# STRINGdb Package Vignette

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## 1 INTRODUCTION

STRING (https://www.string-db.org) is a database of known and predicted protein-protein interactions. The interactions include direct (physical) and indirect (functional) associations. The database contains information from numerous sources, including experimental repositories, computational prediction methods and public text collections. Each interaction is associated with a combined confidence score that integrates the various evidences. STRING v12.5 covers 59.3 million proteins from 12,535 organisms and more than 28 billion interactions. It also provides directed regulatory networks alongside the functional and physical networks.

As you will learn in this guide, the STRING database can be useful to add meaning to lists of genes, for example the best hits coming out from a screen or the most differentially expressed genes coming out from a microarray or RNA-seq experiment.

We provide the STRINGdb R package in order to facilitate our users in accessing the STRING database from R. In this guide we explain, with examples, most of the package's features and functionalities.

In the STRINGdb R package we use the new ReferenceClasses of R (search for "ReferenceClasses" in the R documentation.). Besides we make use of the iGraph package (http://igraph.sourceforge.net) as a data structure to represent our protein-protein interaction network.

To begin, you should first know the NCBI taxonomy identifiers of the organism on which you have performed the experiment (e.g. 9606 for Human, 10090 for mouse). If you don't know that, you can search the NCBI Taxonomy (http://www.ncbi.nlm.nih.gov/taxonomy) or start looking at our species table (that you can also use to verify that your organism is represented in the STRING database).
Hence, if your species is not Human (i.e. our default species), you can find it and their taxonomy identifiers on STRING webpage under the 'organisms' section (https://string-db.org/cgi/input.pl?input_page_active_form=org
or download the full list in the download section of STRING website.

```
> library(STRINGdb)
> string_db <- STRINGdb$new( version="12.5", species=9606,
+                            score_threshold=400, network_type="full", link_data="detailed", input_di
```

As it has been shown in the above commands, you start instantiating the STRINGdb reference class. In the constructor of the class you can also define the STRING version to be used and a threshold for the combined scores of the interactions, such that any interaction below that threshold is not loaded in the object (by default the score threshold is set to 400).

You can also specify the network type "full" (or its alias "functional") for the full functional STRING network, "physical" for the physical subnetwork, or "regulatory" for the directed regulatory subnetwork. In a regulatory network, an edge points from the regulator to its target. Use `network_flavor="typed"` with a full/functional network to show the typed network view in the STRING web interface; this view is not available for a regulatory network. The optional `typed_physical_edges`, `typed_regulatory_edges`, and `show_regulatory_signs` arguments select which typed-edge details are shown.
Besides, if you specify a local directory to the parameter input-directory, the database files will be downloaded into this directory and most of the methods can be used off-line. Otherwise, the database files will be saved and cached in a temporary directory that will be cleaned automatically when the R session is closed. By setting `link_data="detailed"` we also load the evidence score columns, such as the experimental score, in addition to the combined score.

For a better understanding of the package two other commands can be useful:

```
> STRINGdb$methods()              # To list all the methods available.

 [1] ".objectPackage"                          ".objectParent"
 [3] "add_diff_exp_color"                      "add_proteins_description"
 [5] "benchmark_ppi"                           "benchmark_ppi_pathway_view"
 [7] "callSuper"                               "copy"
 [9] "enrichment_heatmap"                      "export"
[11] "field"                                   "get_aliases"
[13] "get_annotations"                         "get_bioc_graph"
[15] "get_clusters"                            "get_enrichment"
[17] "get_enrichment_figure"                   "get_geneset_description"
[19] "get_graph"                               "get_homologs"
[21] "get_homologs_besthits"                   "get_homology_graph"
[23] "get_interaction_partners"                "get_interactions"
[25] "get_link"                                "get_neighbors"
[27] "get_paralogs"                            "get_pathways_benchmarking_blackList"
[29] "get_png"                                 "get_ppi_enrichment"
[31] "get_ppi_enrichment_full"                 "get_proteins"
[33] "get_pubmed"                              "get_pubmed_interaction"
[35] "get_subnetwork"                          "get_summary"
[37] "get_term_proteins"                       "getClass"
[39] "getRefClass"                             "import"
[41] "initFields"                              "initialize"
[43] "load"                                    "load_all"
[45] "map"                                     "mp"
[47] "plot_network"                            "plot_ppi_enrichment"
[49] "post_payload"                            "ppi_enrichment"
[51] "remove_homologous_interactions"          "set_background"
[53] "show"                                    "show#envRefClass"
[55] "trace"                                   "untrace"
[57] "usingMethods"


> STRINGdb$help("get_graph")      # To visualize their documentation.

Call:
$get_graph()
```

```
Description:
  Return an igraph object with the entire STRING network.
  We invite the user to use the functions of the iGraph package to conveniently
  search/analyze the network.

References:
  Csardi G, Nepusz T: The igraph software package for complex network research,
  InterJournal, Complex Systems 1695. 2006.
  http://igraph.sf.net

See Also:
  In order to simplify the most common tasks, we do also provide convenient functions
  that wrap some iGraph functions.
  get_interactions(string_ids)   # returns the interactions in between the input proteins
  get_neighbors(string_ids)      # Get the neighborhoods of a protein (or of a vector of proteins).
  get_subnetwork(string_ids)     # returns a subgraph from the given input proteins

Author(s):
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```

For all the methods that we are going to explain below, you can always use the help function in order to get additional information/parameters with respect to those explained in this guide.

As an example, we use the analyzed data of a microarray study taken from GEO (Gene Expression Omnibus, GSE9008). This study investigates the activity of Resveratrol, a natural phytoestrogen found in red wine and a variety of plants, in A549 lung cancer cells. Microarray gene expression profiling after 48 hours exposure to Revestarol has been performed and compared to a control composed by A549 lung cancer cells threated only with ethanol. This data is already analyzed for differential expression using the limma package: the genes are sorted by fdr corrected pvalues and the log fold change of the differential expression is also reported in the table.

```
> data(diff_exp_example1)
> head(diff_exp_example1)

     pvalue    logFC         gene
1 0.0001018 3.333461       VSTM2L
2 0.0001392 3.822383       TBC1D2
3 0.0001720 3.306056        LENG9
4 0.0001739 3.024605       TMEM27
5 0.0001990 3.854414 LOC100506014
6 0.0002393 3.082052       TSPAN1
```

As a first step, we map the gene names to the STRING database identifiers using the "map" method. In this particular example, we map from gene HUGO names, but our mapping function supports several other common identifiers (e.g. Entrez GeneID, ENSEMBL proteins, RefSeq transcripts ... etc.).

The map function adds an additional column with STRING identifiers to the dataframe that is passed as first parameter.

```
> example1_mapped <- string_db$map( diff_exp_example1, "gene", removeUnmappedRows = TRUE )

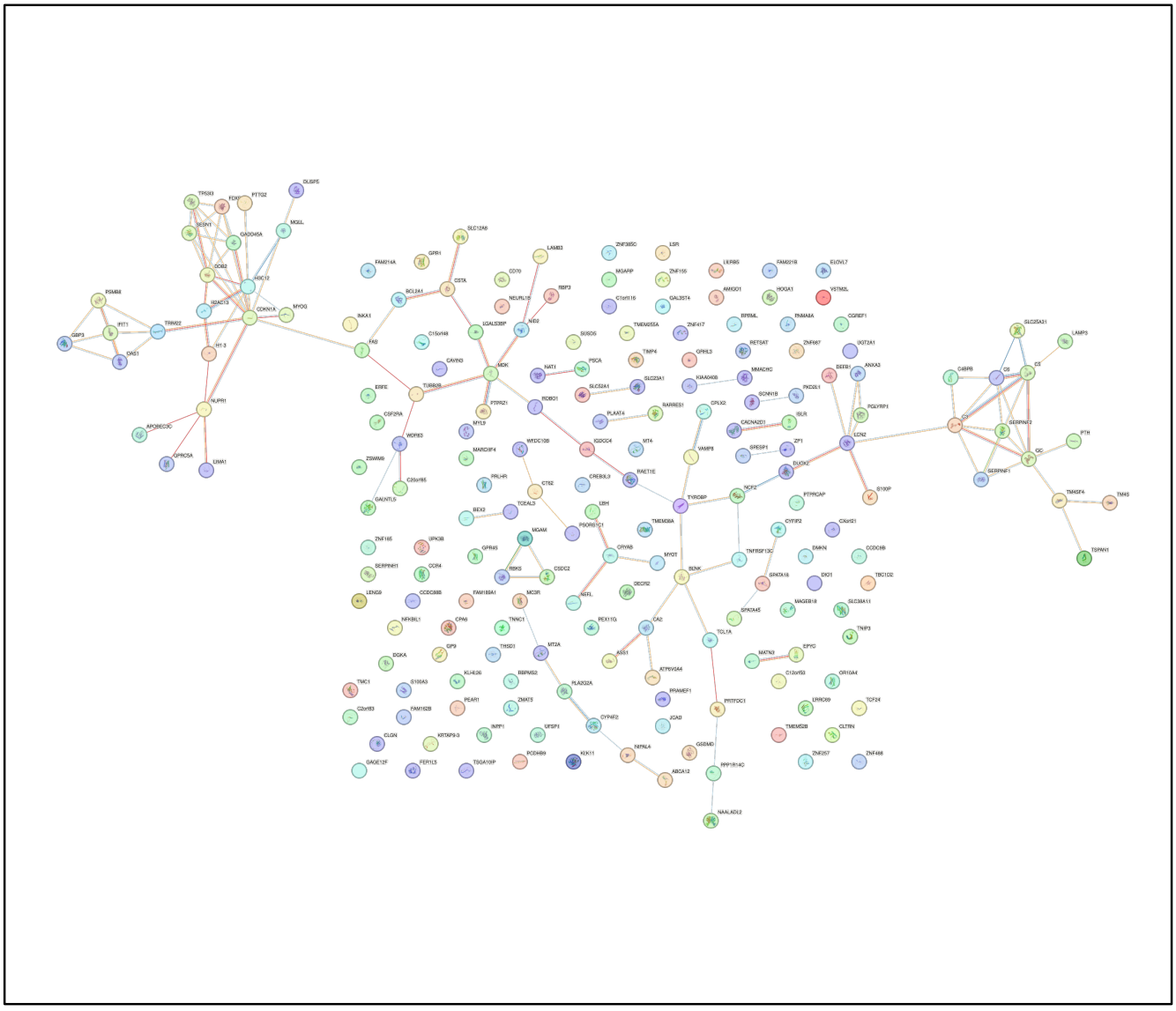
Warning:  we couldn't map to STRING 15% of your identifiers
```

As you may have noticed, the previous command prints a warning showing the number of genes that we failed to map. In this particular example, we cannot map all the probes of the microarray that refer to position of the chromosome that are not assigned to a real gene (i.e. all the LOC genes). If we remove all these LOC genes before the mapping we obtain a much lower percentage of unmapped genes (i.e. $< 6$ %).
If you set to FALSE the "removeUnmappedRows" parameter, than the rows which corresponds to unmapped genes are left and you can manually inspect them.
Finally, we extract the genes with p-value $< 0.05$ and, for the examples below, we focus on the top 200 genes sorted by p-value. The image shows clearly the genes and how they are possibly functionally related. On the top of the plot, we insert a pvalue that represents the probability that you can expect such an equal or greater number of interactions by chance.

```
> example1_mapped_pval05 <- subset(example1_mapped, pvalue < 0.05)
> example1_mapped_pval05 <- example1_mapped_pval05[order(example1_mapped_pval05$pvalue), ]
> hits <- example1_mapped_pval05$STRING_id[1:200]

> string_db$plot_network( hits )
```

proteins: 200
interactions: 127
expected interactions: 67 (p−value: 3.04e−11)



If you want more control over the appearance of the network image, the `get_png` and `get_link` methods expose additional styling options. In particular, you can switch between `evidence` and `confidence` edge styles, hide node labels, hide disconnected proteins, disable the structure pictures inside bubbles, use the flat node design, center node labels, and change the label font size.

## 2 PAYLOAD MECHANISM

This R library provides the ability to interact with the STRING payload mechanism. The payload appears as an additional colored "halo" around the bubbles.

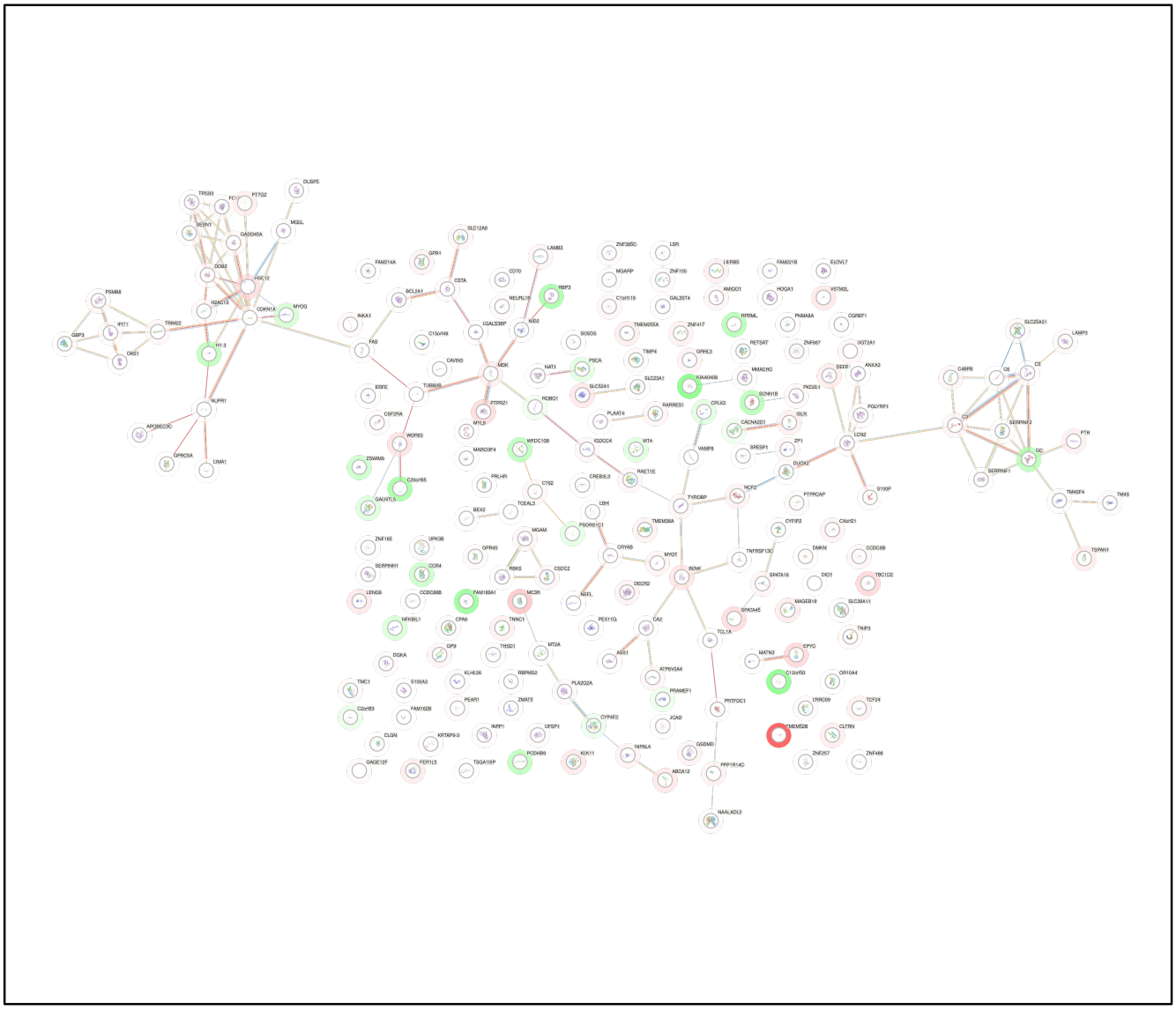
For example, this allows to color in green the genes that are down-regulated and in red the genes that are up-regulated. For this mechanism to work, we provide a function that posts the information on our web server.

```
> # filter by p-value and add a color column
> # (i.e. green down-regulated gened and red for up-regulated genes)
> example1_mapped_pval05 <- string_db$add_diff_exp_color( subset(example1_mapped, pvalue<0.05),
+                                                         logFcColStr="logFC" )

> # post payload information to the STRING server
> payload_id <- string_db$post_payload( example1_mapped_pval05$STRING_id,
+                                       colors=example1_mapped_pval05$color )

> # display a STRING network png with the "halo"
> string_db$plot_network( hits, payload_id=payload_id )
```

proteins: 200
interactions: 127
expected interactions: 67 (p−value: 3.04e−11)



# 3 ENRICHMENT

We provide a method to compute the enrichment in Gene Ontology (Process, Function and Component), KEGG and Reactome pathways, PubMed publications, UniProt Keywords, and PFAM/INTERPRO/SMART domains for your set of proteins all in one simple call. The enrichment itself is computed using an hypergeometric test and the FDR is calculated using Benjamini-Hochberg procedure.

```
> enrichment <- string_db$get_enrichment( hits )
> head(enrichment, n=20)
```

```
       category          term number_of_genes number_of_genes_in_background
1  COMPARTMENTS  GOCC:0005576              44                           1948
2  COMPARTMENTS  GOCC:1903561              19                            552
3       Process    GO:0006952              35                           1486
4     Component    GO:1903561              43                           2075
5     Component    GO:0070062              42                           2045
6     Component    GO:0005615              54                           3120
7     Component    GO:0005576              64                           3988
8       TISSUES   BTO:0000392              11                            187
9       Keyword       KW-0391              18                            630
10      Keyword       KW-0732              55                           3283
11      Keyword       KW-0964              37                           1910
12      Keyword       KW-0180               4                             30
13         KEGG      hsa04115               6                             75
14         PMID PMID:41977132               5                              9
15         PMID PMID:42163716               7                             26
16         PMID PMID:42235939               5                              9
17         PMID PMID:38499035               5                              9
18         PMID PMID:39331229               6                             16
19         PMID PMID:29879226               7                             25
20         PMID PMID:25738893               5                             10
   ncbiTaxonId
1         9606
2         9606
3         9606
4         9606
5         9606
6         9606
7         9606
8         9606
9         9606
10        9606
11        9606
12        9606
13        9606
14        9606
15        9606
16        9606
17        9606
18        9606
19        9606
20        9606
```

```
1
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7  9606.ENSP00000008938,9606.ENSP00000014914,9606.ENSP00000187762,9606.ENSP00000216286,9606.ENSP00000
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6                                                                    PGLYRP1,GPRC5A,TMEM38A,NID2,C5,RARRES1,
7  PGLYRP1,GPRC5A,TMEM38A,NID2,C5,RARRES1,C4BPB,C3,ISLR,SERPINF1,THSD1,LGALS3BP,C6,VAMP8,CSTA,ANXA3,Z
8
9
10                                                                 PGLYRP1,NID2,C5,C4BPB,C3,ISLR,SERPINF1,T
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```

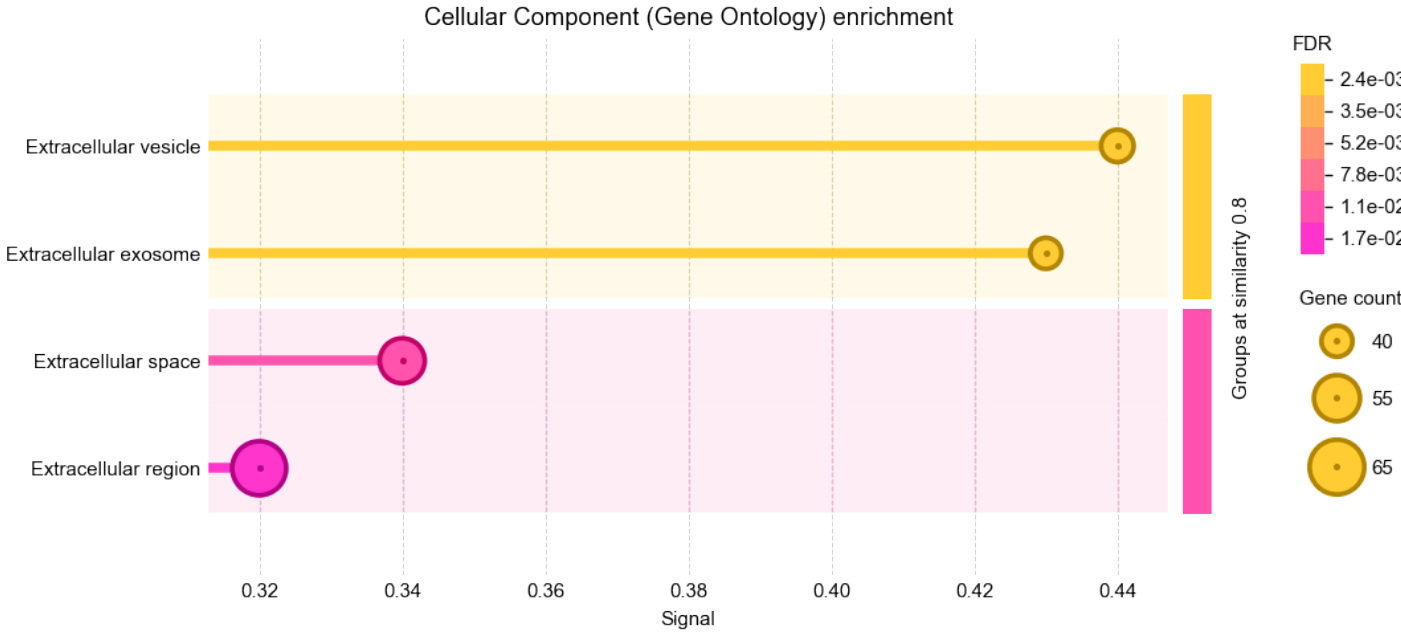
| | p_value | fdr |
|---|---|---|
| 1 | 2.90e-07 | 0.0005 |
| 2 | 3.78e-06 | 0.0022 |
| 3 | 2.40e-06 | 0.0295 |
| 4 | 4.00e-06 | 0.0024 |
| 5 | 6.66e-06 | 0.0029 |
| 6 | 3.66e-05 | 0.0130 |
| 7 | 5.66e-05 | 0.0167 |
| 8 | 3.44e-06 | 0.0031 |

```
9  8.00e-05 0.0208
10 7.51e-05 0.0208
11 9.13e-05 0.0208
12 2.30e-04 0.0392
13 1.10e-04 0.0419
14 1.25e-08 0.0125
15 5.59e-09 0.0125
16 1.25e-08 0.0125
17 1.25e-08 0.0125
18 7.48e-09 0.0125
19 4.12e-09 0.0125
20 2.48e-08 0.0212

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14                                                                                                             (
15 (2026) In vitro screening of compounds for targeting gastric cancer with Y220C p53 mutation: a mol
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17                                                                                                 (2024) Applicabili
18
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20                                                                                             (2015) Time, dose and
```

You can also retrieve an enrichment figure directly from STRING for a selected category. The image can be returned as a PNG array or as SVG markup, and you can customize the category, similarity-based grouping, color palette, the number of terms shown, and the variable displayed on the X-axis.

```
> enrichment_img <- string_db$get_enrichment_figure(
+   hits,
+   category = "Component",
+   group_by_similarity = 0.8,
+   color_palette = "yellow_pink",
+   x_axis = "signal"
+ )
> grid::grid.newpage()
> grid::grid.raster(enrichment_img, interpolate = FALSE)
```




If you have performed your experiment on a predefined set of proteins, it is important to run the enrichment statistics using that set as a background (otherwise you would get a wrong p-value !). Hence, before to launch the method above, you may want to set the background:

```
> backgroundV <- example1_mapped$STRING_id
> string_db$set_background(backgroundV)

IGRAPH 5e04e12 UN-- 17440 1990788 --
+ attr: name (v/c), neighborhood (e/n), fusion (e/n), cooccurence
| (e/n), coexpression (e/n), experimental (e/n), database (e/n),
| textmining (e/n), combined_score (e/n)
+ edges from 5e04e12 (vertex names):
 [1] 9606.ENSP00000000233--9606.ENSP00000054666
 [2] 9606.ENSP00000000233--9606.ENSP00000158762
 [3] 9606.ENSP00000000233--9606.ENSP00000160827
 [4] 9606.ENSP00000000233--9606.ENSP00000216446
 [5] 9606.ENSP00000000233--9606.ENSP00000217402
 [6] 9606.ENSP00000000233--9606.ENSP00000219479
+ ... omitted several edges
```

If you already have a `STRINGdb` object, calling `set_background` is sufficient and you do not need to instantiate a new object again. Passing `backgroundV` to the constructor is simply an alternative when you already know the background at initialization time:

```
> string_db <- STRINGdb$new( score_threshold=400, backgroundV = backgroundV )
```

Once the background has been set, the enrichment results can change substantially because the statistical test is now performed against the selected background rather than the whole proteome. In many cases the enrichment signals become smaller, which is expected: the test is now calibrated against the proteins that were actually measurable or considered in the experiment, rather than against all proteins in the organism.

```
> enrichment_background_corrected <- string_db$get_enrichment( hits )
> head(enrichment_background_corrected, n=20)
```

| | category | term | number_of_genes | number_of_genes_in_background |
|---|---|---|---|---|
| 1 | COMPARTMENTS | GOCC:0005576 | 44 | 1867 |
| 2 | COMPARTMENTS | GOCC:1903561 | 19 | 541 |
| 3 | Component | GO:1903561 | 43 | 2017 |
| 4 | Component | GO:0070062 | 42 | 1988 |
| 5 | TISSUES | BTO:0000392 | 11 | 183 |
| 6 | Keyword | KW-0391 | 18 | 592 |
| 7 | Keyword | KW-0732 | 55 | 2985 |
| 8 | Keyword | KW-0964 | 37 | 1744 |
| 9 | Keyword | KW-0180 | 4 | 28 |
| 10 | Keyword | KW-1015 | 54 | 3005 |
| 11 | PMID | PMID:41977132 | 5 | 9 |
| 12 | PMID | PMID:42163716 | 7 | 26 |
| 13 | PMID | PMID:42235939 | 5 | 9 |
| 14 | PMID | PMID:38499035 | 5 | 9 |
| 15 | PMID | PMID:39331229 | 6 | 15 |
| 16 | PMID | PMID:29879226 | 7 | 25 |
| 17 | PMID | PMID:25738893 | 5 | 10 |
| 18 | Hallmark | M5939 | 11 | 196 |

```
   ncbiTaxonId
1         9606
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3         9606
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7  9606.ENSP00000008938,9606.ENSP00000216286,9606.ENSP00000223642,9606.ENSP00000243611,9606.ENSP00000
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10                      9606.ENSP00000008938,9606.ENSP00000216286,9606.ENSP00000223642,9606.ENSP00000
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1                                                                                          PGLYRP1,GPRC5A,
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3                                                                                PGLYRP1,GPRC5A,TMEM38A,NID2,C5,
4                                                                                    PGLYRP1,GPRC5A,TMEM38A,NID
5
6
7  PGLYRP1,NID2,C5,C4BPB,C3,ISLR,SERPINF1,THSD1,EPYC,TYROBP,LGALS3BP,C6,LAMP3,MARCHF4,ZP1,TIMP4,RETSA
8
9
10               PGLYRP1,NID2,C5,PRLHR,C4BPB,C3,ISLR,THSD1,EPYC,TYROBP,LGALS3BP,C6,LAMP3,ZP1,TIMP4,TN
11
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```

```
13
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    p_value     fdr
1  2.38e-06 0.00410
2  1.54e-05 0.00880
3  3.85e-05 0.02250
4  5.97e-05 0.02620
5  8.65e-06 0.00750
6  1.60e-04 0.03760
7  1.50e-04 0.03760
8  1.70e-04 0.03760
9  2.80e-04 0.04520
10 3.30e-04 0.04520
11 2.28e-08 0.02250
12 1.27e-08 0.02250
13 2.28e-08 0.02250
14 2.28e-08 0.02250
15 9.62e-09 0.02250
16 9.40e-09 0.02250
17 4.51e-08 0.03830
18 1.65e-05 0.00083

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12 (2026) In vitro screening of compounds for targeting gastric cancer with Y220C p53 mutation: a mol
13
14                                                                                                 (2024) Applicabili
15
16
17                                                                                               (2015) Time, dose and
18
```

If you just want to know which terms are assigned to your set of proteins, and not necessarily which ones are enriched, you can use "get_annotations" method. This method outputs the terms from most categories (the exceptions are KEGG terms due to licensing issues and PubMed due to the size of the output) that are associated with your set of proteins.

```
> annotations <- string_db$get_annotations( hits )
> head(annotations, n=20)
```

| | category | term_id | number_of_genes | ratio_in_set | species |
|---|---|---|---|---|---|
| 1 | COMPARTMENTS | GOCC:0000139 | 2 | 0.010 | 9606 |
| 2 | COMPARTMENTS | GOCC:0000151 | 2 | 0.010 | 9606 |
| 3 | COMPARTMENTS | GOCC:0000323 | 6 | 0.030 | 9606 |
| 4 | COMPARTMENTS | GOCC:0000502 | 1 | 0.005 | 9606 |
| 5 | COMPARTMENTS | GOCC:0000785 | 1 | 0.005 | 9606 |
| 6 | COMPARTMENTS | GOCC:0000791 | 1 | 0.005 | 9606 |
| 7 | COMPARTMENTS | GOCC:0001533 | 1 | 0.005 | 9606 |
| 8 | COMPARTMENTS | GOCC:0001650 | 1 | 0.005 | 9606 |
| 9 | COMPARTMENTS | GOCC:0001669 | 1 | 0.005 | 9606 |
| 10 | COMPARTMENTS | GOCC:0001725 | 2 | 0.010 | 9606 |
| 11 | COMPARTMENTS | GOCC:0001726 | 2 | 0.010 | 9606 |
| 12 | COMPARTMENTS | GOCC:0005576 | 44 | 0.220 | 9606 |
| 13 | COMPARTMENTS | GOCC:0005577 | 1 | 0.005 | 9606 |
| 14 | COMPARTMENTS | GOCC:0005579 | 3 | 0.015 | 9606 |
| 15 | COMPARTMENTS | GOCC:0005581 | 1 | 0.005 | 9606 |
| 16 | COMPARTMENTS | GOCC:0005604 | 2 | 0.010 | 9606 |
| 17 | COMPARTMENTS | GOCC:0005610 | 1 | 0.005 | 9606 |
| 18 | COMPARTMENTS | GOCC:0005615 | 23 | 0.115 | 9606 |
| 19 | COMPARTMENTS | GOCC:0005622 | 102 | 0.510 | 9606 |
| 20 | COMPARTMENTS | GOCC:0005634 | 34 | 0.170 | 9606 |

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| 12 | |
| 13 | |
| 14 | |
| 15 | |
| 16 | |
| 17 | |
| 18 | |
| 19 | 9606.ENSP00000008938,9606.ENSP00000078445,9606.ENSP00000187762,9606.ENSP00000219481,9606.ENSP00000 |
| 20 | |

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19 PGLYRP1,CREB3L3,TMEM38A,DECR2,PEX11G,CYP4F2,BLNK,TNNC1,MYOT,MYOG,H1-3,C3,ISLR,PLAAT4,DDB2,THSD1,TU
20
                          description
1                       Golgi membrane
2             Ubiquitin ligase complex
3                         Lytic vacuole
4                   Proteasome complex
5                             Chromatin
6                           Euchromatin
7                   Cornified envelope
8                     Fibrillar center
9                    Acrosomal vesicle
10                        Stress fiber
11                               Ruffle
12                 Extracellular region
13                   Fibrinogen complex
14              Membrane attack complex
15                      Collagen trimer
16                   Basement membrane
17                   laminin-5 complex
18                  Extracellular space
19 Intracellular anatomical structure
20                             Nucleus
```

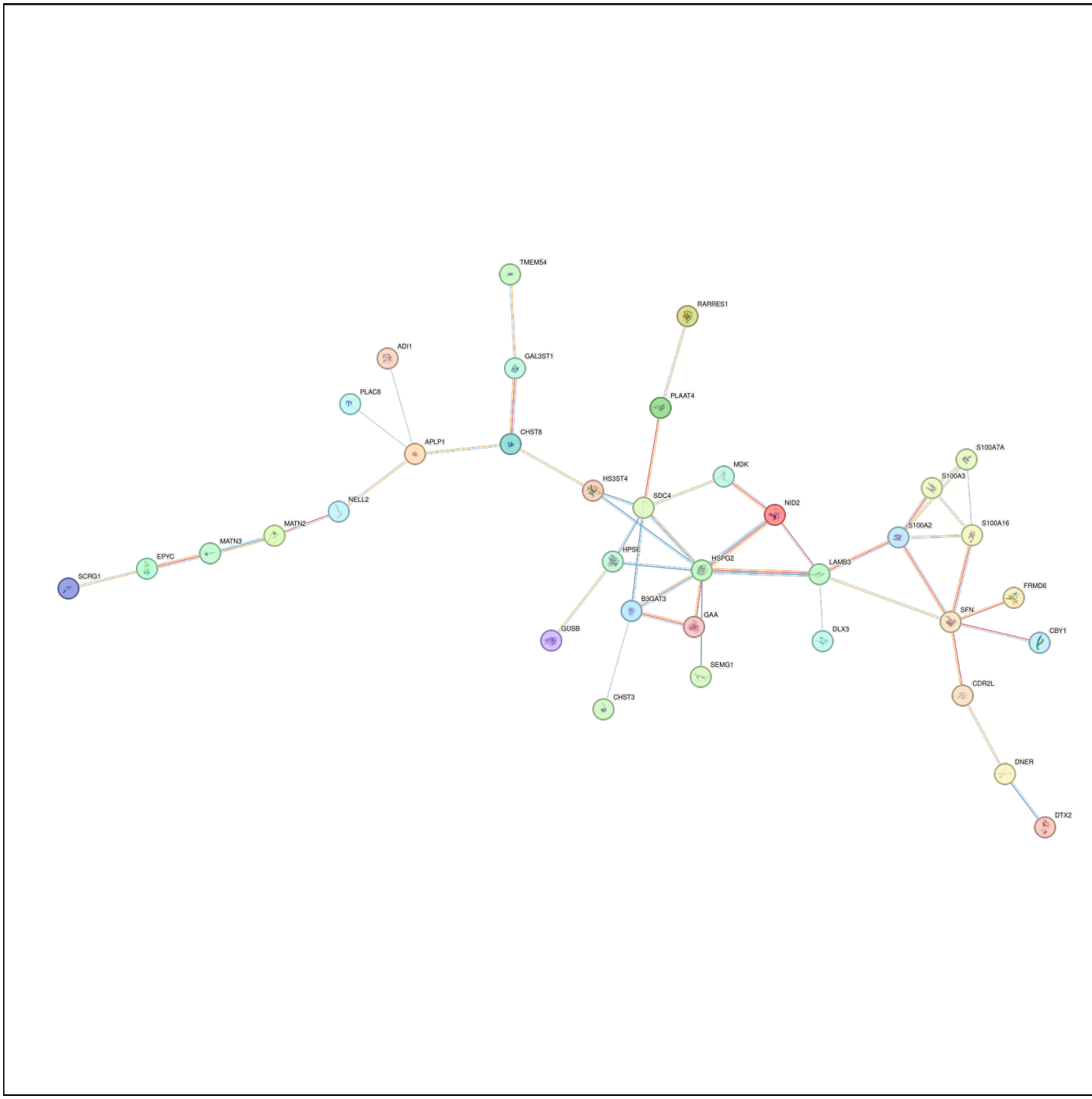
# 4 CLUSTERING

The iGraph package provides several clustering/community algorithms: "fastgreedy", "walktrap", "spinglass", "edge.betweenness". We encapsulate this in an easy-to-use function that returns the clusters in a list.

```
> # get clusters
> clustersList <- string_db$get_clusters(example1_mapped_pval05$STRING_id[1:600])
```

Now we visualize the first four clusters one by one.

```
> par(mar = c(0, 0, 0.5, 0))
> string_db$plot_network(clustersList[[1]], add_summary = FALSE)
> par(mar = c(0, 0, 0.5, 0))
> string_db$plot_network(clustersList[[2]], add_summary = FALSE)
> par(mar = c(0, 0, 0.5, 0))
> string_db$plot_network(clustersList[[3]], add_summary = FALSE)
> par(mar = c(0, 0, 0.5, 0))
> string_db$plot_network(clustersList[[4]], add_summary = FALSE)
```


TMEM54
RARRES1
ADI1
GAL3ST1
PLAC8
PLAAT4
APLP1
CHST8
S100A7A
MDK
S100A3
HS3ST4
SDC4
NID2
NELL2
S100A2
S100A16
MATN2
MATN3
HPSE
HSPG2
LAMB3
EPYC
SCRG1
FRMD6
B3GAT3
SFN
GAA
GUSB
DLX3
CBY1
SEMG1
CDR2L
CHST3
DNER
DTX2

# 5 ADDITIONAL PROTEIN INFORMATION

You can get a table that contains all the proteins that are present in our database of the species of interest. The protein table also includes the preferred name, the size and a short description of each protein.

```
> string_proteins <- string_db$get_proteins()
> head(string_proteins)
```

```
   protein_external_id preferred_name protein_size
1 9606.ENSP00000000233           ARF5          180
2 9606.ENSP00000000412           M6PR          277
3 9606.ENSP00000001008          FKBP4          459
4 9606.ENSP00000001146        CYP26B1          512
5 9606.ENSP00000002125        NDUFAF7          441
6 9606.ENSP00000002165          FUCA2          467

1
2
3 Peptidyl-prolyl cis-trans isomerase FKBP4, N-terminally processed; Immunophilin protein with PPIase
4 Cytochrome P450 26B1; Involved in the metabolism of retinoic acid (RA), rendering this classical mo
5
6
```

In the following section we will show how to query STRING with R on some specific proteins. In the examples, we will use the famous tumor proteins TP53 and ATM .

First we need to get the STRING identifier of those proteins, using our mp method:

```
> tp53 = string_db$mp( "tp53" )
> atm = string_db$mp( "atm" )
> mdm2 = string_db$mp( "mdm2" )
```

The mp method (i.e. map proteins) is an alternative to our map method, to be used when you need to map only one or few proteins.
It takes in input a vector of protein aliases and returns a vector with the STRING identifiers of those proteins.

# 6 TYPED NETWORK VIEWS AND REGULATORY EDGES

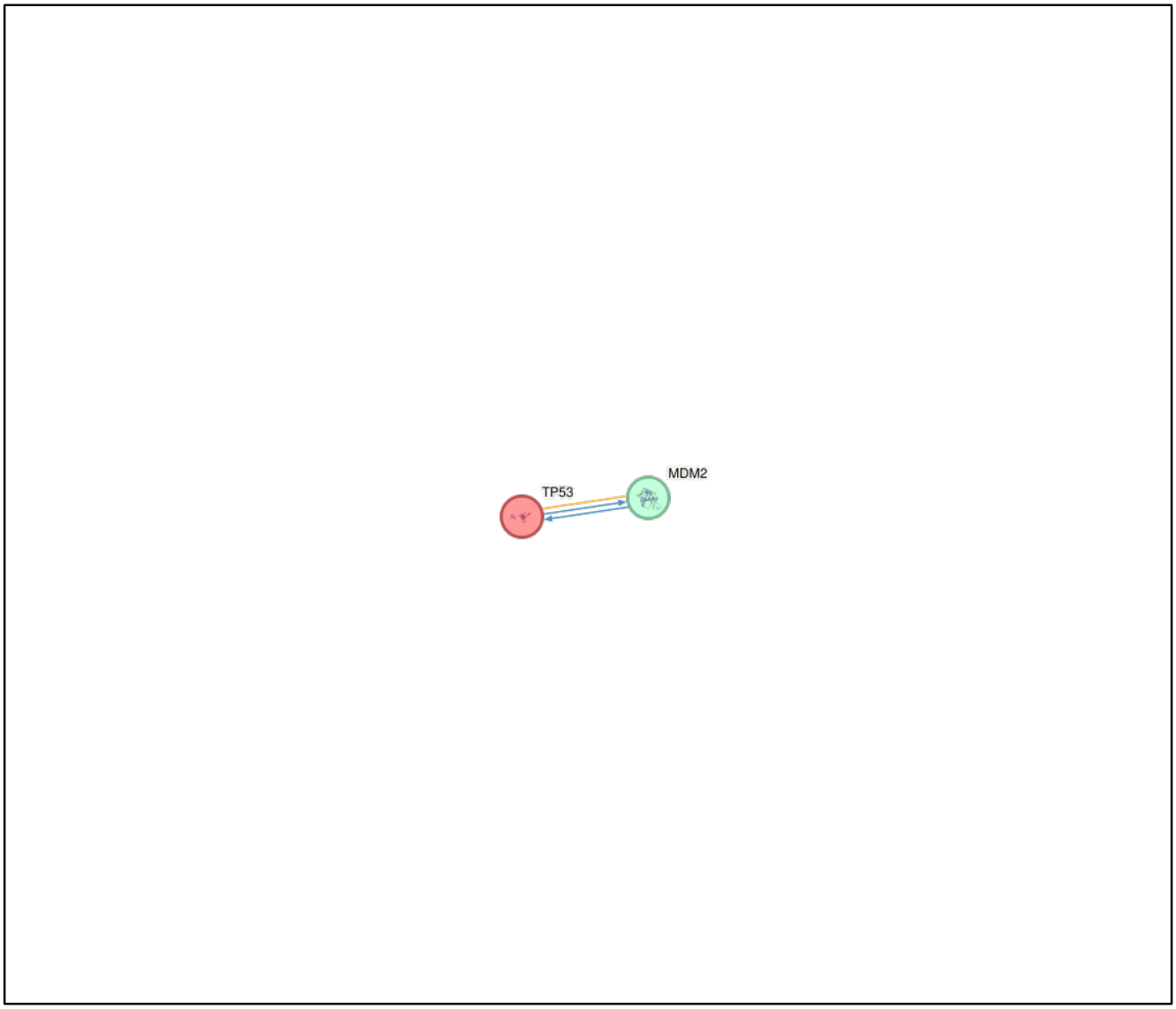
The full functional network, physical network, and regulatory network have different edge semantics. The functional and physical networks are undirected, while the regulatory network is directed from regulator to target. Create a separate `STRINGdb` object for each network type that you need; they can use the same cache directory because their downloaded link files have distinct names. The `string_db` object created above is the functional network object.

```
> functional_db <- string_db
> physical_db <- STRINGdb$new(
+   version = "12.5", species = 9606, score_threshold = 400,
+   network_type = "physical", link_data = "detailed",
+   input_directory = functional_db$input_directory
+ )
> regulatory_db <- STRINGdb$new(
+   version = "12.5", species = 9606, score_threshold = 400,
+   network_type = "regulatory", link_data = "detailed",
+   input_directory = functional_db$input_directory
+ )
```

Use the typed view on the functional object to overlay the physical and regulatory edge types. The three typed-edge arguments control which edge types and regulatory signs are shown:

```
> functional_db$plot_network(
+   c(tp53, mdm2),
+   network_flavor = "typed",
+   typed_physical_edges = TRUE,
+   typed_regulatory_edges = TRUE,
+   show_regulatory_signs = TRUE
+ )
```

proteins: 2
interactions: 1
expected interactions: 1 (p−value: 0.4871)




For analysis of regulation, use the regulatory object itself. Its interaction table retains direction: `from` is the regulator and `to` is its target. For example, the following retrieves regulatory links within the TP53–MDM2 pair and lists them as `from_name -> to_name`.

This pair illustrates a well-characterized negative-feedback circuit: TP53 transcriptionally activates MDM2, whereas MDM2 promotes proteasome-dependent degradation of TP53. Both directions are therefore expected in the regulatory graph.

```
> regulatory_edges <- regulatory_db$get_interactions(c(tp53, mdm2))
> regulatory_edges$regulatory_edge <- paste(
+   regulatory_edges$from_name, "->", regulatory_edges$to_name
+ )
> regulatory_edges[, c("regulatory_edge", "combined_score")]

  regulatory_edge combined_score
1    MDM2 -> TP53            997
2    TP53 -> MDM2            996
```

You can retrieve the interactions that connect certain input proteins between each other.
The "get_interactions" method returns a data frame describing the interactions among the queried proteins. The table contains the STRING identifiers in the `from` and `to` columns, the corresponding preferred names in `from_name` and `to_name`, the `combined_score`, and any additional evidence scores available for the current `link_data` setting. This makes it easy to inspect both the interacting proteins and the supporting scores for each interaction.

```
> string_db$get_interactions( c(tp53, atm) )

                  from                  to combined_score from_name to_name
1 9606.ENSP00000269305 9606.ENSP00000278616            999      TP53     ATM
  neighborhood fusion cooccurence coexpression experimental database textmining
1            0      0           0            0          999      900        954
```

If you want to retrieve the first-shell interaction partners of one or more proteins from the locally loaded graph, you can use the "get_interaction_partners" method.
The output keeps the same interaction-table structure as "get_interactions".

```
> string_db$get_interaction_partners( c(tp53, atm), limit=5 )[, c("from", "to", "combined_score", "ex
```

```
                       from                  to combined_score experimental
32004  9606.ENSP00000269305 9606.ENSP00000212015            999          999
37406  9606.ENSP00000269305 9606.ENSP00000215832            999          999
53636  9606.ENSP00000269305 9606.ENSP00000216911            999          999
55816  9606.ENSP00000269305 9606.ENSP00000217244            999          999
66287  9606.ENSP00000269305 9606.ENSP00000219548            999          999
386993 9606.ENSP00000278616 9606.ENSP00000265433            999          999
419128 9606.ENSP00000278616 9606.ENSP00000269305            999          999
461927 9606.ENSP00000278616 9606.ENSP00000325863            999          992
461988 9606.ENSP00000278616 9606.ENSP00000343741            999          972
462088 9606.ENSP00000278616 9606.ENSP00000361423            999          999
       from_name to_name
32004       TP53   SIRT1
37406       TP53   MAPK1
53636       TP53   AURKA
55816       TP53 CSNK2A1
66287       TP53   STUB1
386993       ATM     NBN
419128       ATM    TP53
```

```
461927       ATM   MRE11
461988       ATM     ATR
462088       ATM    ABL1
```

You can also output only interactions supported by a particular type of evidence. Here we retrieve only experimentally supported interactions, keeping only the protein names together with the experimental score:

```
> interaction_partners <- string_db$get_interaction_partners( c(tp53, atm), limit=5 )
> interaction_partners_exp <- subset(interaction_partners, experimental > 0,
+                                    select = c("from_name", "to_name", "experimental"))
> interaction_partners_exp

       from_name to_name experimental
32004       TP53   SIRT1          999
37406       TP53   MAPK1          999
53636       TP53   AURKA          999
55816       TP53 CSNK2A1          999
66287       TP53   STUB1          999
386993       ATM     NBN          999
419128       ATM    TP53          999
461927       ATM   MRE11          992
461988       ATM     ATR          972
462088       ATM    ABL1          999
```

STRING provides a way to get homologous proteins: in our database we store ALL-AGAINST-ALL alignments within species. You can retrieve all paralogs of the protein using "get_paralogs" method.

```
> # Get all homologs of TP53 in human.
> string_db$get_paralogs(tp53)

  ncbiTaxonId_A           stringId_A ncbiTaxonId_B           stringId_B
1          9606 9606.ENSP00000269305          9606 9606.ENSP00000269305
  bitscore
1    815.8
```

STRING also stores best hits (as measured by bitscore) between proteins from different species. "get_homologs_besthits" lets you retrieve these homologs.

```
> # get the best hits of TP53 in all STRING species
> tp53_besthits <- string_db$get_homologs_besthits(tp53)
> tp53_besthits_100 <- subset(tp53_besthits, bitscore > 100)
> nrow(tp53_besthits_100)

[1] 318
```

```
> head(tp53_besthits_100, n=10)
```

```
   ncbiTaxonId_A           stringId_A ncbiTaxonId_B                stringId_B
1           9606 9606.ENSP00000269305          9597   9597.ENSPPAP00000011040
2           9606 9606.ENSP00000269305          9598   9598.ENSPTRP00000014836
3           9606 9606.ENSP00000269305          9606      9606.ENSP00000269305
4           9606 9606.ENSP00000269305          9601   9601.ENSPPYP00000008923
5           9606 9606.ENSP00000269305         61621  61621.ENSRBIP00000028632
6           9606 9606.ENSP00000269305         61622  61622.ENSRROP00000026843
7           9606 9606.ENSP00000269305        591936 591936.ENSPTEP00000010553
8           9606 9606.ENSP00000269305         60711  60711.ENSCSAP00000007551
9           9606 9606.ENSP00000269305          9541   9541.ENSMFAP00000038526
10          9606 9606.ENSP00000269305          9544   9544.ENSMMUP00000011334
   bitscore
1     815.8
2     815.8
3     815.8
4     799.3
5     786.6
6     786.6
7     785.8
8     785.0
9     783.9
10    783.9
```

... or you can specify the species of interest:

```
> # get the homologs of the following two proteins in the mouse (i.e. species_id=10090)
> string_db$get_homologs_besthits(c(tp53, atm), target_species_id=10090)
```

```
  ncbiTaxonId_A           stringId_A ncbiTaxonId_B                stringId_B
1          9606 9606.ENSP00000269305         10090 10090.ENSMUSP00000104298
2          9606 9606.ENSP00000278616         10090 10090.ENSMUSP00000156344
  bitscore
1    598.2
2   5328.8
```

# 7 CITATION

Please cite:

Szklarczyk D, Nastou K, Koutrouli M, Kirsch R, Mehryary F, Hachilif R, Hu D, Peluso ME, Huang Q, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ, von Mering C. The STRING database in 2025: protein networks with directionality of regulation. Nucleic Acids Res. 2025 Jan 6;53(D1):D730-D737. doi: 10.1093/nar/gkae1113.