

# Package ‘hipathia’

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**Title** HiPathia: High-throughput Pathway Analysis

**Version** 2.3.0

**Description** Hipathia is a method for the computation of signal transduction along signaling pathways from transcriptomic data. The method is based on an iterative algorithm which is able to compute the signal intensity passing through the nodes of a network by taking into account the level of expression of each gene and the intensity of the signal arriving to it. It also provides a new approach to functional analysis allowing to compute the signal arriving to the functions annotated to each pathway.

**Depends** R (>= 3.6), igraph (>= 1.0.1), AnnotationHub(>= 2.6.5), MultiAssayExperiment(>= 1.4.9), SummarizedExperiment(>= 1.8.1)

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<code>annotate_paths</code>	<i>Annotates functions to pathways</i>
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---

**Description**

Annotates functions from a database to each pathway

**Usage**

```
annotate_paths(metainfo, dbannot)
```

**Arguments**

<code>metainfo</code>	Pathways object
<code>dbannot</code>	Either a string indicating which precomputed annotation to use ("uniprot" for Uniprot Keywords or "GO" for Gene Ontology terms), or a dataframe with the annotation of the genes to the functions. First column are gene symbols, second column the functions.

**Value**

Object of annotations from pathways to functions

```
#@examples #pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320", #"hsa04012"))
#annotate_paths(pathways, "GO")
#@export
```

---

<code>brca</code>	<i>BRCA gene expression dataset as SummarizedExperiment</i>
-------------------	---

---

**Description**

A dataset containing a matrix with the Gene expression of 40 samples from the BRCA-US project from The Cancer Genome Atlas (TCGA), and their experimental design, containing 20 "Tumor" samples 20 "Normal" samples.

**Usage**

```
data(brca)
```

**Format**

SummarizedExperiment. The assay is a matrix with 40 columns and 18638 rows. Row names are Entrez IDs and column names are the TCGA identifiers of the samples. The `colData()` is a data.frame with 1 column and 40 rows, including the experimental design of the 40 samples from the BRCA-US project from TCGA. Field `group` is the type of sample, either "Tumor" or "Normal".

**Details**

The gene expression matrix includes 40 samples. The data has been log-transformed and normalized with TMM.

**Value**

SummarizedExperiment including a matrix with 40 columns and 18638 rows. Row names are Entrez IDs and column names are the TCGA identifiers of the samples.

**Source**

<https://cancergenome.nih.gov/>

---

brca\_data

*BRCA gene expression dataset*

---

**Description**

Gene expression of 40 samples from the BRCA-US project from The Cancer Genome Atlas (TCGA).

**Usage**

```
data(brca_data)
```

**Format**

Matrix with 40 columns and 18638 rows. Row names are Entrez IDs and column names are the TCGA identifiers of the samples.

**Details**

Gene expression matrix with 40 samples taken from the BRCA-US project from The Cancer Genome Atlas (TCGA). The data has been log-transformed and normalized with TMM.

**Value**

Matrix with 40 columns and 18638 rows. Row names are Entrez IDs and column names are the TCGA identifiers of the samples.

**Source**

<https://cancergenome.nih.gov/>

---

brca_design	<i>BRCA experimental design</i>
-------------	---------------------------------

---

**Description**

Experimental design of the gene expression matrix `brca_data` with 40 samples taken from the BRCA-US project from The Cancer Genome Atlas (TCGA). 20 samples are "Tumor" samples and 20 samples are "Normal" samples.

**Usage**

```
data(brca_design)
```

**Format**

Dataframe with 1 column and 40 rows, including the experimental design of the 40 samples from the BRCA-US project from TCGA. Field `group` is the type of sample, either "Tumor" or "Normal".

**Value**

Dataframe with 1 column and 40 rows, including the experimental design of the 40 samples from the BRCA-US project from TCGA. Field `group` is the type of sample, either "Tumor" or "Normal".

**Source**

<https://cancergenome.nih.gov/>

---

comp	<i>Wilcoxon comparison of pathways object</i>
------	---

---

**Description**

Comparison object returned by `hipathia::do_wilcoxon` function, after calling `comp <-do_wilcoxon(path_vals, sample_group = "Tumor", g2 = "Normal")` `path_names <-get_path_names(pathways, rownames(comp))` `comp <-cbind(path_names, comp)`

**Usage**

```
data(comp)
```

**Format**

Table with 1868 rows and 5 columns

**Value**

Pathway comparison result

---

create_report	<i>Create visualization HTML</i>
---------------	----------------------------------

---

## Description

Saves the results of a Wilcoxon comparison for the Hipathia pathway values into a folder, and creates a HTML from which to visualize the results on top of the pathways. The results are stored into the specified folder. If this folder does not exist, it will be created. The parent folder must exist.

## Usage

```
create_report(comp, metainfo, output_folder = NULL, path = NULL,
  node_colors = NULL, group_by = "pathway", conf = 0.05,
  verbose = FALSE)
```

## Arguments

comp	Comparison object as given by the <code>do_wilcoxon</code> function
metainfo	Pathways object as returned by the <code>load_pathways</code> function
output_folder	Name of the folder in which the report will be stored.
path	Absolute path to the parent directory in which 'output_folder' will be saved. If it is not provided, it will be created in a temp folder.
node_colors	List of colors with which to paint the nodes of the pathways, as returned by the <code>node_color_per_de</code> function. Default is white.
group_by	How to group the subpathways to be visualized. By default they are grouped by the pathway to which they belong. Available groupings include "uniprot", to group subpathways by their annotated Uniprot functions, "GO", to group subpathways by their annotated GO terms, and "genes", to group subpathways by the genes they include. Default is set to "pathway".
conf	Level of significance. By default 0.05.
verbose	Boolean, whether to show details about the results of the execution

## Value

Saves the results and creates a report to visualize them through a server in the specified `output_folder`. Returns the folder where the report has been stored.

## Examples

```
data(comp)
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
  "hsa04012"))
report <- create_report(comp, pathways, "save_results")

## Not run:
data(results)
data(brca)
sample_group <- colData(brca)[,1]
colors_de <- node_color_per_de(results, pathways,
  sample_group, "Tumor", "Normal")
```

```
report_colors <- create_report(comp, pathways, "save_results",
node_colors = colors_de)

## End(Not run)
```

---

do\_pca *Performs a Principal Components Analysis*

---

### Description

Performs a Principal Components Analysis

### Usage

```
do_pca(data, sel_assay = 1, cor = FALSE)
```

### Arguments

data	SummarizedExperiment or matrix of values to be analyzed. Samples must be represented in the columns.
sel_assay	Character or integer, indicating the assay to be normalized in the Summarized-Experiment. Default is 1.
cor	A logical value indicating whether the calculation should use the correlation matrix or the covariance matrix. (The correlation matrix can only be used if there are no constant variables.)

### Value

do\_pca returns a list with class `princomp`.

### Examples

```
data(path_vals)
pca_model <- do_pca(path_vals[seq_len(ncol(path_vals)),])
```

---

do\_wilcoxon *Apply Wilcoxon test*

---

### Description

Performs a Wilcoxon test for the values in `sel_vals` comparing conditions `g1` and `g2`

### Usage

```
do_wilcoxon(data, group, g1, g2, paired = FALSE, adjust = TRUE,
sel_assay = 1, order = FALSE)
```

**Arguments**

data	Either a SummarizedExperiment object or a matrix, containing the values. Columns represent samples.
group	Either a character indicating the name of the column in colData including the classes to compare, or a character vector with the class to which each sample belongs. Samples must be ordered as in data
g1	String, label of the first group to be compared
g2	String, label of the second group to be compared
paired	Boolean, whether the samples to be compared are paired. If TRUE, function wilcoxsign_test from package coin is used. If FALSE, function wilcox.test from package stats is used.
adjust	Boolean, whether to adjust the p.value with Benjamini-Hochberg FDR method
sel_assay	Character or integer, indicating the assay to be normalized in the SummarizedExperiment. Default is 1.
order	Boolean, whether to order the results table by the FDRp.value column. Default is FALSE.

**Value**

Dataframe with the result of the comparison

**Examples**

```
data(path_vals)
data(brca_design)
sample_group <- brca_design[colnames(path_vals), "group"]
comp <- do_wilcoxon(path_vals, sample_group, g1 = "Tumor", g2 = "Normal")
```

---

exp\_data

*Normalized BRCA gene expression dataset*

---

**Description**

Experimental design matrix once expression matrix brca\_data has been translated to Entrez genes with translate\_matrix and normalized using normalize\_data.

**Usage**

```
data(exp_data)
```

**Format**

Matrix with 40 columns and 3184 rows. Row names are Entrez IDs and column names are the TCGA identifiers of the samples.

**Details**

To create the data, the following functions have been called: trans\_data <- translate\_matrix(brca\_data, "hsa")  
exp\_data <- normalize\_data(trans\_data)



**Value**

Matrix with 40 columns and 3184 rows. Row names are Entrez IDs and column names are the TCGA identifiers of the samples.

---

get_go_names	<i>Tranlates GO IDs to GO names</i>
--------------	-------------------------------------

---

**Description**

Translates the GO IDs to readable and comprehensible names.

**Usage**

```
get_go_names(names, species, maxchar = NULL)
```

**Arguments**

names	Character vector with the GO IDs to be translated.
species	Species of the samples.
maxchar	Integer, describes the number of maximum characters to be shown. By default no filter is applied.

**Value**

A character vector including the readable names of the GO IDs, in the same order as provided.

**Examples**

```
data(go_vals)
get_go_names(rownames(go_vals), "hsa")
```

---

get_highest_sig_ancestor	<i>Get highest common GO ancestor of GO annotations</i>
--------------------------	---

---

**Description**

Get highest common GO ancestor of GO annotations

**Usage**

```
get_highest_sig_ancestor(go_terms, go_comp, metainfo, unique = TRUE,
  pval = 0.05)
```

**Arguments**

go_terms	GO terms for which the highest common ancestors are to be looked for.
go_comp	Wilcoxon comparison of the matrix of GO values as returned by do_wilcoxon.
metainfo	Pathways object
unique	Boolean, whether to return only one highest significant GO ancestor or all of them. By default, TRUE.
pval	P-value cut-off. Default values is set to 0.05.

**Value**

highest common ancestors  
#@export

---

get\_nodes\_data      *Gets the object of node activation values*

---

**Description**

This function returns the object with the levels of activation of each node for each sample. Rows represent the nodes and columns represent the samples. Each cell is the value of activation of a node in a sample.

Rownames are the IDs of the nodes In order to transform IDs into readable names, use get\_node\_names.

Effector subpathways are subgraphs of a pathway including all the paths leading to an effector protein. Effector proteins are defined as final nodes in the graph. Each effector protein (final node) in a pathway defines its own effector subpathway as the nodes and edges in a path leading to it.

Decomposed subpathways are subgraphs of a pathway including all the paths starting in a receptor protein and ending in an effector protein. Receptor proteins are defined as initial nodes and effector proteins are defined as final nodes in the graph. Each effector subpathway can be decomposed in as many decomposed subpathways as initial nodes it includes.

**Usage**

```
get_nodes_data(results, matrix = FALSE)
```

**Arguments**

results	Results object as returned by hipathia.
matrix	Boolean, if TRUE the function returns a matrix object, if FALSE (as default) returns a SummarizedExperiment object.

**Value**

Object, either a SummarizedExperiment or a matrix, with the levels of activation of each decomposed subpathway for each sample.

**Examples**

```
data(results)
path_vals <- get_paths_data(results)
```

---

get_node_names	<i>Translates node IDs to node names</i>
----------------	--

---

### Description

Translates the node IDs to readable and comprehensible names.

The names of the nodes are encoded as "pathway: name", where "pathway" is the pathway to which the node belongs and "node" is the name of the node. Nodes may include more genes than the one depicted in the name.

### Usage

```
get_node_names(metainfo, names, maxchar = NULL)
```

### Arguments

metainfo	Pathways object
names	Character vector with the subpathway IDs to be translated
maxchar	Integer, describes the number of maximum characters to be shown. By default no filter is applied.

### Value

A character vector including the readable names of the subpathways IDs, in the same order as provided.

### Examples

```
data(results)
pathways_list <- c("hsa03320", "hsa04012")
pathways <- load_pathways(species = "hsa", pathways_list)
node_vals <- get_nodes_data(results)
translated_names <- get_node_names(pathways, rownames(node_vals))
```

---

get_paths_data	<i>Gets the object of subpathway activation values</i>
----------------	--

---

### Description

This function returns the object with the levels of activation of each subpathway for each sample. Rows represent the subpathways and columns represent the samples. Each cell is the value of activation of a subpathway in a sample.

Rownames are the IDs of the subpathways. In order to transform IDs into readable names, use `get_path_names`.

Effector subpathways are subgraphs of a pathway including all the paths leading to an effector protein. Effector proteins are defined as final nodes in the graph. Each effector protein (final node) in a pathway defines its own effector subpathway as the nodes and edges in a path leading to it.

Decomposed subpathways are subgraphs of a pathway including all the paths starting in a receptor protein and ending in an effector protein. Receptor proteins are defined as initial nodes and effector proteins are defined as final nodes in the graph. Each effector subpathway can be decomposed in as many decomposed subpathways as initial nodes it includes.

### Usage

```
get_paths_data(results, matrix = FALSE)
```

### Arguments

results	Results object as returned by hipathia.
matrix	Boolean, if TRUE the function returns a matrix object, if FALSE (as default) returns a SummarizedExperiment object.

### Value

Object, either a SummarizedExperiment or a matrix, with the levels of activation of each decomposed subpathway for each sample.

### Examples

```
data(results)
path_vals <- get_paths_data(results)
```

---

```
get_pathways_annotations
```

*Get Pathways functional annotations*

---

### Description

Get functional annotation of the pathways, either for a particular annotation or a stored one.

### Usage

```
get_pathways_annotations(pathway_names, metainfo, dbannot,
  collapse = FALSE)
```

### Arguments

pathway_names	Character vector of the names of the pathways
metainfo	Pathways object
dbannot	Either a string indicating which precomputed annotation to use ("uniprot" for Uniprot Keywords or "GO" for Gene Ontology terms), or a dataframe with the annotation of the genes to the functions. First column are gene symbols, second column the functions.
collapse	Boolean, whether to collapse all functions of the same path in a single character string.

**Value**

2-columns matrix with the annotations of each pathway ID in the annotation dbannot.

**Examples**

```
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
pathway_names <- c("P-hsa03320-37", "P-hsa03320-61", "P-hsa03320-46",
"P-hsa03320-57", "P-hsa03320-64", "P-hsa03320-47", "P-hsa03320-65")
## Not run: get_pathways_annotations(pathway_names, pathways, "GO")
get_pathways_annotations(pathway_names, pathways, "uniprot")
```

---

get\_pathways\_list      *Lists the IDs of the pathways in a pathways object*

---

**Description**

Lists the IDs of the pathways included in the pathways object metainfo

**Usage**

```
get_pathways_list(metainfo)
```

**Arguments**

metainfo      Pathways object

**Value**

List of the pathway IDs included in the pathways object

**Examples**

```
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
pathways_list <- get_pathways_list(pathways)
```

---

get\_pathways\_summary      *Compute pathway summary*

---

**Description**

Computes a summary of the results, summarizing the number and proportion of up- and down-regulated subpathways in each pathway.

**Usage**

```
get_pathways_summary(comp, metainfo, conf = 0.05)
```

**Arguments**

comp	Comparison data frame as returned by the do_wilcoxon function.
metainfo	Pathways object
conf	Level of significance of the comparison for the adjusted p-value. Default is 0.05.

**Value**

Table with the summarized information for each of the pathways. Rows are the analyzed pathways. Columns are: \* num\_total\_paths Number of total subpathways in which each pathway is decomposed. \* num\_significant\_paths Number of significant subpathways in the provided comparison. \* percent\_significant\_paths Percentage of significant subpathways from the total number of subpathways in a pathway. \* num\_up\_paths Number of significant up-regulated subpathways in the provided comparison. \* percent\_up\_paths Percentage of significant up-regulated subpathways from the total number of subpathways in a pathway. \* num\_down\_paths Number of significant down-regulated subpathways in the provided comparison. \* percent\_down\_paths Percentage of significant down-regulated subpathways from the total number of subpathways in a pathway.

**Examples**

```
data(comp)
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
get_pathways_summary(comp, pathways)
```

---

get\_pathway\_functions *Returns functions related to a pathway*

---

**Description**

Returns functions related to a pathway

**Usage**

```
get_pathway_functions(pathigraph, dbannot, entrez2hgnc,
  use_last_nodes = TRUE, unique = TRUE)
```

**Arguments**

pathigraph	Pathway object
dbannot	Dataframe with the annotation of the genes to the functions. First column are gene symbols, second column the functions.
entrez2hgnc	Relation between Entrez and HGNC genes.
use_last_nodes	Boolean, whether to annotate functions to the last nodes of the pathways or not. If FALSE, functions will refer to all the nodes of the pathway.
unique	Boolean, whether to return the first function for each path.

**Value**

List of annotations from pathways to functions

---

get_path_names	<i>Tranlates path IDs to path names</i>
----------------	---

---

### Description

Translates the subpathway IDs to readable and comprehensible names.

For effector subpathways, the names of the subpathways are encoded as "pathway: effector\_protein", where "pathway" is the pathway to which the subpathway belongs and "effector\_protein" is the name of the last node in the subpathway.

For decomposed subpathways, the names of the subpathways are encoded as "pathway: receptor\_protein - effector\_protein", where "pathway" is the pathway to which the subpathway belongs, "receptor\_protein" is the name of the initial node of the subpathway and "effector\_protein" is the name of the last node in the subpathway.

### Usage

```
get_path_names(metainfo, names, maxchar = NULL)
```

### Arguments

metainfo	Pathways object
names	Character vector with the subpathway IDs to be translated
maxchar	Integer, describes the number of maximum characters to be shown. By default no filter is applied.

### Value

A character vector including the readable names of the subpathways IDs, in the same order as provided.

### Examples

```
data(path_vals)
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
translated_names <- get_path_names(pathways, rownames(path_vals))
```

---

go_vals	<i>Gene Ontology matrix of the BRCA gene expression dataset</i>
---------	---

---

### Description

Matrix of Gene Ontology terms activation values for the BRCA dataset. This matrix is computed from the Results object returned by the hipathia function by means of the quantify\_terms function.

### Usage

```
data(go_vals)
```

**Format**

Matrix with 40 columns and 1654 rows. Row names are Gene Ontology terms and column names are the TCGA identifiers of the samples.

**Details**

```
go_vals <- quantify_terms(results, pathways, "GO")
```

**Value**

Matrix with 40 columns and 1654 rows. Row names are Gene Ontology terms and column names are the TCGA identifiers of the samples.

---

heatmap_plot	<i>Plots subpathways heatmap</i>
--------------	----------------------------------

---

**Description**

Plots a heatmap with the values of the subpathways.

**Usage**

```
heatmap_plot(data, group = NULL, sel_assay = 1, colors = "classic",
  sample_clust = TRUE, variable_clust = FALSE, labRow = NULL,
  labCol = NULL, sample_colors = NULL, scale = TRUE,
  save_png = NULL, legend = TRUE, legend_xy = "topright", pch = 15,
  main = NULL)
```

**Arguments**

data	Either a SummarizedExperiment or a matrix with the values to be plotted. Rows are features and columns are samples.
group	Either a character indicating the name of the column in colData including the classes to plot, or a character vector with the class to which each sample belongs. Samples must be ordered as in data. By default, all samples will be assigned to the same class.
sel_assay	Character or integer, indicating the assay to be normalized in the Summarized-Experiment. Default is 1.
colors	Either a character vector with colors or a key name indicating the color scheme to be used in the heatmap. If a character vector is provided, it is recommended to provide at least 3 colors. Three different predefined color schemes may be selected by providing a key name. Options are: * classic Blue for lower values, white for medium values, red for higher values. * hipathia Hipathia predefined color scheme: Green for lower values, white for medium values, orange for higher values. * redgreen Green for lower values, black for medium values, red for higher values. By default classic color scheme is applied.
sample_clust	Boolean, whether to cluster samples (columns). By default TRUE.
variable_clust	Boolean, whether to cluster variables (rows). By default FALSE. If TRUE, rows with 0 variance are removed.



labRow, labCol	Character vectors with row and column labels to be used. By default rownames(data) or colnames(data) are used, respectively.
sample_colors	Named character vector of colors. The names of the colors must be the classes in group. Each sample will be assigned the color corresponding to its class, taken from the group vector. By default a color will be assigned automatically to each class.
scale	Boolean, whether to scale each row to the interval [0,1]. Default is TRUE.
save_png	Path to the file where the image as PNG will be saved. By default, the image is not saved.
legend	Boolean, whether to display a legend.
legend_xy	Position for the legend, in case legend is TRUE.
pch	Graphical parameter from par() function.
main	Main title of the image

**Value**

Heatmap of the values of the subpathways

**Examples**

```
data(brca_design)
data(path_vals)
sample_group <- brca_design[colnames(path_vals), "group"]
heatmap_plot(path_vals, group = sample_group)
heatmap_plot(path_vals, group = "group", colors = "hipathia",
variable_clust = TRUE)
```

---

hhead	<i>Head function for SummarizedExperiment, data.frames and matrix objects</i>
-------	---

---

**Description**

Shows the first  $n$  rows and the first  $n$  columns of a matrix, in case the matrix has more than  $n+5$  rows or columns. Otherwise, it shows all the rows or columns, respectively.

**Usage**

```
hhead(mat, n = 5, sel_assay = 1)
```

**Arguments**

mat	Object to be shown
n	Number of rows and columns
sel_assay	Character or integer, indicating the assay to be translated in the SummarizedExperiment. Default is 1.

**Value**

Matrix with as much as n rows and n columns.

**Examples**

```
mat <- matrix(rnorm(100), ncol = 10)
hhead(mat)
hhead(mat, 3)
hhead(mat, 7)
```

---

hipathia	<i>Computes the level of activation of the subpathways for each of the samples</i>
----------	--

---

**Description**

```
#@importFrom igraph
```

**Usage**

```
hipathia(genes_vals, metainfo, sel_assay = 1, decompose = FALSE,
         maxnum = 100, verbose = TRUE, tol = 1e-06, test = TRUE)
```

**Arguments**

genes_vals	A SummarizedExperiment or matrix with the normalized expression values of the genes. Rows represent genes and columns represent samples. Rownames() must be accepted gene IDs.
metainfo	Pathways object
sel_assay	Character or integer, indicating the assay to be processed in the SummarizedExperiment. Only applied if genes_vals is a SummarizedExperiment. Default is 1.
decompose	Boolean, whether to compute the values for the decomposed subpathways. By default, effector subpathways are computed.
maxnum	Number of maximum iterations when iterating the signal through the loops into the pathways
verbose	Boolean, whether to show details about the results of the execution of hipathia
tol	Tolerance for the difference between two iterations when iterating the signal through the loops into the pathways
test	Boolean, whether to test the input objects. Default is TRUE.

**Value**

A MultiAssayExperiment object with the level of activation of the subpathways from the pathways in pathigraphs for the experiment with expression values in genes\_vals.

**Examples**

```

data(exp_data)
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
results <- hipathia(exp_data, pathways, verbose = TRUE)
## Not run: results <- hipathia(exp_data, pathways, decompose = TRUE,
verbose = FALSE)
## End(Not run)

```

---

igraphs_upgrade	<i>Upgrade igraphs to current version</i>
-----------------	---

---

**Description**

Upgrades the igraph objects in metainfo object to the corresponding version of the igraph package.

**Usage**

```
igraphs_upgrade(metainfo)
```

**Arguments**

metainfo      Pathways object

**Value**

The pathways object with the upgraded igraph objects

---

is_accepted_species	<i>Checks whether a species is accepted</i>
---------------------	---

---

**Description**

Checks whether a species is accepted

**Usage**

```
is_accepted_species(species)
```

**Arguments**

species      Species of the samples.  
#@examples #is\_accepted\_species("hsa") #is\_accepted\_species("fca")

**Value**

Boolean, whether species is accepted or not.

---

load_annotfuns	<i>Loads annotations object</i>
----------------	---------------------------------

---

**Description**

Loads annotations object

**Usage**

```
load_annotfuns(db, species)
```

**Arguments**

db	Database to be used. Either "GO" or "uniprot".
species	Species of the samples. #@examples #load_annotfuns("GO", "hsa") #load_annotfuns("uniprot", "hsa")

**Value**

Annotations object

---

load_annots	<i>Loads functional annotations to genes</i>
-------------	--

---

**Description**

Loads functional annotations from HGNC to the selected database.

**Usage**

```
load_annots(db, species)
```

**Arguments**

db	Database to be used. Either "GO" or "uniprot".
species	Species of the samples. #@examples #load_annots("GO", "hsa")

**Value**

Functional annotations from HGNC to the selected database.

---

load_entrez_hgnc	<i>Loads table of translation from HGNC to Entrez</i>
------------------	---

---

**Description**

Loads table of translation from HGNC to Entrez

**Usage**

```
load_entrez_hgnc(species)
```

**Arguments**

species	Species of the samples. #@examples #load_entrez_hgnc("hsa")
---------	--

**Value**

Table of translation from HGNC to Entrez

---

load_gobp_frame	<i>Loads GO graph information</i>
-----------------	-----------------------------------

---

**Description**

```
#@examples #load_gobp_frame()
```

**Usage**

```
load_gobp_frame()
```

**Value**

GO graph information

---

load_gobp_net	<i>Loads GO graph</i>
---------------	-----------------------

---

**Description**

```
#@examples #load_gobp_net()
```

**Usage**

```
load_gobp_net()
```

**Value**

GO graph

---

load_mgi	<i>Loads object with graph information</i>
----------	--

---

**Description**

Loads object with graph information

**Usage**

```
load_mgi(species)
```

**Arguments**

species	Species of the samples. #@examples #load_mgi("hsa")
---------	--

**Value**

Graph information object

---

load_pathways	<i>Loads the pathways object.</i>
---------------	-----------------------------------

---

**Description**

Loads the pathways object, which includes information about the pathways to be analyzed.

**Usage**

```
load_pathways(species, pathways_list = NULL)
```

**Arguments**

species	Species of the samples.
pathways_list	Vector of the IDs of the pathways to load. By default all available pathways are load.

**Details**

The object of pathways includes information about the pathways and the subpathways which will be analyzed. This object must be provided to some of the functions (like `hipathia` or `quantify_terms`) in the package. These functions will analyze all the pathways included in this object. By default, all available pathways are load. In order to restrict the analysis to a predefined set of pathways, specify the set of pathways to load with the parameter `pathways_list`.

**Value**

An pathways object including

- \* `species` Species to which the pathways are related.
- \* `pathigraphs` List of Pathigraph objects. Each Pathigraph contains the necessary information of a pathway for it to be analyzed with `Hipathia`.
- \* `all_genes` List of all the genes included in the selection of pathways stored in `pathigraphs`.
- \* `eff_norm` Vector of normalization values for effector subpathways.
- \* `path_norm` Vector of normalization values for decomposed subpathways.

**Examples**

```
## Not run: pathways <- load_pathways("hsa") # Loads all pathways for human
pathways <- load_pathways("mmu", c("mmu03320", "mmu04024", "mmu05200"))
# Loads pathways 03320, 04024 and 05200 for mouse
```

---

load_pseudo_mgi	<i>Loads object with pseudo graph information</i>
-----------------	---

---

**Description**

Loads object with pseudo graph information

**Usage**

```
load_pseudo_mgi(species, group_by)
```

**Arguments**

species	Species of the samples.
group_by	How to group the subpathways to be visualized. By default they are grouped by the pathway to which they belong. Available groupings include "uniprot", to group subpathways by their annotated Uniprot functions, "GO", to group subpathways by their annotated GO terms, and "genes", to group subpathways by the genes they include. #@examples #load_pseudo_mgi("hsa", "uniprot")

**Value**

Pseudo graph information object

---

load_xref	<i>Loads table of references</i>
-----------	----------------------------------

---

**Description**

Loads table of references

**Usage**

```
load_xref(species)
```

**Arguments**

species	Species of the samples. #@examples #load_xref("hsa")
---------	---

**Value**

Table of references

---

`mgf_from_sif`                      *Create a Pathways object from SIF files*

---

### Description

Creates a Pathways object from the information of a pathway stored in a SIF file with some attributes. This pathways object can be used by function `hipathia` to analyze data.

### Usage

```
mgf_from_sif(sif.folder, spe, entrez_symbol = NULL, dbannot = NULL)
```

### Arguments

<code>sif.folder</code>	Path to the folder in which SIF and ATT files are stored.
<code>spe</code>	Species
<code>entrez_symbol</code>	Relation between Entrez (NCBI) genes and gene symbols. Data.frame with 2 columns: First column is the EntrezGene ID, second column is the gene Symbol. The genes in the nodes of the pathways should be defined by Entrez IDs in the SIF and ATT files of the pathways. In order to be more readable, gene names are used when plotting the pathways.
<code>dbannot</code>	Functional annotation of the genes in the pathways to create function nodes.

### Value

A pathways object with the same structure of that returned by function `load_pathways`.

---

`multiple_pca_plot`                      *Plots multiple components of a PCA*

---

### Description

Plots multiple components of a PCA analysis computed with `do_pca`

### Usage

```
multiple_pca_plot(fit, group = NULL, sample_colors = NULL,
  comps = seq_len(3), plot_variance = FALSE, legend = TRUE,
  cex = 2, pch = 20, main = "Multiple PCA plot", save_png = NULL)
```

### Arguments

<code>fit</code>	princomp object as returned by <code>do_pca</code>
<code>group</code>	Vector with the group to which each sample belongs. The samples must be ordered as in <code>path_vals</code> . By default, all samples will be assigned to the same class.



sample_colors	Named character vector of colors. The names of the colors must be the classes in group. Each sample will be assigned the color corresponding to its class, taken from the group vector. By default a color will be assigned automatically to each class.
comps	Vector with the components to be plot
plot_variance	Logical, whether to plot the cumulative variance.
legend	Boolean, whether to plot a legend in the plot. Default is TRUE.
cex	Graphical parameter from par() function.
pch	Graphical parameter from par() function.
main	Main title of the image
save_png	Path to the file where the image as PNG will be saved. By default, the image is not saved.

**Value**

Plots multiple components of a PCA

**Examples**

```
data(path_vals)
sample_group <- brca_design[colnames(path_vals), "group"]
pca_model <- do_pca(path_vals[seq_len(ncol(path_vals)),])
multiple_pca_plot(pca_model, sample_group, cex = 3, plot_variance = TRUE)
```

---

node\_color

*Get colors of the nodes from a comparison file*


---

**Description**

Computes the colors of the nodes depending on the sign and p.value from the provided file. Significant up- and down-regulated nodes are depicted with the selected color, with a gradient towards the non-significant color depending on the value of the p-value. Smaller p-values give rise to purer colors than higher p-values.

**Usage**

```
node_color(comp, metainfo, group_by = "pathway", colors = "classic",
  conf = 0.05, adjust = TRUE)
```

**Arguments**

comp	Comparison file as returned by do_wilcoxon. Must include a column named "UP/DOWN" with the sign of the comparison coded as UP or DOWN, a column named "p.value" of raw p.values and a column named "FDRp.value" of adjusted p.values.
metainfo	Object of pathways.

group_by	How to group the subpathways to be visualized. By default they are grouped by the pathway to which they belong. Available groupings include "uniprot", to group subpathways by their annotated Uniprot functions, "GO", to group subpathways by their annotated GO terms, and "genes", to group subpathways by the genes they include. Default is set to "pathway".
colors	Either a character vector with 3 colors (indicating, in this order, down-regulation, non-significance and up-regulation colors) or a key name indicating the color scheme to be used. Options are:
conf	Level of significance of the comparison for the adjusted p-value.
adjust	Boolean, whether to adjust the p.value from the comparison. Default is TRUE.

### Value

List of color vectors, named by the pathways to which they belong. The color vectors represent the differential expression of the nodes in each pathway.

### Slots

classic ColorBrewer blue, white and colorBrewer red.

hipathia Hipathia predefined color scheme: Green, white and orange. By default classic color scheme is applied.

### Examples

```
data(results)
data(brca)
pathways_list <- c("hsa03320", "hsa04012")
pathways <- load_pathways(species = "hsa", pathways_list)
comp <- do_wilcoxon(results[["nodes"]], "group", "Tumor", "Normal")
colors_de <- node_color(comp, pathways)
```

---

node_color_per_de	<i>Colors of the nodes by its differential expression</i>
-------------------	---

---

### Description

Performs a Limma differential expression on the nodes and computes the colors of the nodes depending on it\_ Significant up- and down-regulated nodes are depicted with the selected color, with a gradient towards the non-significant color depending on the value of the p-value. Smaller p-values give rise to purer colors than higher p-values.

### Usage

```
node_color_per_de(results, metainfo, group, expdes, g2 = NULL,
  group_by = "pathway", colors = "classic", conf = 0.05,
  adjust = TRUE)
```

**Arguments**

results	Object of results as provided by the hipathia function_
metainfo	Object of pathways_
group	Character indicating the column in which the group variable is stored, in case the object provided to hipathia was a SummarizedExperiment, or a vector with the class to which each sample belongs. Samples must be ordered as in results.
expdes	String, either the comparison to be performed or the label of the first group to be compared.
g2	String, label of the second group to be compared. Only necessary in case expdes is the name of the first group, not the comparison.
group_by	How to group the subpathways to be visualized. By default they are grouped by the pathway to which they belong. Available groupings include "uniprot", to group subpathways by their annotated Uniprot functions, "GO", to group subpathways by their annotated GO terms, and "genes", to group subpathways by the genes they include. Default is set to "pathway".
colors	Either a character vector with 3 colors (indicating, in this order, down-regulation, non-significance and up-regulation colors) or a key name indicating the color scheme to be used. Options are:
conf	Level of significance of the comparison for the adjusted p-value.
adjust	Boolean, whether to adjust the p.value from the comparison. Default is TRUE.

**Value**

List of color vectors, named by the pathways to which they belong. The color vectors represent the differential expression of the nodes in each pathway.

**Slots**

`classic` ColorBrewer blue, white and colorBrewer red.

`hipathia` Hipathia predefined color scheme: Green, white and orange. By default classic color scheme is applied.

**Examples**

```
data(results)
data(brca)
pathways_list <- c("hsa03320", "hsa04012")
pathways <- load_pathways(species = "hsa", pathways_list)
colors_de <- node_color_per_de(results, pathways, "group", "Tumor - Normal")
colors_de <- node_color_per_de(results, pathways, "group", "Tumor", "Normal")
```

---

normalize_data	<i>Normalize expression data from a SummarizedExperiment or matrix to be used in hipathia</i>
----------------	---

---

### Description

Transforms the rank of the SummarizedExperiment or matrix of gene expression to [0,1] in order to be processed by hipathia. The transformation may be performed in two different ways. If `percentil = FALSE`, the transformation is a re-scaling of the rank of the matrix. If `percentil = TRUE`, the transformation is performed assigning to each cell its percentil in the corresponding distribution. This option is recommended for distributions with very long tails.

### Usage

```
normalize_data(data, sel_assay = 1, by_quantiles = FALSE,
              by_gene = FALSE, percentil = FALSE, truncation_percentil = NULL)
```

### Arguments

<code>data</code>	Either a SummarizedExperiment or a matrix of gene expression.
<code>sel_assay</code>	Character or integer, indicating the assay to be normalized in the Summarized-Experiment. Default is 1.
<code>by_quantiles</code>	Boolean, whether to normalize the data by quantiles. Default is FALSE.
<code>by_gene</code>	Boolean, whether to transform the rank of each row of the matrix to [0,1]. Default is FALSE.
<code>percentil</code>	Boolean, whether to take as value the percentil of each sample in the corresponding distribution.
<code>truncation_percentil</code>	Real number $p$ in [0,1]. When provided, values beyond percentil $p$ are truncated to the value of percentil $p$ , and values beyond $1-p$ are truncated to percentil $1-p$ . By default no truncation is performed.

### Details

This transformation may be applied either to the whole matrix (by setting `by_gene = FALSE`), which we strongly recommend, or to each of the rows (by setting `by_gene = TRUE`), allowing each gene to have its own scale.

A previous quantiles normalization may be applied by setting `by_quantiles = TRUE`. This is recommended for noisy data.

For distributions with extreme outlayer values, a percentil  $p$  may be given to the parameter `truncation_percentil`. When provided, values beyond percentil  $p$  are truncated to the value of percentil  $p$ , and values beyond  $1-p$  are truncated to percentil  $1-p$ . This step is performed before any other tranformation. By default no truncation is performed.

### Value

Matrix of gene expression whose values are in [0,1].

## Examples

```
data("brca_data")
trans_data <- translate_data(brca_data, "hsa")
exp_data <- normalize_data(trans_data)
exp_data <- normalize_data(trans_data, by_quantiles = TRUE,
truncation_percentil=0.95)
```

---

normalize_paths	<i>Normalize the pathway matrix by rows</i>
-----------------	---

---

## Description

Due to the nature of the Hipathia method, the length of a pathway may influence its signal rank. In order to compare signal values among subpathways, we strongly recommend to normalize the matrix with this normalization.

## Usage

```
normalize_paths(path_vals, metainfo)
```

## Arguments

path_vals	SummarizedExperiment or matrix of the pathway values
metainfo	Pathways object

## Details

This function removes the bias caused by the length of the subpathways by dividing by the value obtained from running the method with a basal value of 0.5 at each node.

## Value

SummarizedExperiment or matrix of normalized pathway values, depending on the class of path\_vals.

## Examples

```
data(path_vals)
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
path_normalized <- normalize_paths(path_vals, pathways)
```

---

paths\_to\_go\_ancestor *Create path results table with highest significant GO ancestors*

---

### Description

Create table of results with the comparison of the paths together with the GO functional annotation and the highest significant GO ancestor (HSGOA).

### Usage

```
paths_to_go_ancestor(pathways, comp_paths, comp_go, pval = 0.05)
```

### Arguments

pathways	Pathways object
comp_paths	Wilcoxon comparison of the matrix of pathways values as returned by do_wilcoxon.
comp_go	Wilcoxon comparison of the matrix of GO values as returned by do_wilcoxon.
pval	P-value cut-off. Default values is set to 0.05.

### Details

The table returns in each row: the name of a pathway and its Wilcoxon comparison information (direction, adjusted p-value), the GO term to which the path is related (not necessarily unique), the Wilcoxon comparison information for this GO (direction, adjusted p-value), the HSGOA of this GO and its Wilcoxon comparison information (direction, adjusted p-value).

The HSGOA is computed as the GO term with minimum level from all the significant (with respect to value pval) ancestors of a GO. The level of a GO term is computed as the number of nodes in the shortest path from this GO term to the term "GO:0008150". The ancestors of a node are defined as all the nodes from which a path can be defined from the ancestor to the node.

### Value

Table of comparisons with Highest common ancestors

### Examples

```
data(comp)
data(go_vals)
data(brca_design)
data(path_vals)
sample_group <- brca_design[colnames(path_vals),"group"]
comp_go <- do_wilcoxon(go_vals, sample_group, g1 = "Tumor", g2 = "Normal")
## Not run: pathways <- load_pathways(species = "hsa", pathways_list =
c("hsa03320", "hsa04012"))
table <- paths_to_go_ancestor(pathways, comp, comp_go)
## End(Not run)
```

---

 pathway\_comparison\_plot

*Plots pathway with colored significant paths*


---

### Description

Plots the layout of a pathway, coloring the significant subpathways in different colors depending on whether they are significantly up- or down-regulated. Nodes may be also colored providing a suitable list of colors for each node. Function `node_color_per_de` assigns colors to the nodes depending on their differential expression.

### Usage

```
pathway_comparison_plot(comp, metainfo, pathway, conf = 0.05,
  node_colors = NULL, colors = "classic")
```

### Arguments

<code>comp</code>	Comparison data frame as returned by the <code>do_wilcox</code> function.
<code>metainfo</code>	Pathways object.
<code>pathway</code>	Name of the pathway to be plotted.
<code>conf</code>	Level of significance of the comparison for the adjusted p-value. Default is 0.05.
<code>node_colors</code>	List, named by the pathway name, including the color of each node for each pathway.
<code>colors</code>	Either a character vector with 3 colors (indicating, in this order, down-regulation, non-significance and up-regulation colors) or a key name indicating the color scheme to be used. Options are:

### Value

Image in which a pathway is plotted. Edges are colored so that the UP- and DOWN-activated subpathways are identified.

### Slots

`classic` ColorBrewer blue, white and colorBrewer red.

`hipathia` Hipathia predefined color scheme: Green, white and orange. By default classic color scheme is applied.

### Examples

```
data(comp)
pathways_list <- c("hsa03320", "hsa04012")
pathways <- load_pathways(species = "hsa", pathways_list)
pathway_comparison_plot(comp, metainfo = pathways, pathway = "hsa03320")

## Not run:
data(results)
data(brca)
colors_de <- node_color_per_de(results, pathways, group, "Tumor", "Normal")
```

```
pathway_comparison_plot(comp, metainfo = pathways, pathway = "hsa04012",
node_colors = colors_de)
```

```
## End(Not run)
```

---

path_vals	<i>Pathways matrix of the BRCA gene expression dataset</i>
-----------	--

---

### Description

Matrix of pathway activation values for the BRCA dataset. This matrix is extracted from the Results object returned by the hipathia function by means of the get\_paths\_matrix function.

### Usage

```
data(path_vals)
```

### Format

Matrix with 40 columns and 1868 rows. Row names are Pathway IDs and column names are the TCGA identifiers of the samples.

### Details

```
path_vals <- get_paths_matrix(results)
```

### Value

Matrix with 40 columns and 1868 rows. Row names are Pathway IDs and column names are the TCGA identifiers of the samples.

---

pca_plot	<i>Plots two components of a PCA</i>
----------	--------------------------------------

---

### Description

Plots two components of a PCA computed with do\_pca

### Usage

```
pca_plot(fit, group = NULL, sample_colors = NULL, cp1 = 1, cp2 = 2,
legend = TRUE, legend_xy = "bottomleft", cex = 2, pch = 20,
mgp = c(3, 1, 0), main = "PCA plot", save_png = NULL)
```



**Arguments**

fit	princomp object as returned by do_pca
group	Vector with the group to which each sample belongs. The samples must be ordered as in rownames(fit\$scores). By default, all samples will be assigned to the same class.
sample_colors	Named character vector of colors. The names of the colors must be the classes in group. Each sample will be assigned the color corresponding to its class, taken from the group vector. By default a color will be assigned automatically to each class.
cp1	Integer, number of the component in the X-axis. Default is 1, the first component.
cp2	Integer, number of the component in the Y-axis. Default is 2, the second component.
legend	Boolean, whether to plot a legend in the plot. Default is TRUE.
legend_xy	Situation of the legend in the plot. Available options are: "bottomright", "bottom", "bottomleft", "left", "topleft", "top", "topright", "right" and "center".
cex	Graphical parameter from par() function.
pch	Graphical parameter from par() function.
mgp	Graphical parameter from par() function.
main	Title of the graphics
save_png	Path to the file where the image as PNG will be saved. By default, the image is not saved.

**Value**

Plots two components of a PCA

**Examples**

```
data(path_vals)
sample_group <- brca_design[colnames(path_vals),"group"]
pca_model <- do_pca(path_vals[seq_len(ncol(path_vals)),])
pca_plot(pca_model, sample_group)
```

---

quantify_terms	<i>Computes the level of activation of the functions related to the previously computed subpathways</i>
----------------	---

---

**Description**

Computes the level of activation of the functions related to the previously computed subpathways

**Usage**

```
quantify_terms(results, metaginfo, dbannot, out_matrix = FALSE,
  normalize = TRUE)
```

**Arguments**

<code>results</code>	List of results as returned by the <code>hipathia</code> function
<code>metainfo</code>	Pathways object
<code>dbannot</code>	Either a string indicating which precomputed annotation to use ("uniprot" for Uniprot Keywords or "GO" for Gene Ontology terms), or a dataframe with the annotation of the genes to the functions. First column are gene symbols, second column the functions.
<code>out_matrix</code>	Boolean, whether the output object should be a matrix object. Default is FALSE, returning a SummarizedExperiment object.
<code>normalize</code>	Boolean, whether to normalize the matrix of pathway values with <code>normalize_paths</code> before quantifying the signal. Due to the nature of the Hipathia method, in which the length of each pathway may alter its signal rank, we strongly recommend to perform this normalization. This normalization removes the bias. Default is set to TRUE.

**Value**

Matrix with the level of activation of the functions in `dbannot`

**Examples**

```
data(results)
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
go_values <- quantify_terms(results, pathways, "GO")
uniprot_values <- quantify_terms(results, pathways, "uniprot")
```

---

<code>results</code>	<i>Results object</i>
----------------------	-----------------------

---

**Description**

Results object returned by `hipathia::hipathia` function, after calling `results <- hipathia(exp_data, pathways, ver`

**Usage**

```
data(results)
```

**Format**

Object of results, including pathways information.

**Value**

Object of results, including pathways information.

---

save_results	<i>Save results to folder</i>
--------------	-------------------------------

---

**Description**

Saves results to a folder. In particular, it saves the matrix of subpathway values, a table with the results of the provided comparison, the accuracy of the results and the .SIF and attributes of the pathways.

**Usage**

```
save_results(results, comp, metainfo, output_folder = NULL,
             path = NULL)
```

**Arguments**

results	Results object as returned by the hipathia function.
comp	Comparison as returned by the do_wilcoxon function.
metainfo	Pathways object
output_folder	Name of the folder in which the results will be stored.
path	Absolute path to the parent directory in which 'output_folder' will be saved. If it is not provided, it will be created in a temp folder.

**Value**

Creates a folder in disk in which all the information to browse the pathway results is stored.

**Examples**

```
data(results)
data(comp)
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
save_results(results, comp, pathways, "output_results")
```

---

top_pathways	<i>Computes pathway significance</i>
--------------	--------------------------------------

---

**Description**

Performs a test for each pathway checking if the number of significant paths is significant, compared to not having any of the paths as significant.

**Usage**

```
top_pathways(comp)
```

**Arguments**

comp                      Comparison data frame as returned by the do\_wilcoxon function.

**Value**

Table with the names of the pathways and their p-value for the Fisher test comparing the proportion of significant subpaths vs. 0.

**Examples**

```
data(comp)
top_pathways(comp)
```

---

translate_data	<i>Translation of the rownames IDs of a SummarizedExperiment to Entrez IDs.</i>
----------------	---

---

**Description**

Translates the IDs in the rownames of a SummarizedExperiment to Entrez IDs. For accepted IDs to be transformed see the DOCUMENTATION.

**Usage**

```
translate_data(data, species, sel_assay = 1, verbose = TRUE)
```

**Arguments**

data                      Either a SummarizedExperiment object or a matrix of gene expression.  
species                    Species of the samples.  
sel\_assay                  Character or integer, indicating the assay to be translated in the SummarizedExperiment. Default is 1.  
verbose                    Boolean, whether to show details about the results of the execution.

**Value**

Either a SummarizedExperiment or a matrix (depending on the input type) of gene expression with Entrez IDs as rownames.

**Examples**

```
data("brca_data")
trans_data <- translate_data(brca_data, "hsa")
```

---

translate_matrix	<i>Translation of the rownames IDs of a matrix to Entrez IDs.</i>
------------------	---

---

**Description**

Translates the IDs in the rownames of a matrix to Entrez IDs. For accepted IDs to be transformed see the DOCUMENTATION.

**Usage**

```
translate_matrix(exp, species, verbose = TRUE)
```

**Arguments**

exp	Matrix of gene expression.
species	Species of the samples.
verbose	Boolean, whether to show details about the results of the execution.

**Value**

Matrix of gene expression with Entrez IDs as rownames.

---

visualize_report	<i>Visualize a HiPathia report</i>
------------------	------------------------------------

---

**Description**

Visualize a HiPathia report

**Usage**

```
visualize_report(output_folder, port = 4000)
```

**Arguments**

output_folder	Folder in which results to visualize are stored
port	Port to use

**Value**

The instructions to visualize a HiPathia report in a web browser

**Examples**

```
data(comp)
pathways <- load_pathways(species = "hsa", pathways_list = c("hsa03320",
"hsa04012"))
report <- create_report(comp, pathways, "save_results")
visualize_report(report)

## Not run:
data(results)
data(brca)
sample_group <- colData(brca)[,1]
colors_de <- node_color_per_de(results, pathways,
sample_group, "Tumor", "Normal")
report <- create_report(comp, pathways, "save_results",
node_colors = colors_de)
visualize_report(report)
visualize_report(report, port = 5000)

## End(Not run)
```

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