", "features"),
  bb_method = c("sdsm", "fdsm", "fixedrow"),
  bb_extract_alpha = 0.05,
  bb_extract_fwer = c("none", "bonferroni", "holm"),
  bb_fullinfo = FALSE,
  bb_remove_singletons = TRUE,
  color_graph = TRUE,
  color_by_geneset = "z_score",
  color_by_feature = "log2FoldChange",
  ...
)

```

**Arguments**

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
res_de	A DESeqResults object.
annotation_obj	A data.frame object with the feature annotation information, with at least two columns, gene_id and gene_name.

gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
n_gs	Integer value, corresponding to the maximal number of gene sets to be included
gs_ids	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be included in addition to the top ones (via n_gs)
bb_on	A character string, either "genesets" or "features", to specify which entity should be based the backbone graph on.
bb_method	A character string, referring to the function to be called ( from the backbone package) for computing the backbone of the specified bipartite graph. Defaults to "sdsm", as recommended in the backbone package.
bb_extract_alpha	A numeric value, specifying the significance level to use when detecting the backbone of the network
bb_extract_fwer	A character string, defaulting to "none", specifying which method to use for the multiple testing correction for controlling the family-wise error rate
bb_fullinfo	Logical value, determining what will be returned as output: either a simple igraph object with the graph backbone (if set to FALSE), or a list object containing also the backbone object, and the gene-geneset graph used for the computation (if TRUE)
bb_remove_singletons	Logical value, defines whether to remove or leave in the returned graph the nodes that are not connected to other vertices
color_graph	Logical value, specifies whether to use information about genesets or features to colorize the nodes, e.g. for this info to be used in interactive versions of the graph
color_by_geneset	Character string, corresponding to the column in res_enrich to be used for coloring the nodes if bb_on is set to "genesets". Defaults to the "z_score", which can be obtained via get_aggrscores()
color_by_feature	Character string, corresponding to the column in res_de to be used for coloring the nodes if bb_on is set to "features". Defaults to the "log2FoldChange", which should be normally included in a DESeqResults object.
...	Additional parameters to be passed internally

### Value

According to the bb\_fullinfo, either a simple igraph object with the graph backbone, or a named list object containing:

- the igraph of the extracted backbone
- the backbone object itself
- the gene-geneset graph used for the computation



**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

ggs_bbg <- ggs_backbone(res_enrich,
  res_de,
  anno_df,
  n_gs = 50,
  bb_on = "genesets",
  color_graph = TRUE,
  color_by_geneset = "z_score"
)
plot(ggs_bbg)

# if desired, one can also plot the interactive version
visNetwork::visIgraph(ggs_bbg)
```

**Description**

Construct a gene-geneset-graph from the results of a functional enrichment analysis

**Usage**

```
ggs_graph(
  res_enrich,
  res_de,
  annotation_obj = NULL,
  gtl = NULL,
  n_gs = 15,
  gs_ids = NULL,
  prettify = TRUE,
  geneset_graph_color = "gold",
  genes_graph_colpal = NULL
)
```

**Arguments**

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
res_de	A DESeqResults object.
annotation_obj	A data.frame object with the feature annotation information, with at least two columns, gene_id and gene_name.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
n_gs	Integer value, corresponding to the maximal number of gene sets to be included
gs_ids	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be included in addition to the top ones (via n_gs)
prettify	Logical, controlling the aspect of the returned graph object. If TRUE (default value), different shapes of the nodes are returned, based on the node type
geneset_graph_color	Character value, specifying which color should be used for the fill of the shapes related to the gene sets.
genes_graph_colpal	A vector of colors, also provided with their hex string, to be used as a palette for coloring the gene nodes. If unspecified, defaults to a color ramp palette interpolating from blue through yellow to red.

**Value**

An igraph object to be further manipulated or processed/plotted (e.g. via [igraph::plot.igraph\(\)](#) or [visNetwork::visIgraph\(\)](#))

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

ggs <- ggs_graph(
  res_enrich,
  res_de,
  anno_df
)

ggs

#' # could be viewed interactively with
# library(visNetwork)
# library(magrittr)
# ggs %>%
#   visIgraph() %>%
#   visOptions(highlightNearest = list(enabled = TRUE,
#     degree = 1,
#     hover = TRUE),
#   nodesIdSelection = TRUE)
```

---

gostres\_macrophage      *A sample output from g:Profiler*

---

### Description

A sample output object as created from a call to `g:Profiler`, with the interface provided by `gprofiler2` - using the `gost()` function

### Details

This object has been created on the data from the `macrophage` package by analyzing downstream the differentially expressed genes when comparing IFN $\gamma$  treated samples vs naive samples, accounting for the different cell lines included.

Details on how this object has been created are included in the `create_gt_data.R` script, included in the `scripts` folder of the `GeneTonic` package.

### References

Alasoo, et al. "Shared genetic effects on chromatin and gene expression indicate a role for enhancer priming in immune response", *Nature Genetics*, January 2018 doi: 10.1038/s41588-018-0046-7.

### See Also

Other pathway-analysis-results: [enrichr\\_output\\_macrophage](#), [topgoDE\\_macrophage\\_IFN \$\gamma\$ \\_vs\\_naive](#)

---

go\_2\_html      *Information on a GeneOntology identifier*

---

### Description

Assembles information, in HTML format, regarding a Gene Ontology identifier

### Usage

```
go_2_html(go_id, res_enrich = NULL)
```

### Arguments

<code>go_id</code>	Character, specifying the GeneOntology identifier for which to retrieve information
<code>res_enrich</code>	A <code>data.frame</code> object, storing the result of the functional enrichment analysis. If not provided, the experiment-related information is not shown, and only some generic info on the identifier is displayed. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).

**Details**

Also creates a link to the AmiGO database

**Value**

HTML content related to a GeneOntology identifier, to be displayed in web applications (or inserted in Rmd documents)

**Examples**

```
go_2_html("GO:0002250")
go_2_html("GO:0043368")
```

---

gs\_alluvial

*Alluvial (sankey) plot for a set of genesets and the associated genes*

---

**Description**

Generate an interactive alluvial plot linking genesets to their associated genes

**Usage**

```
gs_alluvial(
  res_enrich,
  res_de,
  annotation_obj,
  gtl = NULL,
  n_gs = 5,
  gs_ids = NULL
)
```

```
gs_sankey(
  res_enrich,
  res_de,
  annotation_obj,
  gtl = NULL,
  n_gs = 5,
  gs_ids = NULL
)
```

**Arguments**

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
res_de	A DESeqResults object.

**annotation\_obj** A data.frame object with the feature annotation information, with at least two columns, gene\_id and gene\_name.  
**gtl** A GeneTonic-list object, containing in its slots the arguments specified above: dds, res\_de, res\_enrich, and annotation\_obj - the names of the list *must* be specified following the content they are expecting  
**n\_gs** Integer value, corresponding to the maximal number of gene sets to be displayed  
**gs\_ids** Character vector, containing a subset of gs\_id as they are available in res\_enrich. Lists the gene sets to be displayed.

### Value

A plotly object

### Examples

```

library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOTableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

gs_alluvial(
  res_enrich = res_enrich,
  res_de = res_de,
  annotation_obj = anno_df,

```

```

    n_gs = 4
  )
# or using the alias...
gs_sankey(
  res_enrich = res_enrich,
  res_de = res_de,
  annotation_obj = anno_df,
  n_gs = 4
)

```

---

gs\_dendro

*Dendrogram of the gene set enrichment results*


---

### Description

Calculate (and plot) the dendrogram of the gene set enrichment results

### Usage

```

gs_dendro(
  res_enrich,
  gtl = NULL,
  n_gs = nrow(res_enrich),
  gs_ids = NULL,
  gs_dist_type = "kappa",
  clust_method = "ward.D2",
  color_leaves_by = "z_score",
  size_leaves_by = "gs_pvalue",
  color_branches_by = "clusters",
  create_plot = TRUE
)

```

### Arguments

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to see the formatting requirements.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
n_gs	Integer value, corresponding to the maximal number of gene sets to be included (from the top ranked ones). Defaults to the number of rows of res_enrich
gs_ids	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be included, additionally to the ones specified via n_gs. Defaults to NULL.
gs_dist_type	Character string, specifying which type of similarity (and therefore distance measure) will be used. Defaults to kappa, which uses <a href="#">create_kappa_matrix()</a>

`clust_method` Character string defining the agglomeration method to be used for the hierarchical clustering. See `stats::hclust()` for details, defaults to `ward.D2`

`color_leaves_by` Character string, which columns of `res_enrich` will define the color of the leaves. Defaults to `z_score`

`size_leaves_by` Character string, which columns of `res_enrich` will define the size of the leaves. Defaults to the `gs_pvalue`

`color_branches_by` Character string, which columns of `res_enrich` will define the color of the branches. Defaults to `clusters`, which calls `dynamicTreeCut::cutreeDynamic()` to define the clusters

`create_plot` Logical, whether to create the plot as well.

### Value

A dendrogram object is returned invisibly, and a plot can be generated as well on that object.

### Examples

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)
```



```
gs_dendro(res_enrich,
  n_gs = 100
)
```

---

gs\_fuzzyclustering      *Compute fuzzy clusters of gene sets*

---

## Description

Compute fuzzy clusters of different gene sets, aiming to identify grouped categories that can better represent the distinct biological themes in the enrichment results

## Usage

```
gs_fuzzyclustering(
  res_enrich,
  gtl = NULL,
  n_gs = nrow(res_enrich),
  gs_ids = NULL,
  similarity_matrix = NULL,
  similarity_threshold = 0.35,
  fuzzy_seeding_initial_neighbors = 3,
  fuzzy_multilinkage_rule = 0.5
)
```

## Arguments

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
n_gs	Integer value, corresponding to the maximal number of gene sets to be displayed
gs_ids	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be displayed.
similarity_matrix	A similarity matrix between gene sets. Can be e.g. computed with <a href="#">create_kappa_matrix()</a> or <a href="#">create_jaccard_matrix()</a> or a similar function, returning a symmetric matrix with numeric values (max = 1). If not provided, this will be computed on the fly with <a href="#">create_kappa_matrix()</a>
similarity_threshold	A numeric value for the similarity matrix, used to determine the initial seeds as in the implementation of DAVID. Higher values will lead to more genesets being initially unclustered, leading to a functional classification result with fewer groups and fewer geneset members. Defaults to 0.35, recommended to not go below 0.3 (see DAVID help pages)

fuzzy\_seeding\_initial\_neighbors

Integer value, corresponding to the minimum geneset number in a seeding group. Lower values will lead to the inclusion of more genesets in the functional groups, and may generate a lot of small size groups. Defaults to 3

fuzzy\_multilinkage\_rule

Numeric value, comprised between 0 and 1. This parameter will determine how the seeding groups merge with each other, by specifying the percentage of shared genesets required to merge the two subsets into one group. Higher values will give sharper separation between the groups of genesets. Defaults to 0.5 (50%)

### Value

A data frame, shaped in a similar way as the originally provided `res_enrich` object, containing two extra columns: `gs_fuzzycluster`, to specify the identifier of the fuzzy cluster of genesets, and `gs_cluster_status`, which can specify whether the geneset is the "Representative" for that cluster or a simple "Member". Notably, the number of rows in the returned object can be higher than the original number of rows in `res_enrich`.

### References

See [https://david.ncifcrf.gov/helps/functional\\_classification.html#clustering](https://david.ncifcrf.gov/helps/functional_classification.html#clustering) for details on the original implementation

### Examples

```
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
# taking a smaller subset
res_enrich_subset <- res_enrich[1:100, ]

fuzzy_subset <- gs_fuzzyclustering(
  res_enrich = res_enrich_subset,
  n_gs = nrow(res_enrich_subset),
  gs_ids = NULL,
  similarity_matrix = NULL,
  similarity_threshold = 0.35,
  fuzzy_seeding_initial_neighbors = 3,
  fuzzy_multilinkage_rule = 0.5
)

# show all genesets members of the first cluster
fuzzy_subset[fuzzy_subset$gs_fuzzycluster == "1", ]

# list only the representative clusters
head(fuzzy_subset[fuzzy_subset$gs_cluster_status == "Representative", ], 10)
```

gs\_heatmap

*Plot a heatmap of the gene signature on the data***Description**

Plot a heatmap for the selected gene signature on the provided data, with the possibility to compactly display also DE only genes

**Usage**

```
gs_heatmap(
  se,
  res_de,
  res_enrich,
  annotation_obj = NULL,
  gtl = NULL,
  geneset_id = NULL,
  genelist = NULL,
  FDR = 0.05,
  de_only = FALSE,
  cluster_rows = TRUE,
  cluster_columns = FALSE,
  center_mean = TRUE,
  scale_row = FALSE,
  anno_col_info = NULL,
  plot_title = NULL,
  ...
)
```

**Arguments**

se	A SummarizedExperiment object, or an object derived from this class, such as a DESeqTransform object (variance stabilized transformed data, or regularized logarithm transformed), in where the transformation has been applied to make the data more homoscedastic and thus a better fit for visualization.
res_de	A DESeqResults object.
res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
annotation_obj	A data.frame object with the feature annotation information, with at least two columns, gene_id and gene_name.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
geneset_id	Character specifying the gene set identifier to be plotted

genelist	A vector of character strings, specifying the identifiers contained in the row names of the se input object.
FDR	Numeric value, specifying the significance level for thresholding adjusted p-values. Defaults to 0.05.
de_only	Logical, whether to include only differentially expressed genes in the plot
cluster_rows	Logical, determining if rows should be clustered, as specified by <code>ComplexHeatmap::Heatmap()</code>
cluster_columns	Logical, determining if columns should be clustered, as specified by <code>ComplexHeatmap::Heatmap()</code>
center_mean	Logical, whether to perform mean centering on the row-wise
scale_row	Logical, whether to standardize by row the expression values
anno_col_info	A character vector of names in <code>colData(dds)</code> to use for decorating the heatmap as annotation.
plot_title	Character string, to specify the title of the plot, displayed over the heatmap. If left to NULL as by default, it tries to use the information on the geneset identifier provided
...	Additional arguments passed to other methods, e.g. in the call to <code>ComplexHeatmap::Heatmap()</code>

### Value

A plot returned by the `ComplexHeatmap::Heatmap()` function

### Examples

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

vst_macrophage <- vst(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)
```

```

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOTableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

gs_heatmap(vst_macrophage,
  res_de,
  res_enrich,
  anno_df,
  geneset_id = res_enrich$gs_id[1],
  cluster_columns = TRUE,
  anno_col_info = "condition"
)

```

---

gs\_horizon

*Plots a summary of enrichment results*


---

## Description

Plots a summary of enrichment results - horizon plot to compare one or more sets of results

## Usage

```

gs_horizon(
  res_enrich,
  compared_res_enrich_list,
  n_gs = 20,
  p_value_column = "gs_pvalue",
  color_by = "z_score",
  ref_name = "ref_scenario",
  sort_by = c("clustered", "first_set")
)

```

## Arguments

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
compared_res_enrich_list	A named list, where each element is a data.frame formatted like the standard res_enrich objects used by GeneTonic. The names of the list are the names of the scenarios.
n_gs	Integer value, corresponding to the maximal number of gene sets to be displayed

p_value_column	Character string, specifying the column of <code>res_enrich</code> where the p-value to be represented is specified. Defaults to <code>gs_pvalue</code> (it could have other values, in case more than one p-value - or an adjusted p-value - have been specified).
color_by	Character, specifying the column of <code>res_enrich</code> to be used for coloring the plotted gene sets. Defaults sensibly to <code>z_score</code> .
ref_name	Character, defining the name of the scenario to compare against (the one in <code>res_enrich</code> ) - defaults to <code>"ref_scenario"</code> .
sort_by	Character string, either <code>"clustered"</code> , or <code>"first_set"</code> . This controls the sorting order of the included terms in the final plot. <code>"clustered"</code> presents the terms grouped by the scenario where they assume the highest values. <code>"first_set"</code> sorts the terms by the significance value in the reference scenario.

### Details

It makes sense to have the results in `res_enrich` sorted by increasing `gs_pvalue`, to make sure the top results are first sorted by the significance (when selecting the common gene sets across the `res_enrich` elements provided in `compared_res_enrich_list`)

The gene sets included are a subset of the ones in common to all different scenarios included in `res_enrich` and the elements of `compared_res_enrich_list`.

### Value

A ggplot object

### See Also

[gs\\_summary\\_overview\(\)](#), [gs\\_summary\\_overview\\_pair\(\)](#)

### Examples

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
```

```
    row.names = rownames(dds_macrophage)
  )

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

res_enrich2 <- res_enrich[1:42, ]
res_enrich3 <- res_enrich[1:42, ]
res_enrich4 <- res_enrich[1:42, ]

set.seed(2 * 42)
shuffled_ones_2 <- sample(seq_len(42)) # to generate permuted p-values
res_enrich2$gs_pvalue <- res_enrich2$gs_pvalue[shuffled_ones_2]
res_enrich2$z_score <- res_enrich2$z_score[shuffled_ones_2]
res_enrich2$aggr_score <- res_enrich2$aggr_score[shuffled_ones_2]

set.seed(3 * 42)
shuffled_ones_3 <- sample(seq_len(42)) # to generate permuted p-values
res_enrich3$gs_pvalue <- res_enrich3$gs_pvalue[shuffled_ones_3]
res_enrich3$z_score <- res_enrich3$z_score[shuffled_ones_3]
res_enrich3$aggr_score <- res_enrich3$aggr_score[shuffled_ones_3]

set.seed(4 * 42)
shuffled_ones_4 <- sample(seq_len(42)) # to generate permuted p-values
res_enrich4$gs_pvalue <- res_enrich4$gs_pvalue[shuffled_ones_4]
res_enrich4$z_score <- res_enrich4$z_score[shuffled_ones_4]
res_enrich4$aggr_score <- res_enrich4$aggr_score[shuffled_ones_4]

compa_list <- list(
  scenario2 = res_enrich2,
  scenario3 = res_enrich3,
  scenario4 = res_enrich4
)

gs_horizon(res_enrich,
  compared_res_enrich_list = compa_list,
  n_gs = 50,
  sort_by = "clustered"
)

gs_horizon(res_enrich,
  compared_res_enrich_list = compa_list,
  n_gs = 20,
  sort_by = "first_set"
)
```

gs\_mds

*Multi Dimensional Scaling plot for gene sets***Description**

Multi Dimensional Scaling plot for gene sets, extracted from a `res_enrich` object

**Usage**

```
gs_mds(
  res_enrich,
  res_de,
  annotation_obj,
  gtl = NULL,
  n_gs = nrow(res_enrich),
  gs_ids = NULL,
  similarity_measure = "kappa_matrix",
  mds_k = 2,
  mds_labels = 0,
  mds_colorby = "z_score",
  gs_labels = NULL,
  plot_title = NULL,
  return_data = FALSE
)
```

**Arguments**

<code>res_enrich</code>	A <code>data.frame</code> object, storing the result of the functional enrichment analysis. See more in the main function, <code>GeneTonic()</code> , to check the formatting requirements (a minimal set of columns should be present).
<code>res_de</code>	A <code>DESeqResults</code> object.
<code>annotation_obj</code>	A <code>data.frame</code> object with the feature annotation information, with at least two columns, <code>gene_id</code> and <code>gene_name</code> .
<code>gtl</code>	A <code>GeneTonic-list</code> object, containing in its slots the arguments specified above: <code>dds</code> , <code>res_de</code> , <code>res_enrich</code> , and <code>annotation_obj</code> - the names of the list <i>must</i> be specified following the content they are expecting
<code>n_gs</code>	Integer value, corresponding to the maximal number of gene sets to be included (from the top ranked ones). Defaults to the number of rows of <code>res_enrich</code>
<code>gs_ids</code>	Character vector, containing a subset of <code>gs_id</code> as they are available in <code>res_enrich</code> . Lists the gene sets to be included, additionally to the ones specified via <code>n_gs</code> . Defaults to <code>NULL</code> .
<code>similarity_measure</code>	Character, currently defaults to <code>kappa_matrix</code> , to specify how to compute the similarity measure between gene sets



mds_k	Integer value, number of dimensions to compute in the multi dimensional scaling procedure
mds_labels	Integer, defines the number of labels to be plotted on top of the scatter plot for the provided gene sets.
mds_colorby	Character specifying the column of res_enrich to be used for coloring the plotted gene sets. Defaults sensibly to z_score.
gs_labels	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be labeled.
plot_title	Character string, used as title for the plot. If left NULL, it defaults to a general description of the plot and of the DE contrast
return_data	Logical, whether the function should just return the data.frame of the MDS coordinates, related to the original res_enrich object. Defaults to FALSE.

**Value**

A ggplot object

**See Also**

[create\\_kappa\\_matrix\(\)](#) is used to calculate the similarity between gene sets

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive
```

```
# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

gs_mds(res_enrich,
  res_de,
  anno_df,
  n_gs = 200,
  mds_labels = 10
)
```

---

gs\_radar

*Radar (spider) plot for gene sets*


---

## Description

Radar (spider) plot for gene sets, either for one or more results from functional enrichment analysis.

## Usage

```
gs_radar(
  res_enrich,
  res_enrich2 = NULL,
  n_gs = 20,
  p_value_column = "gs_pvalue"
)

gs_spider(
  res_enrich,
  res_enrich2 = NULL,
  n_gs = 20,
  p_value_column = "gs_pvalue"
)
```

## Arguments

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <code>GeneTonic()</code> , to check the formatting requirements (a minimal set of columns should be present).
res_enrich2	Analogous to res_enrich1, another data.frame object, storing the result of the functional enrichment analysis, but for a different setting (e.g. another contrast). Defaults to NULL (in this case, a single set of enrichment results is plotted).
n_gs	Integer value, corresponding to the maximal number of gene sets to be displayed
p_value_column	Character string, specifying the column of res_enrich where the p-value to be represented is specified. Defaults to gs_pvalue (it could have other values, in case more than one p-value - or an adjusted p-value - have been specified).

**Value**

A plotly object

**Examples**

```

library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)
gs_radar(res_enrich = res_enrich)
# or using the alias...
gs_spider(res_enrich = res_enrich)

# with more than one set
res_enrich2 <- res_enrich[1:60, ]
set.seed(42)
shuffled_ones <- sample(seq_len(60)) # to generate permuted p-values
res_enrich2$gs_pvalue <- res_enrich2$gs_pvalue[shuffled_ones]
# ideally, I would also permute the z scores and aggregated scores
gs_radar(
  res_enrich = res_enrich,
  res_enrich2 = res_enrich2
)

```

---

gs_scores	<i>Compute gene set scores</i>
-----------	--------------------------------

---

### Description

Compute gene set scores for each sample, by transforming the gene-wise change to a geneset-wise change

### Usage

```
gs_scores(se, res_de, res_enrich, annotation_obj = NULL, gtl = NULL)
```

### Arguments

se	A SummarizedExperiment object, or an object derived from this class, such as a DESeqTransform object (variance stabilized transformed data, or regularized logarithm transformed), in where the transformation has been applied to make the data more homoscedastic and thus a better fit for visualization.
res_de	A DESeqResults object.
res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
annotation_obj	A data.frame object with the feature annotation information, with at least two columns, gene_id and gene_name.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting

### Value

A matrix with the geneset Z scores, e.g. to be plotted with [gs\\_scoresheat\(\)](#)

### See Also

[gs\\_scoresheat\(\)](#) plots these scores

### Examples

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
```

```

dds_macrophage <- estimateSizeFactors(dds_macrophage)

vst_macrophage <- vst(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

scores_mat <- gs_scores(
  vst_macrophage,
  res_de,
  res_enrich[1:50, ],
  anno_df
)

```

---

 gs\_scoresheat

*Plots a matrix of geneset scores*


---

## Description

Plots a matrix of geneset Z scores, across all samples

## Usage

```

gs_scoresheat(
  mat,
  n_gs = nrow(mat),
  gs_ids = NULL,
  clustering_distance_rows = "euclidean",
  clustering_distance_cols = "euclidean",
  cluster_rows = TRUE,
  cluster_cols = TRUE
)

```

**Arguments**

<code>mat</code>	A matrix, e.g. returned by the <code>gs_scores()</code> function
<code>n_gs</code>	Integer value, corresponding to the maximal number of gene sets to be displayed.
<code>gs_ids</code>	Character vector, containing a subset of <code>gs_id</code> as they are available in <code>res_enrich</code> . Lists the gene sets to be displayed.
<code>clustering_distance_rows</code>	Character, a distance measure used in clustering rows
<code>clustering_distance_cols</code>	Character, a distance measure used in clustering columns
<code>cluster_rows</code>	Logical, determining if rows should be clustered
<code>cluster_cols</code>	Logical, determining if columns should be clustered

**Value**

A ggplot object

**See Also**

`gs_scores()` computes the scores plotted by this function

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

vst_macrophage <- vst(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
```

```
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

scores_mat <- gs_scores(
  vst_macrophage,
  res_de,
  res_enrich[1:30, ],
  anno_df
)
gs_scoreshat(scores_mat,
  n_gs = 30
)
```

---

gs\_simplify

*Simplify results from functional enrichment analysis*

---

## Description

Simplify results from functional enrichment analysis, removing genesets that are redundant to enhance interpretation of the results

## Usage

```
gs_simplify(res_enrich, gs_overlap = 0.75)
```

## Arguments

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
gs_overlap	Numeric value, which defines the threshold for removing terms that present an overlap greater than the specified value. Changing its value can control the granularity of how redundant terms are removed from the original res_enrich for the next steps, e.g. plotting this via <a href="#">gs_volcano()</a>

## Value

A data.frame with a subset of the original gene sets

## See Also

[gs\\_volcano\(\)](#) and [ggs\\_graph\(\)](#) can e.g. show an overview on the simplified table of gene sets

**Examples**

```
# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)

dim(res_enrich)
res_enrich_simplified <- gs_simplify(res_enrich)
dim(res_enrich_simplified)
# and then use this further for all other functions expecting a res_enrich
```

---

<code>gs_summary_heat</code>	<i>Plots a heatmap for genes and genesets</i>
------------------------------	---

---

**Description**

Plots a heatmap for genes and genesets, useful to spot out intersections across genesets and an overview of them

**Usage**

```
gs_summary_heat(res_enrich, res_de, annotation_obj, gtl = NULL, n_gs = 80)
```

**Arguments**

<code>res_enrich</code>	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <a href="#">GeneTonic()</a> , to check the formatting requirements (a minimal set of columns should be present).
<code>res_de</code>	A DESeqResults object.
<code>annotation_obj</code>	A data.frame object with the feature annotation information, with at least two columns, <code>gene_id</code> and <code>gene_name</code> .
<code>gtl</code>	A GeneTonic-list object, containing in its slots the arguments specified above: <code>dds</code> , <code>res_de</code> , <code>res_enrich</code> , and <code>annotation_obj</code> - the names of the list <i>must</i> be specified following the content they are expecting
<code>n_gs</code>	Integer value, corresponding to the maximal number of gene sets to be displayed

**Value**

A ggplot object

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
```



```
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

gs_summary_heat(
  res_enrich = res_enrich,
  res_de = res_de,
  annotation_obj = anno_df,
  n_gs = 20
)
```

---

gs\_summary\_overview *Plots a summary of enrichment results*

---

## Description

Plots a summary of enrichment results for one set

## Usage

```
gs_summary_overview(
  res_enrich,
  gtl = NULL,
  n_gs = 20,
  p_value_column = "gs_pvalue",
  color_by = "z_score",
  return_barchart = FALSE
)
```

**Arguments**

<code>res_enrich</code>	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <code>GeneTonic()</code> , to check the formatting requirements (a minimal set of columns should be present).
<code>gtl</code>	A GeneTonic-list object, containing in its slots the arguments specified above: <code>dds</code> , <code>res_de</code> , <code>res_enrich</code> , and <code>annotation_obj</code> - the names of the list <i>must</i> be specified following the content they are expecting
<code>n_gs</code>	Integer value, corresponding to the maximal number of gene sets to be displayed
<code>p_value_column</code>	Character string, specifying the column of <code>res_enrich</code> where the p-value to be represented is specified. Defaults to <code>gs_pvalue</code> (it could have other values, in case more than one p-value - or an adjusted p-value - have been specified).
<code>color_by</code>	Character, specifying the column of <code>res_enrich</code> to be used for coloring the plotted gene sets. Defaults sensibly to <code>z_score</code> .
<code>return_barchart</code>	Logical, whether to return a barchart (instead of the default dot-segment plot); defaults to FALSE.

**Value**

A ggplot object

**See Also**

[gs\\_summary\\_overview\\_pair\(\)](#), [gs\\_horizon\(\)](#)

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)
```

```

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

gs_summary_overview(res_enrich)

# if desired, it can also be shown as a barplot
gs_summary_overview(res_enrich, n_gs = 30, return_barchart = TRUE)

```

---

gs\_summary\_overview\_pair

*Plots a summary of enrichment results*

---

## Description

Plots a summary of enrichment results - for two sets of results

## Usage

```

gs_summary_overview_pair(
  res_enrich,
  res_enrich2,
  n_gs = 20,
  p_value_column = "gs_pvalue",
  color_by = "z_score",
  alpha_set2 = 1
)

```

## Arguments

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <code>GeneTonic()</code> , to check the formatting requirements (a minimal set of columns should be present).
res_enrich2	As res_enrich, the result of functional enrichment analysis, in a scenario/contrast different than the first set.
n_gs	Integer value, corresponding to the maximal number of gene sets to be displayed
p_value_column	Character string, specifying the column of res_enrich where the p-value to be represented is specified. Defaults to gs_pvalue (it could have other values, in case more than one p-value - or an adjusted p-value - have been specified).

color_by	Character, specifying the column of res_enrich to be used for coloring the plotted gene sets. Defaults sensibly to z_score.
alpha_set2	Numeric value, between 0 and 1, which specified the alpha transparency used for plotting the points for gene set 2.

**Value**

A ggplot object

**See Also**

[gs\\_summary\\_overview\(\)](#), [gs\\_horizon\(\)](#)

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

res_enrich2 <- res_enrich[1:42, ]
set.seed(42)
shuffled_ones <- sample(seq_len(42)) # to generate permuted p-values
res_enrich2$gs_pvalue <- res_enrich2$gs_pvalue[shuffled_ones]
res_enrich2$z_score <- res_enrich2$z_score[shuffled_ones]
```

```

res_enrich2$aggr_score <- res_enrich2$aggr_score[shuffled_ones]
# ideally, I would also permute the z scores and aggregated scores
gs_summary_overview_pair(
  res_enrich = res_enrich,
  res_enrich2 = res_enrich2
)

```

---

gs\_volcano

*Volcano plot for gene sets*


---

## Description

Volcano plot for gene sets, to summarize visually the functional enrichment results

## Usage

```

gs_volcano(
  res_enrich,
  gtl = NULL,
  p_threshold = 0.05,
  color_by = "aggr_score",
  volcano_labels = 10,
  scale_circles = 1,
  gs_ids = NULL,
  plot_title = NULL
)

```

## Arguments

res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <code>GeneTonic()</code> , to check the formatting requirements (a minimal set of columns should be present). This object needs to be processed first by a function such as <code>get_aggrscores()</code> to compute the term-wise z_score or aggr_score, which will be used for plotting
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting
p_threshold	Numeric, defines the threshold to be used for filtering the gene sets to display. Defaults to 0.05
color_by	Character specifying the column of res_enrich to be used for coloring the plotted gene sets. Defaults to aggr_score.
volcano_labels	Integer, maximum number of labels for the gene sets to be plotted as labels on the volcano scatter plot.
scale_circles	A numeric value, to define the scaling factor for the circle sizes. Defaults to 1.
gs_ids	Character vector, containing a subset of gs_id as they are available in res_enrich. Lists the gene sets to be labeled.
plot_title	Character string, used as title for the plot. If left NULL, it defaults to a general description of the plot and of the DE contrast

## Details

It is also possible to reduce the redundancy of the input `res_enrich` object, if it is passed in advance to the `gs_simplify()` function.

## Value

A ggplot object

## See Also

`gs_simplify()` can be applied in advance to `res_enrich` to reduce the redundancy of the displayed gene sets

## Examples

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

gs_volcano(res_enrich)
```

---

happy_hour	<i>Happy hour!</i>
------------	--------------------

---

## Description

Start the happy hour, creating a report containing a document full of goodies derived from the provided objects.

## Usage

```
happy_hour(
  dds,
  res_de,
  res_enrich,
  annotation_obj,
  gtl = NULL,
  project_id,
  mygenesets,
  mygenes,
  usage_mode = "batch_mode",
  input_rmd = NULL,
  output_file = "my_first_GeneTonic_happyhour.html",
  output_dir = tempdir(),
  output_format = NULL,
  force_overwrite = FALSE,
  knitr_show_progress = FALSE,
  ignore_pandoc = FALSE,
  open_after_creating = TRUE,
  ...
)
```

## Arguments

dds	A DESeqDataSet object, normally obtained after running your data through the DESeq2 framework.
res_de	A DESeqResults object. As for the dds parameter, this is also commonly used in the DESeq2 framework.
res_enrich	A data.frame object, storing the result of the functional enrichment analysis. See <a href="#">GeneTonic()</a> for the formatting requirements.
annotation_obj	A data.frame object with the feature annotation information, with at least two columns, gene_id and gene_name. See <a href="#">GeneTonic()</a> for the formatting requirements.
gtl	A GeneTonic-list object, containing in its slots the arguments specified above: dds, res_de, res_enrich, and annotation_obj - the names of the list <i>must</i> be specified following the content they are expecting

project_id	A character string, which can be considered as an identifier for the set/session, and will be e.g. used in the title of the report created via <code>happy_hour()</code>
mygenesets	A vector of character strings, containing...
mygenes	A vector of character strings, containing...
usage_mode	A character string, which controls the behavior of the Rmd document, based on whether the rendering is triggered while using the app ("shiny_mode"), or offline, in batch mode. Defaults to "batch_mode".
input_rmd	Character string with the path to the RMarkdown (.Rmd) file that will be used as the template for generating the report. Defaults to NULL, which will then use the one provided with the GeneTonic package.
output_file	Character string, specifying the file name of the output report. The file name extension must be either .html or .pdf, and consistent with the value of output_format.
output_dir	Character, defining the path to the output directory where the report will be generated. Defaults to the temp directory ( <code>tempdir()</code> ).
output_format	The format of the output report. Either <code>html_document</code> or <code>pdf_document</code> . The file name extension of <code>output_file</code> must be consistent with this choice. Can also be left empty and determined accordingly.
force_overwrite	Logical, whether to force overwrite an existing report with the same name in the output directory. Defaults to FALSE.
knitr_show_progress	Logical, whether to display the progress of knitr while generating the report. Defaults to FALSE.
ignore_pandoc	Logical, controlling how the report generation function will behave if <code>pandoc</code> or <code>pandoc-citeproc</code> are missing.
open_after_creating	Logical, whether to open the report in the default browser after being generated. Defaults to TRUE.
...	Other arguments that will be passed to <code>rmarkdown::render()</code> .

## Details

When `happy_hour` is called, a RMarkdown template file will be copied into the output directory, and `rmarkdown::render()` will be called to generate the final report.

As a default template, `happy_hour` uses the one delivered together with the GeneTonic package, which provides a comprehensive overview of what the user can extract. Experienced users can take that as a starting point to further edit and customize.

If there is already a .Rmd file with the same name in the output directory, the function will raise an error and stop, to avoid overwriting the existing file. The reason for this behaviour is that the copied template in the output directory will be deleted once the report is generated.

Credits to the original implementation proposed by Charlotte Sonesson, upon which this function is **heavily** inspired.



**Value**

Generates a fully fledged report in the `output_dir` directory, called `output_file` and returns (invisibly) the name of the generated report.

**See Also**

[GeneTonic\(\)](#), [shake\\_topG0tableResult\(\)](#), [shake\\_enrichResult\(\)](#)

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topG0tableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)
## Not run:
happy_hour(
  dds = dds_macrophage,
  res_de = res_de,
  res_enrich = res_enrich,
  annotation_obj = anno_df,
  project_id = "examplerun",
  mygenesets = res_enrich$gs_id[c(1:5, 11, 31)],
  mygenes = c(
    "ENSG00000125347",
    "ENSG00000172399",
```

```
      "ENSG00000137496"  
    )  
  )  
  
## End(Not run)
```

---

map2color

*Maps numeric values to color values*

---

## Description

Maps numeric continuous values to values in a color palette

## Usage

```
map2color(x, pal, limits = NULL)
```

## Arguments

x	A character vector of numeric values (e.g. log2FoldChange values) to be converted to a vector of colors
pal	A vector of characters specifying the definition of colors for the palette, e.g. obtained via <a href="#">brewer.pal</a>
limits	A vector containing the limits of the values to be mapped. If not specified, defaults to the range of values in the x vector.

## Value

A vector of colors, each corresponding to an element in the original vector

## Examples

```
a <- 1:9  
pal <- RColorBrewer::brewer.pal(9, "Set1")  
map2color(a, pal)  
plot(a, col = map2color(a, pal), pch = 20, cex = 4)  
  
b <- 1:50  
pal2 <- grDevices::colorRampPalette(  
  RColorBrewer::brewer.pal(name = "RdYlBu", 11)  
) (50)  
plot(b, col = map2color(b, pal2), pch = 20, cex = 3)
```

---

overlap\_coefficient *Calculate overlap coefficient*

---

**Description**

Calculate similarity coefficient between two sets, based on the overlap

**Usage**

```
overlap_coefficient(x, y)
```

**Arguments**

x                    Character vector, corresponding to set 1  
y                    Character vector, set 2

**Value**

A numeric value between 0 and 1

**See Also**

[https://en.wikipedia.org/wiki/Overlap\\_coefficient](https://en.wikipedia.org/wiki/Overlap_coefficient)

**Examples**

```
a <- seq(1, 21, 2)  
b <- seq(1, 11, 2)  
overlap_coefficient(a, b)
```

---

overlap\_jaccard\_index *Calculate Jaccard Index between two sets*

---

**Description**

Calculate similarity coefficient with the Jaccard Index

**Usage**

```
overlap_jaccard_index(x, y)
```

**Arguments**

x                    Character vector, corresponding to set 1  
y                    Character vector, corresponding to set 2

**Value**

A numeric value between 0 and 1

**Examples**

```
a <- seq(1, 21, 2)
b <- seq(1, 11, 2)
overlap_jaccard_index(a, b)
```

---

res\_macrophage\_IFNg\_vs\_naive

*A sample DESeqResults object*

---

**Description**

A sample DESeqResults object, generated in the DESeq2 framework

**Details**

This DESeqResults object on the data from the macrophage package has been created comparing IFNg treated samples vs naive samples, accounting for the different cell lines included.

Details on how this object has been created are included in the create\_gt\_data.R script, included in the scripts folder of the GeneTonic package.

**References**

Alasoo, et al. "Shared genetic effects on chromatin and gene expression indicate a role for enhancer priming in immune response", Nature Genetics, January 2018 doi: 10.1038/s41588-018-0046-7.

---

shake\_davidResult      *Convert the output of DAVID*

---

**Description**

Convert the output of DAVID for straightforward use in [GeneTonic\(\)](#)

**Usage**

```
shake_davidResult(david_output_file)
```

**Arguments**

david\_output\_file

The location of the text file output, as exported from DAVID

**Value**

A data.frame compatible for use in [GeneTonic\(\)](#) as res\_enrich

**See Also**

Other shakers: [shake\\_enrichResult\(\)](#), [shake\\_enrichrResult\(\)](#), [shake\\_fgseaResult\(\)](#), [shake\\_gprofilerResult\(\)](#), [shake\\_gsenrichResult\(\)](#), [shake\\_topG0tableResult\(\)](#)

**Examples**

```
david_output_file <- system.file("extdata",
  "david_output_chart_BPonly_ifng_vs_naive.txt",
  package = "GeneTonic"
)
res_enrich <- shake_davidResult(david_output_file)
```

---

shake\_enrichResult      *Convert an enrichResult object*

---

**Description**

Convert an enrichResult object for straightforward use in [GeneTonic\(\)](#)

**Usage**

```
shake_enrichResult(obj)
```

**Arguments**

obj                    An enrichResult object, obtained via clusterProfiler (or also via reactomePA)

**Details**

This function is able to handle the output of clusterProfiler and reactomePA, as they both return an object of class enrichResult - and this in turn contains the information required to create correctly a res\_enrich object.

**Value**

A data.frame compatible for use in [GeneTonic\(\)](#) as res\_enrich

**See Also**

Other shakers: [shake\\_davidResult\(\)](#), [shake\\_enrichrResult\(\)](#), [shake\\_fgseaResult\(\)](#), [shake\\_gprofilerResult\(\)](#), [shake\\_gsenrichResult\(\)](#), [shake\\_topG0tableResult\(\)](#)

**Examples**

```

# dds
library("macrophage")
library("DESeq2")
data(gse)
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive
de_symbols_IFNg_vs_naive <- res_macrophage_IFNg_vs_naive[
  (!(is.na(res_macrophage_IFNg_vs_naive$padj))) &
  (res_macrophage_IFNg_vs_naive$padj <= 0.05), "SYMBOL"
]
bg_ids <- rowData(dds_macrophage)$SYMBOL[rowSums(counts(dds_macrophage)) > 0]
## Not run:
library("clusterProfiler")
library("org.Hs.eg.db")
ego_IFNg_vs_naive <- enrichGO(
  gene = de_symbols_IFNg_vs_naive,
  universe = bg_ids,
  keyType = "SYMBOL",
  OrgDb = org.Hs.eg.db,
  ont = "BP",
  pAdjustMethod = "BH",
  pvalueCutoff = 0.01,
  qvalueCutoff = 0.05,
  readable = FALSE
)

res_enrich <- shake_enrichrResult(ego_IFNg_vs_naive)
head(res_enrich)

## End(Not run)

```

---

shake\_enrichrResult     *Convert the output of Enrichr*

---

**Description**

Convert the output of Enrichr for straightforward use in [GeneTonic\(\)](#)

**Usage**

```
shake_enrichrResult(enrichr_output_file, enrichr_output = NULL)
```

**Arguments**

- enrichr\_output\_file  
The location of the text file output, as exported from Enrichr
- enrichr\_output A data.frame with the output of enrichr, related to a specific set of genesets.  
Usually it is one of the members of the list returned by the initial call to enrichr.

**Value**

A data.frame compatible for use in [GeneTonic\(\)](#) as res\_enrich

**See Also**

Other shakers: [shake\\_davidResult\(\)](#), [shake\\_enrichResult\(\)](#), [shake\\_fgseaResult\(\)](#), [shake\\_gprofilerResult\(\)](#), [shake\\_gsenrichResult\(\)](#), [shake\\_topG0tableResult\(\)](#)

**Examples**

```
# library("enrichR")
# dbs <- c("GO_Molecular_Function_2018",
#         "GO_Cellular_Component_2018",
#         "GO_Biological_Process_2018",
#         "KEGG_2019_Human",
#         "Reactome_2016",
#         "WikiPathways_2019_Human")
# degenes <- (deseqresult2df(res_macrophage_IFNg_vs_naive, FDR = 0.01)$SYMBOL)
# if called directly within R...
# enrichr_output_macrophage <- enrichr(degenes, dbs)
# or alternatively, if downloaded from the website in tabular format
enrichr_output_file <- system.file("extdata",
  "enrichr_tblexport_IFNg_vs_naive.txt",
  package = "GeneTonic"
)
res_from_enrichr <- shake_enrichrResult(enrichr_output_file = enrichr_output_file)
# res_from_enrichr2 <- shake_enrichrResult(
#   enrichr_output = enrichr_output_macrophage[["GO_Biological_Process_2018"]])
```

---

shake\_fgseaResult      *Convert the output of fgsea*

---

**Description**

Convert the output of fgsea for straightforward use in [GeneTonic\(\)](#)

**Usage**

```
shake_fgseaResult(fgsea_output)
```

**Arguments**

fgsea\_output    A data.frame with the output of fgsea() in fgsea.

**Value**

A data.frame compatible for use in [GeneTonic\(\)](#) as res\_enrich

**See Also**

Other shakers: [shake\\_davidResult\(\)](#), [shake\\_enrichResult\(\)](#), [shake\\_enrichrResult\(\)](#), [shake\\_gprofilerResult\(\)](#), [shake\\_gsenrichResult\(\)](#), [shake\\_topG0tableResult\(\)](#)

**Examples**

```
data(fgseaRes, package = "GeneTonic")
res_from_fgsea <- shake_fgseaResult(fgseaRes)
```

---

shake\_gprofilerResult    *Convert the output of g:Profiler*

---

**Description**

Convert the output of g:Profiler for straightforward use in [GeneTonic\(\)](#)

**Usage**

```
shake_gprofilerResult(gprofiler_output_file, gprofiler_output = NULL)
```

**Arguments**

gprofiler\_output\_file

The location of the text file output, as exported from g:Profiler

gprofiler\_output

A data.frame with the output of gost() in gprofiler2. Usually it is one of the members of the list returned by the initial call to gost.

**Value**

A data.frame compatible for use in [GeneTonic\(\)](#) as res\_enrich

**See Also**

Other shakers: [shake\\_davidResult\(\)](#), [shake\\_enrichResult\(\)](#), [shake\\_enrichrResult\(\)](#), [shake\\_fgseaResult\(\)](#), [shake\\_gsenrichResult\(\)](#), [shake\\_topG0tableResult\(\)](#)



## Examples

```
# degenes <- (deseqresult2df(res_macrophage_IFNg_vs_naive, FDR = 0.01)$SYMBOL)
# if called directly within R...
# enrichr_output_macrophage <- enrichr(degenes, dbs)
# or alternatively, if downloaded from the website in tabular format
gprofiler_output_file <- system.file(
  "extdata",
  "gProfiler_hsapiens_5-25-2020_tblexport_IFNg_vs_naive.csv",
  package = "GeneTonic"
)
res_from_gprofiler <- shake_gprofilerResult(gprofiler_output_file = gprofiler_output_file)

data(gostres_macrophage, package = "GeneTonic")
res_from_gprofiler_2 <- shake_gprofilerResult(
  gprofiler_output = gostres_macrophage$result
)
```

---

shake\_gsenrichResult *Convert a gseaResult object*

---

## Description

Convert a gseaResult object for straightforward use in [GeneTonic\(\)](#)

## Usage

```
shake_gsenrichResult(obj)
```

## Arguments

obj                    A gseaResult object, obtained via clusterProfiler

## Details

This function is able to handle the output of clusterProfiler's gseGO and GSEA, as they both return an object of class gseaResult - and this in turn contains the information required to create correctly a res\_enrich object.

## Value

A data.frame compatible for use in [GeneTonic\(\)](#) as res\_enrich

## See Also

Other shakers: [shake\\_davidResult\(\)](#), [shake\\_enrichResult\(\)](#), [shake\\_enrichrResult\(\)](#), [shake\\_fgseaResult\(\)](#), [shake\\_gprofilerResult\(\)](#), [shake\\_topGOtableResult\(\)](#)

**Examples**

```

# dds
library("macrophage")
library("DESeq2")
data(gse)
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)

# res object
data(res_de_macrophage, package = "GeneTonic")
sorted_genes <- sort(
  setNames(res_macrophage_IFNg_vs_naive$log2FoldChange,
    res_macrophage_IFNg_vs_naive$SYMBOL),
  decreasing = TRUE
)
## Not run:
library("clusterProfiler")
library("org.Hs.eg.db")
gsego_IFNg_vs_naive <- gseGO(
  geneList = sorted_genes,
  ont = "BP",
  OrgDb = org.Hs.eg.db,
  keyType = "SYMBOL",
  minGSSize = 10,
  maxGSSize = 500,
  pvalueCutoff = 0.05,
  verbose = TRUE
)

res_enrich <- shake_gsenrichResult(gsego_IFNg_vs_naive)
head(res_enrich)
gtl_macrophage <- GeneTonic_list(
  dds = dds_macrophage,
  res_de = res_macrophage_IFNg_vs_naive,
  res_enrich = res_enrich,
  annotation_obj = anno_df
)

## End(Not run)

```

---

shake\_topGOTableResult

*Convert a topGOTableResult object*


---

**Description**

Convert a topGOTableResult object for straightforward use in [GeneTonic\(\)](#)

**Usage**

```
shake_topGOTableResult(obj, p_value_column = "p.value_elim")
```

**Arguments**

`obj` A topGOtableResult object

`p_value_column` Character, specifying which column the p value for enrichment has to be used. Example values are "p.value\_elim" or "p.value\_classic"

**Value**

A data.frame compatible for use in `GeneTonic()` as `res_enrich`

**See Also**

Other shakers: `shake_davidResult()`, `shake_enrichResult()`, `shake_enrichrResult()`, `shake_fgseaResult()`, `shake_gprofilerResult()`, `shake_gsenrichResult()`

**Examples**

```
# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")

res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
```

---

signature\_volcano      *Plot a volcano plot of a geneset*

---

**Description**

Plot a volcano plot for the geneset of the provided data, with the remaining genes as shaded dots in the background of the plot.

**Usage**

```
signature_volcano(
  res_de,
  res_enrich,
  annotation_obj = NULL,
  gtl = NULL,
  geneset_id = NULL,
  genelist = NULL,
  FDR = 0.05,
  color = "#1a81c2",
  volcano_labels = 25,
  plot_title = NULL
)
```

**Arguments**

<code>res_de</code>	A DESeqResults object.
<code>res_enrich</code>	A data.frame object, storing the result of the functional enrichment analysis. See more in the main function, <code>GeneTonic()</code> , to check the formatting requirements (a minimal set of columns should be present).
<code>annotation_obj</code>	A data.frame object with the feature annotation information, with at least two columns, <code>gene_id</code> and <code>gene_name</code> .
<code>gtl</code>	A GeneTonic-list object, containing in its slots the arguments specified above: <code>dds</code> , <code>res_de</code> , <code>res_enrich</code> , and <code>annotation_obj</code> - the names of the list <i>must</i> be specified following the content they are expecting.
<code>geneset_id</code>	Character specifying the gene set identifier to be plotted.
<code>genelist</code>	A vector of character strings, specifying the identifiers contained in the rownames of the <code>res_de</code> input object.
<code>FDR</code>	Numeric value, specifying the significance level for thresholding adjusted p-values. Defaults to 0.05.
<code>color</code>	Character string to specify color of filtered points in the plot. Defaults to #1a81c2 (shade of blue).
<code>volcano_labels</code>	Integer, maximum number of labels for the gene sets to be plotted as labels on the volcano scatter plot. Defaults to 25.
<code>plot_title</code>	Character string, to specify the title of the plot, displayed over the volcano plot. If left to NULL as by default, it tries to use the information on the geneset identifier provided.

**Value**

A plot returned by the `ggplot()` function

**Examples**

```
library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object
data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
```

```

    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOTableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

signature_volcano(res_de,
  res_enrich,
  anno_df,
  geneset_id = res_enrich$gs_id[1]
)

# alternatively

chemokine_list <- c(
  "ENSG00000108702",
  "ENSG00000172156",
  "ENSG00000181374",
  "ENSG00000276409"
)

signature_volcano(res_de,
  res_enrich,
  anno_df,
  genelist = chemokine_list
)

```

---

styleColorBar\_divergent

*Style DT color bars*

---

### Description

Style DT color bars for values that diverge from 0.

### Usage

```
styleColorBar_divergent(data, color_pos, color_neg)
```

**Arguments**

data	The numeric vector whose range will be used for scaling the table data from 0-100 before being represented as color bars. A vector of length 2 is acceptable here for specifying a range possibly wider or narrower than the range of the table data itself.
color_pos	The color of the bars for the positive values
color_neg	The color of the bars for the negative values

**Details**

This function draws background color bars behind table cells in a column, with the width of bars being proportional to the column values *and* the color dependent on the sign of the value.

A typical usage is for values such as log2FoldChange for tables resulting from differential expression analysis. Still, the functionality of this can be quickly generalized to other cases - see in the examples.

The code of this function is heavily inspired from styleColorBar, and borrows at full hands from an excellent post on StackOverflow - <https://stackoverflow.com/questions/33521828/stylecolorbar-center-and-shift-left-right-dependent-on-sign/33524422#33524422>

**Value**

This function generates JavaScript and CSS code from the values specified in R, to be used in DT tables formatting.

**Examples**

```
data(res_de_macrophage, package = "GeneTonic")
res_df <- deseqresult2df(res_macrophage_IFNg_vs_naive)
library("magrittr")
library("DT")
DT::datatable(res_df[1:50, ],
  options = list(
    pageLength = 25,
    columnDefs = list(
      list(className = "dt-center", targets = "_all")
    )
  )
) %>%
formatRound(columns = c("log2FoldChange"), digits = 3) %>%
formatStyle(
  "log2FoldChange",
  background = styleColorBar_divergent(
    res_df$log2FoldChange,
    scales::alpha("navyblue", 0.4),
    scales::alpha("darkred", 0.4)
  ),
  backgroundSize = "100% 90%",
  backgroundRepeat = "no-repeat",
  backgroundPosition = "center"
```

```

)

simplest_df <- data.frame(
  a = c(rep("a", 9)),
  value = c(-4, -3, -2, -1, 0, 1, 2, 3, 4)
)

# or with a very simple data frame
DT::datatable(simplest_df) %>%
  formatStyle(
    "value",
    background = styleColorBar_divergent(
      simplest_df$value,
      scales::alpha("forestgreen", 0.4),
      scales::alpha("gold", 0.4)
    ),
    backgroundSize = "100% 90%",
    backgroundRepeat = "no-repeat",
    backgroundPosition = "center"
  )

```

---

summarize\_ggs\_hubgenes

*Summarize information on the hub genes*


---

## Description

Summarize information on the hub genes in the Gene-Geneset graph

## Usage

```
summarize_ggs_hubgenes(g)
```

## Arguments

**g** An igraph object, as generated by the `ggs_graph()` function

## Value

A data.frame object, formatted for use in `DT::datatable()`

## Examples

```

library("macrophage")
library("DESeq2")
library("org.Hs.eg.db")
library("AnnotationDbi")

# dds object

```

```

data("gse", package = "macrophage")
dds_macrophage <- DESeqDataSet(gse, design = ~ line + condition)
rownames(dds_macrophage) <- substr(rownames(dds_macrophage), 1, 15)
dds_macrophage <- estimateSizeFactors(dds_macrophage)

# annotation object
anno_df <- data.frame(
  gene_id = rownames(dds_macrophage),
  gene_name = mapIds(org.Hs.eg.db,
    keys = rownames(dds_macrophage),
    column = "SYMBOL",
    keytype = "ENSEMBL"
  ),
  stringsAsFactors = FALSE,
  row.names = rownames(dds_macrophage)
)

# res object
data(res_de_macrophage, package = "GeneTonic")
res_de <- res_macrophage_IFNg_vs_naive

# res_enrich object
data(res_enrich_macrophage, package = "GeneTonic")
res_enrich <- shake_topGOtableResult(topgoDE_macrophage_IFNg_vs_naive)
res_enrich <- get_aggrscores(res_enrich, res_de, anno_df)

ggs <- ggs_graph(
  res_enrich,
  res_de,
  anno_df
)
dt_df <- summarize_ggs_hubgenes(ggs)
DT::datatable(dt_df, escape = FALSE)

```

---

```

topgoDE_macrophage_IFNg_vs_naive
  A sample res_enrich object

```

---

## Description

A sample `res_enrich` object, generated with the `topGOtable` function (from the `pcaExplorer` package).

## Details

This `res_enrich` object on the data from the `macrophage` package has been created by analyzing downstream the differentially expressed genes when comparing IFNg treated samples vs naive samples, accounting for the different cell lines included.

Details on how this object has been created are included in the `create_gt_data.R` script, included in the `scripts` folder of the `GeneTonic` package.



**References**

Alasoo, et al. "Shared genetic effects on chromatin and gene expression indicate a role for enhancer priming in immune response", Nature Genetics, January 2018 doi: 10.1038/s41588-018-0046-7.

**See Also**

Other pathway-analysis-results: [enrichr\\_output\\_macrophage](#), [gostres\\_macrophage](#)

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