STRINGdb Package Vignette

Andrea Franceschini

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1 INTRODUCTION

STRING (http://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. We currently cover 5'214'234 proteins from 1133 organisms.

As you will learn in this guide, the STRING database can be usefull to add meaning to list of genes (e.g. the best hits coming out from a screen or the most differentially expressed genes coming out from a Microarray/RNAseq 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 use this function to retrieve/search our species table:

```
> get_STRING_species(version="10", species_name=NULL)
```

```
> library(STRINGdb)
> string_db <- STRINGdb$new( version="10", species=9606,
+                            score_threshold=0, input_directory="" )
```

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 !).

Besides, if you specify a local directory to the parameter input-directory, all the database files will be downloaded into this directory and the package can then 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.

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"                                   "getClass"
[13] "getRefClass"                             "get_aliases"
[15] "get_annotations"                         "get_annotations_desc"
[17] "get_bioc_graph"                          "get_clusters"
[19] "get_enrichment"                          "get_graph"
[21] "get_homologs"                            "get_homologs_besthits"
[23] "get_homology_graph"                      "get_interactions"
[25] "get_link"                                "get_neighbors"
[27] "get_pathways_benchmarking_blackList" "get_png"
[29] "get_ppi_enrichment"                      "get_ppi_enrichment_full"
[31] "get_proteins"                            "get_pubmed"
[33] "get_pubmed_interaction"                  "get_subnetwork"
[35] "get_summary"                             "get_term_proteins"
[37] "import"                                  "initFields"
[39] "initialize"                              "load"
[41] "load_all"                                "map"
[43] "mp"                                      "plot_network"
[45] "plot_ppi_enrichment"                     "post_payload"
[47] "remove_homologous_interactions"          "set_background"
[49] "show"                                    "show#envRefClass"
[51] "trace"                                   "untrace"
[53] "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):
   Andrea Franceschini
```

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 14% 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 best 200 genes and we produce an image of the STRING network for those. 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. Besides, on the bottom, there is a short-url that points to the relative page on our web-interface. You can copy and paste that url on your favorite browser and/or send it via e-mail to your colleagues or insert it in publications.

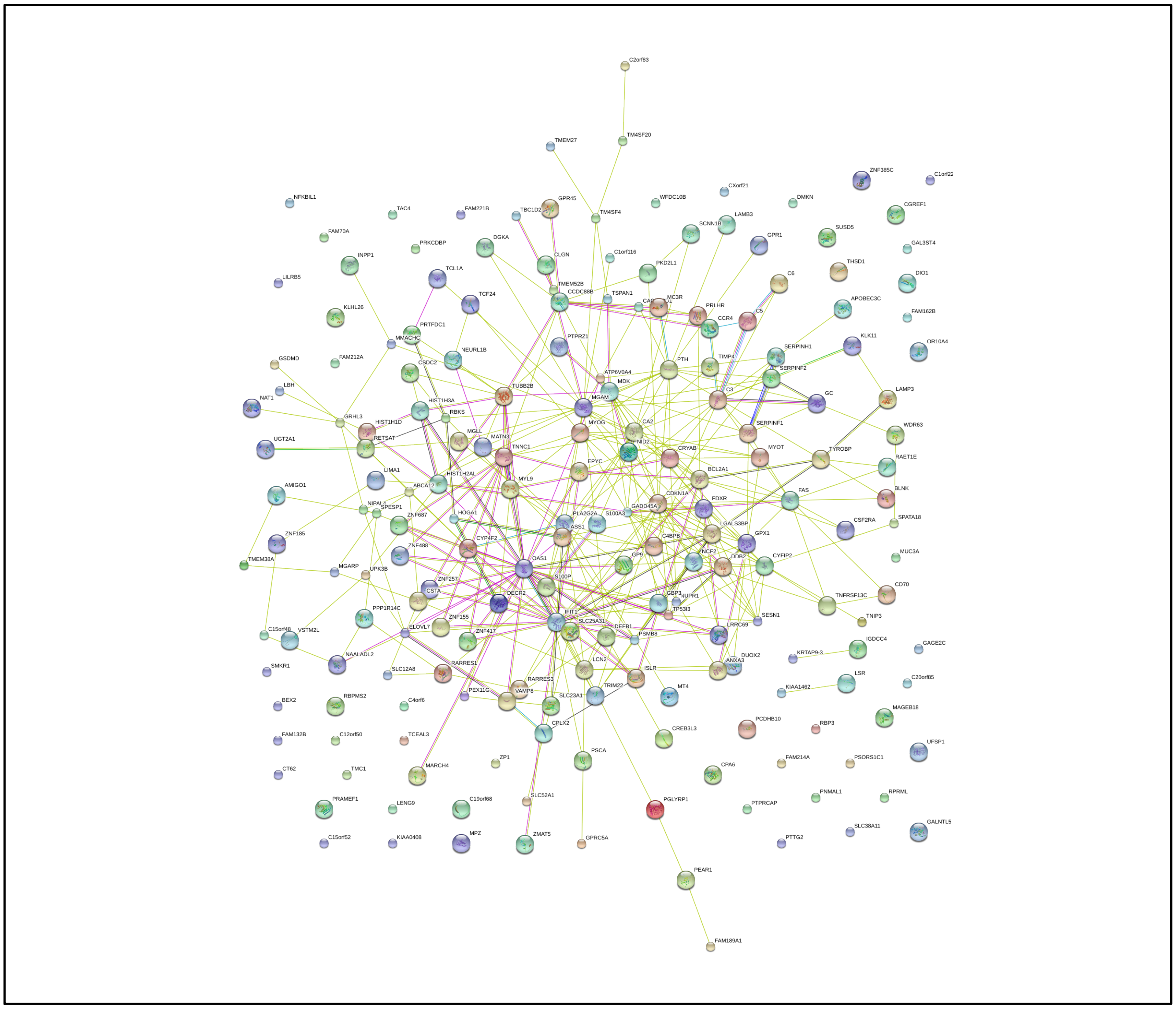
```
> hits <- example1_mapped$STRING_id[1:200]
```

```
> string_db$plot_network( hits )
```

proteins: 200

interactions: 353

expected interactions: 266 (p−value: 2.60548724706489e−07)



http://version10.string−db.org/10/p/1741348753

2 PAYLOAD MECHANISM

This R library provides the ability to interact with the STRING payload mechanism. The payload appears as an additional colored "halo" that starts from the boder of the bubbles.

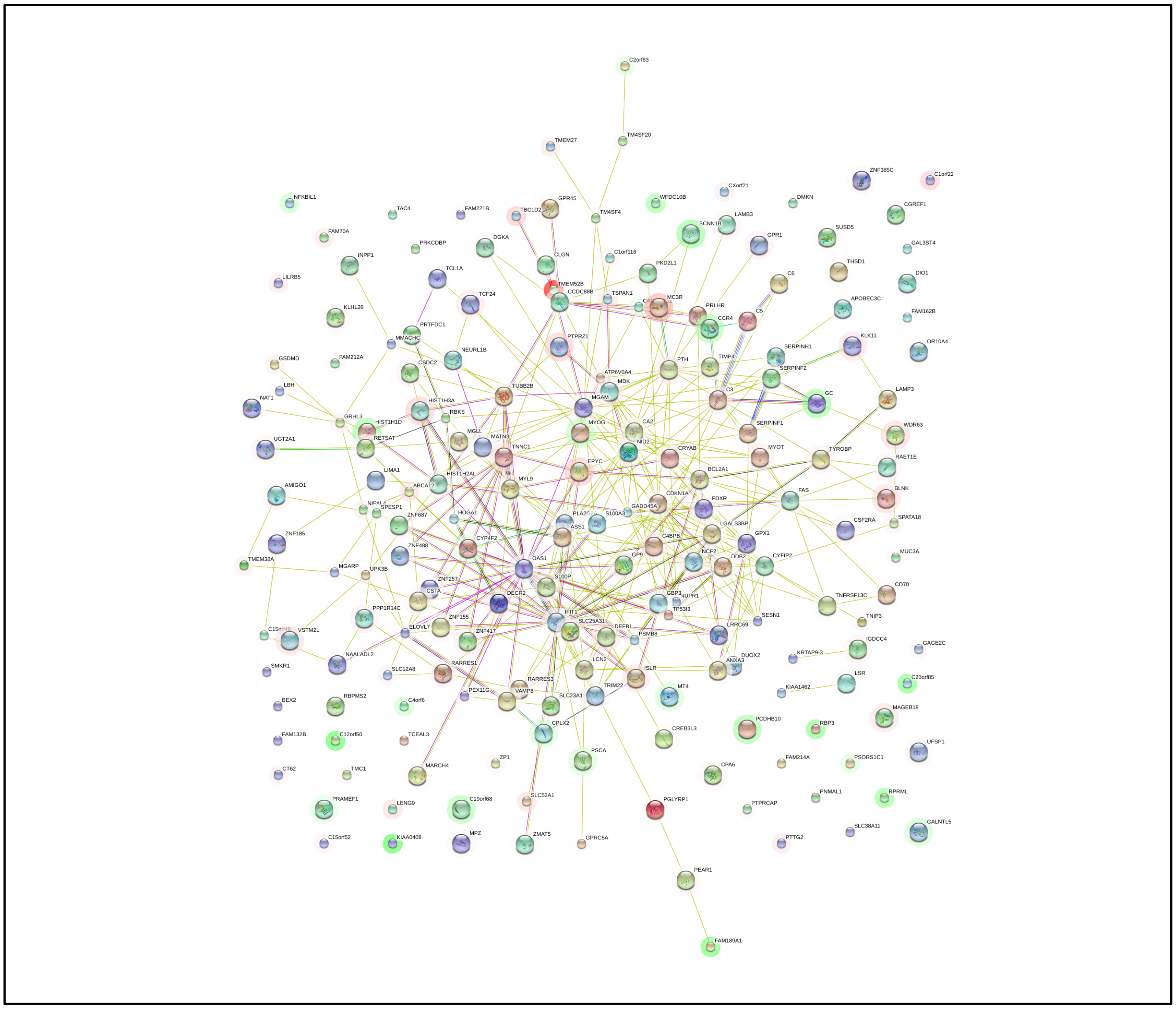
For example, this allows to color in green the genes that are down-regulated and in red the genes that are up-regulated. For this mechanism to work, we provide a function that posts the information on our web server.

```
> # 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: 353
expected interactions: 266 (p−value: 2.60548724706489e−07)

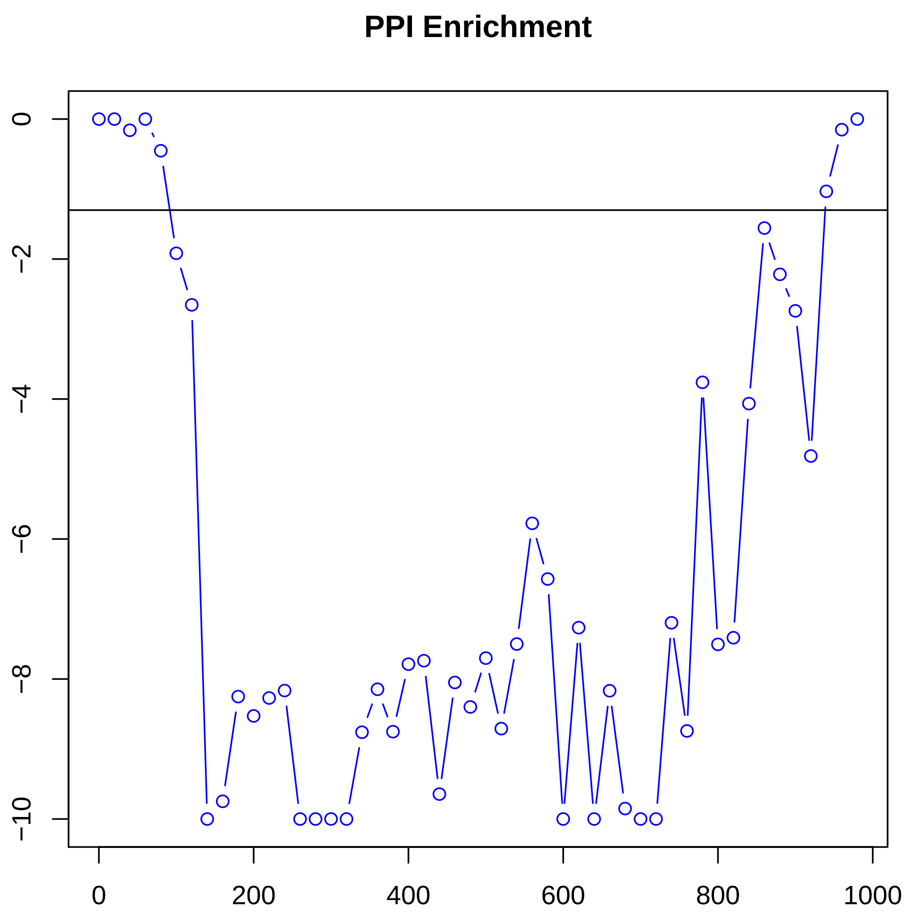


http://version10.string−db.org/10/p/7729348754

3 ENRICHMENT

We provide a method to compute (and visualize) the enrichment in protein-protein interactions along a sorted list of proteins. In the context of genome-wide screens (e.g. RNAi or proteomics), it can be useful to visualize the distribution of the enrichment in the resulting sorted list of genes. If the experiment was successful, and the top hits have protein-protein interactions, you should see more enrichment at the beginning of the list than at the end. Besides, you can also use the enrichment graph to help you to define a threshold on the number of proteins to consider (for example, if you see a strong enrichment up to position 600 on your list, this means that the signal is probably sparsed to cover the best 600 genes).

```
> # plot the enrichment for the best 1000 genes
> string_db$plot_ppi_enrichment( example1_mapped$STRING_id[1:1000], quiet=TRUE )
```



We also provide a method to compute the enrichment in Gene Ontology, KEGG pathway and Interpro domains, similar to the ”enrichment” widget in our web-interface. The enrichment is computed using an hypergeometric test. The multiple testing correction method can be changed (setting the methodMT parameter) and it is also possible to specify whether to use the ”Inferred from Elettronic Annotations” or only the manually curated annotations.

```
> enrichmentGO <- string_db$get_enrichment( hits, category = "Process", methodMT = "fdr", iea = TRUE
> enrichmentKEGG <- string_db$get_enrichment( hits, category = "KEGG", methodMT = "fdr", iea = TRUE )
> head(enrichmentGO, n=7)

     term_id proteins hits       pvalue  pvalue_fdr
1 GO:0048545      383   15 2.337593e-07 0.000368020
2 GO:0006952     1286   28 3.806997e-07 0.000368020
3 GO:0002252      405   15 4.756829e-07 0.000368020
4 GO:0010466      234   11 1.767763e-06 0.001025745
5 GO:0045861      330   12 8.407161e-06 0.003765892
6 GO:0010951      228   10 9.735179e-06 0.003765892
7 GO:0002443      141    8 1.224368e-05 0.003839606
                              term_description
1                   response to steroid hormone
2                              defense response
3                       immune effector process
4     negative regulation of peptidase activity
5           negative regulation of proteolysis
6 negative regulation of endopeptidase activity
7                   leukocyte mediated immunity


> head(enrichmentKEGG, n=7)

  term_id proteins hits       pvalue   pvalue_fdr
1   04115       66    6 9.969204e-08 1.475442e-05
2   04610       68    5 3.577966e-06 2.647695e-04
3   05168      174    5 3.254067e-04 1.605340e-02
4   01100     1161   12 5.277721e-04 1.952757e-02
5   04380      123    4 8.399948e-04 2.486385e-02
6   00590       61    3 1.197959e-03 2.638087e-02
7   05161      139    4 1.322517e-03 2.638087e-02
                    term_description
1               p53 signaling pathway
2 Complement and coagulation cascades
3            Herpes simplex infection
4                  Metabolic pathways
5          Osteoclast differentiation
6         Arachidonic acid metabolism
7                         Hepatitis B
```

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 methods explained above, you should set the background:

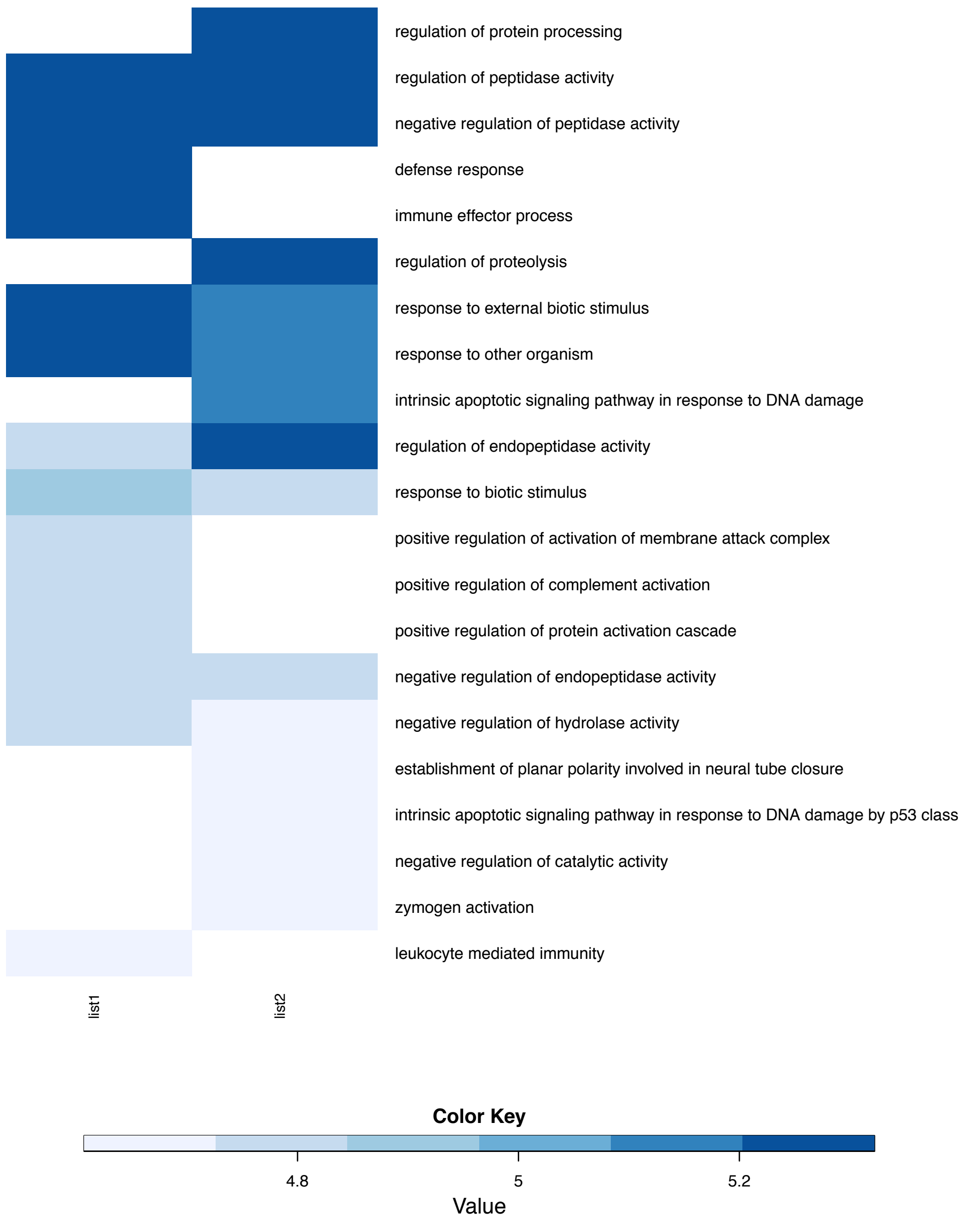
```
> backgroundV <- example1_mapped$STRING_id[1:2000]   # as an example, we use the first 2000 genes
> string_db$set_background(backgroundV)
```

You can also set the background when you instantiate the STRINGdb object:

```
> string_db <- STRINGdb$new( score_threshold=0, backgroundV = backgroundV )
```

If you want to compare the enrichment of two or more lists of genes (e.g. the results of two experiments), you can use our HEATMAP visualization option:

```
> eh <- string_db$enrichment_heatmap( list( hits[1:100], hits[101:200]),
+                                     list("list1","list2"), title="My Lists" )
```



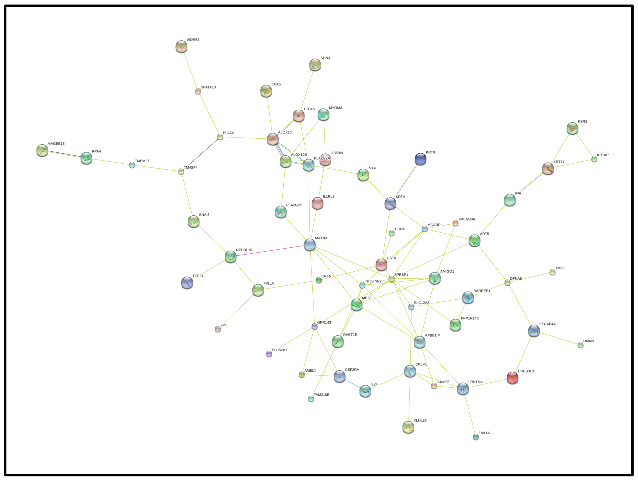
4 CLUSTERING

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$get_clusters(example1_mapped$STRING_id[1:600])
```

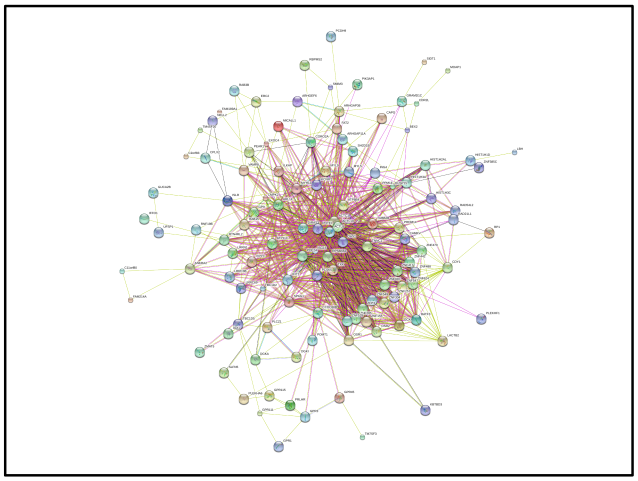
```
> # plot first 4 clusters
> par(mfrow=c(2,2))
> for(i in seq(1:4)){
+  string_db$plot_network(clustersList[[i]])
+ }
```

proteins: 61
interactions: 91
expected interactions: 7 (p−value: 0)



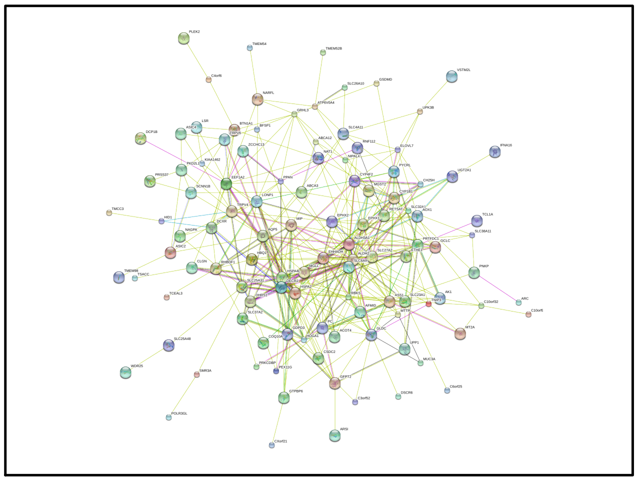
http://version10.string−db.org/10/p/2183348759

proteins: 120
interactions: 881
expected interactions: 457 (p−value: 0)



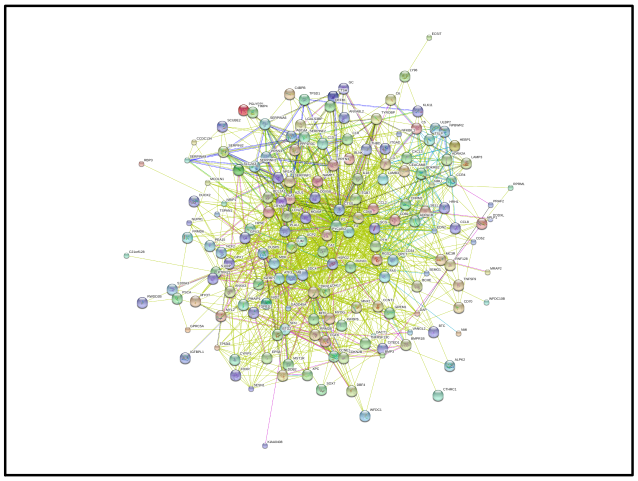
http://version10.string−db.org/10/p/2278348760

proteins: 109
interactions: 356
expected interactions: 82 (p−value: 0)



http://version10.string−db.org/10/p/6843348761

proteins: 166
interactions: 1250
expected interactions: 407 (p−value: 0)



http://version10.string−db.org/10/p/3964348763

5 ADDITIONAL PROTEIN INFORMATION

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

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

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" )
```

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.

Using the following method, you can see the proteins that interact with one or more of your proteins:

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

It is also possible to retrieve the interactions that connect certain input proteins between each other. Using the "get_interactions" method we can clearly see that TP53 and ATM interact with each other with a good evidence/score.

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

                  from                   to neighborhood
1 9606.ENSP00000269305 9606.ENSP00000278616            0
  neighborhood_transferred fusion cooccurence homology coexpression
1                        0      0           0        0            0
  coexpression_transferred experiments experiments_transferred database
1                        0         999                       0      900
  database_transferred textmining textmining_transferred combined_score
1                    0        973                     80            999
```

Using the get_pubmed_interactions method we can retrieve the pubmed identifiers of the articles that contain the name of both the proteins (if any). Such articles could support to the interaction.

```
> string_db$get_pubmed_interaction( tp53, atm )
```

STRING also provides a way to get homologous proteins: in our database we store an ALL-AGAINST-ALL blast alignment of all our 5 million proteins. The method ”get_homologs_besthits” can be used to get the best hits of your proteins in all the >1000 STRING species (with the ”symbets” parameter you can limit the search to the reciprocal_best_hits. This increase the confidence to get orthologs and not paralogous proteins)

```
> # get the reciprocal best hits of the following protein in all the STRING species
> string_db$get_homologs_besthits(tp53, symbets = TRUE)
```

In addition, you can get all the homologous in a given species (i.e. all the blast hits):

```
> # get the homologs of the following two proteins in the mouse (i.e. species_id=10090)
> string_db$get_homologs(c(tp53, atm), target_species_id=10090, bitscore_threshold=60 )
```

6 BENCHMARKING PROTEIN-PROTEIN INTERACTIONS

When a new methodology/algorithm to infer protein-protein interactions is developed, the outcome should be carefully benchmarked against a gold standard. Hence, we suggest to benchmark agains the KEGG pathway database (or other high quality pathway databases), and we provide suitable functions to perform this task.

First of all, you need to provide as input a sorted interaction data frame (with the columns ”proteinA”, ”proteinB”, ”score”).

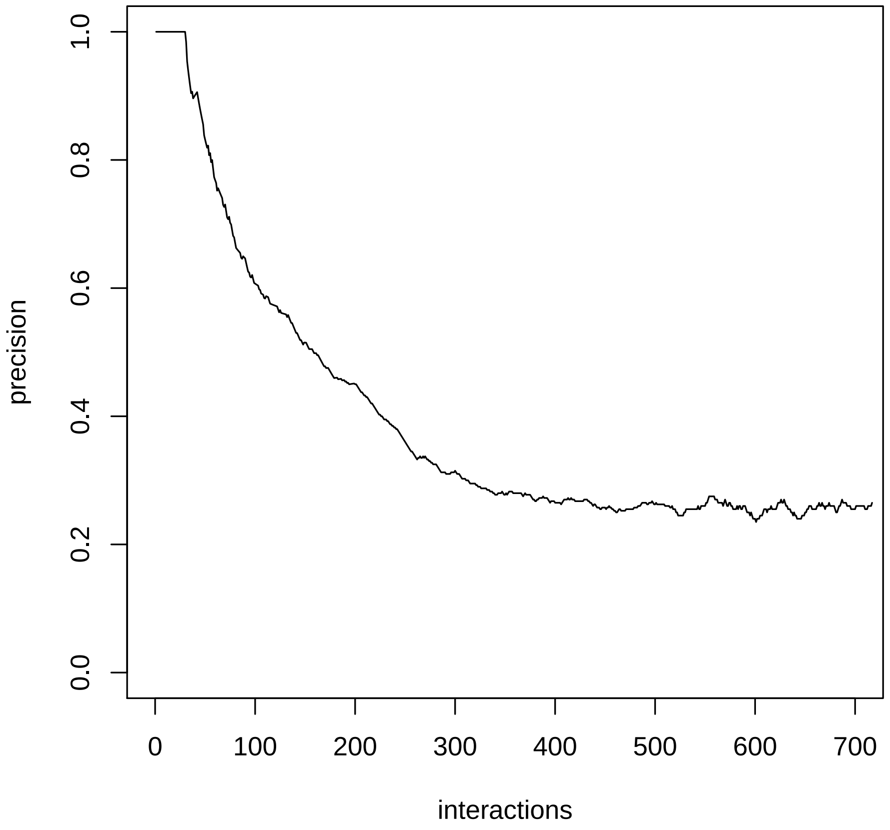
```
> data(interactions_example)
> interactions_benchmark = string_db$benchmark_ppi(interactions_example, pathwayType = "KEGG",
+                  max_homology_bitscore = 60, precision_window = 400, exclude_pathways = "blacklist")

Discarded 55844 interactions ( 79.77714 % )  between homologous proteins
NOTE:  717 interactions ( 5.1 % ) have been mapped to KEGG
```

The function benchmark_ppi will return a data frame containing the sorted list of protein interactions mapped to the benchmark, and the precision computed in a sliding window of 400 interactions (that automatically expands/shrink at the beginning/end of the sorted interactions list). The precision is defined as the number of TP interactions (where the pair of proteins are both present together in at least one pathway) vs the total number of interactions in the window. When benchmarking PPI it is often important to remove the interactions composed by a pair of homologous protein. You can set a parameter in the function to automatically perform this task (max_homology_bitscore). The user may also want to remove several uninformative pathway from the gold standard (i.e. pathways that are too big or too generic). The STRING team manually curates a black list of KEGG pathways that in our opinion should be removed in order to provide a reliable gold standard. With the ”exclude_pathways” parameter it is possible to automatically remove those pathways from the analysis (setting the parameter to ”blacklist”) or to specify a vector contaning the term identifiers of the pathways that the user wants to remove.

It is finally very easy to plot the precision vs the number of sorted interactions:

```
> plot(interactions_benchmark$precision, ylim=c(0,1), type="l", xlim=c(0,700),
+          xlab="interactions", ylab="precision")
```



We also provide a function to list the pathways to which the various TP interactions belong, together with some accompanying statistic:

```
> interactions_pathway_view = string_db$benchmark_ppi_pathway_view(interactions_benchmark, precision_
> head(interactions_pathway_view)

  pathways coverage proteins interactions total_representation
1    00190    0.009      130           73             0.277567
2    00280    0.026       44           25             0.095057
3    00100    0.084       20           16             0.060837
4    04630    0.001      154           15             0.057034
5    05130    0.011       52           14             0.053232
6    04725    0.002      107           11             0.041825
                            term_description
1                  Oxidative phosphorylation
2 Valine, leucine and isoleucine degradation
3                       Steroid biosynthesis
4                  Jak-STAT signaling pathway
5      Pathogenic Escherichia coli infection
6                        Cholinergic synapse
```

For each pathway for which a TP interaction is found in the sorted interaction list, we report its ”total_representation” (i.e. which percentages of interactions belong to that pathway), the ”coverage” (i.e. number of interactions of the input list in the pathway / total number of possible interactions in the pathway) and the size of the pathway in number of proteins.

7 CITATION

Please cite:

Franceschini, A et al. (2013). STRING v9.1: protein-protein interaction networks, with increased coverage and integration. In:’Nucleic Acids Res. 2013 Jan;41(Database issue):D808-15. doi: 10.1093/nar/gks1094. Epub 2012 Nov 29