

# Home

---

MIENTURNET (MIcroRNAENrichmentTURnedNETwork) is an easy-to-use web tool devised for:

- receiving in input a list of genes and inferring possible evidences (computational or experimental) of miRNA regulation based on a statistical analysis for over-representation of miRNA-target interactions;
- receiving in input a list of mature miRNAs and inferring possible evidences (computational or experimental) of their regulation on target genes based on a statistical analysis for over-representation of miRNA-target interactions.

The resulting miRNA-target interactions are visualized as a network.

MIENTURNET makes use of computationally predicted or experimentally validated miRNA-target interactions downloaded from TargetScan [1] website Release 7.2 (March 2018) and miRTarBase [2] website Release 7.0 (September 2017), respectively (Figure 1).

Currently, MIENTURNET supports the choice of six organisms: Homo sapiens, Mus musculus, Rattus norvegicus, Caenorhabditis elegans, Drosophila melanogaster, Danio rerio.

MIENTURNET is especially meant for non-expert users through the development of a simple and intuitive web interface as well as the delivery of an exhaustive and well-documented set of output information (Figure 2).

## Availability and requirements

MIENTURNET was created using the shiny package from RStudio (<https://cran.r-project.org/web/packages/shiny>), it has been developed by Valerio Licursi, Federica Conte, Giulia Fiscon and Paola Paci of Institute for Systems Analysis and Computer Science “Antonio Ruberti”, National Research Council, Rome, Italy. The interactive MIENTURNET web application is available at <http://userver.bio.uniroma1.it/apps/mienturnet/>. This website is free and open to all users and there is no login requirement.

## Browser compatibility

| OS      | Version                | Chrome | Firefox | Microsoft Edge | Internet Explorer | Safari |
|---------|------------------------|--------|---------|----------------|-------------------|--------|
| Linux   | Ubuntu 16.04           | 70.0   | 62.0    | n/a            | n/a               | n/a    |
| MacOS   | High Sierra<br>10.13.6 | 70.0   | 62.0    | n/a            | n/a               | 12.0.2 |
| Windows | 10                     | 70.0   | 62.0    | 17.17134       | 11                | n/a    |

## How to cite MIENTURNET

If you use MIENTURNET, please cite the following article.

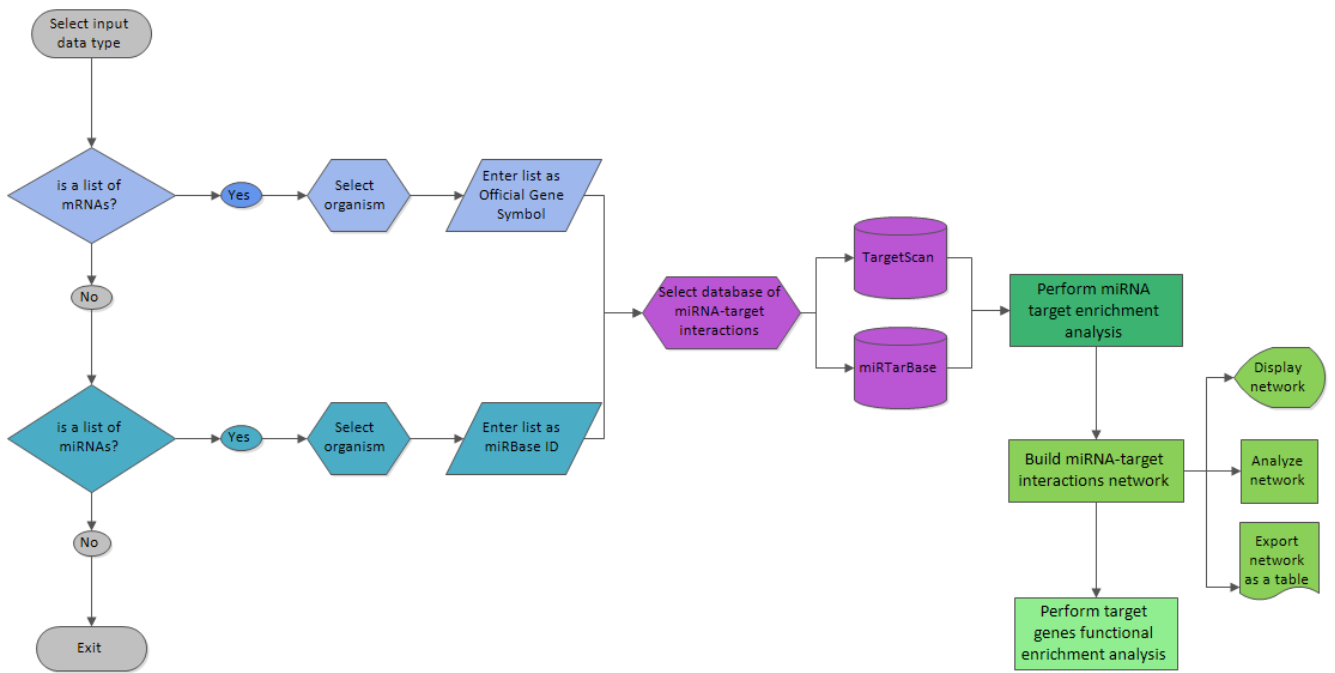


Figure 1. Flowchart of MIENTURNET web tool.

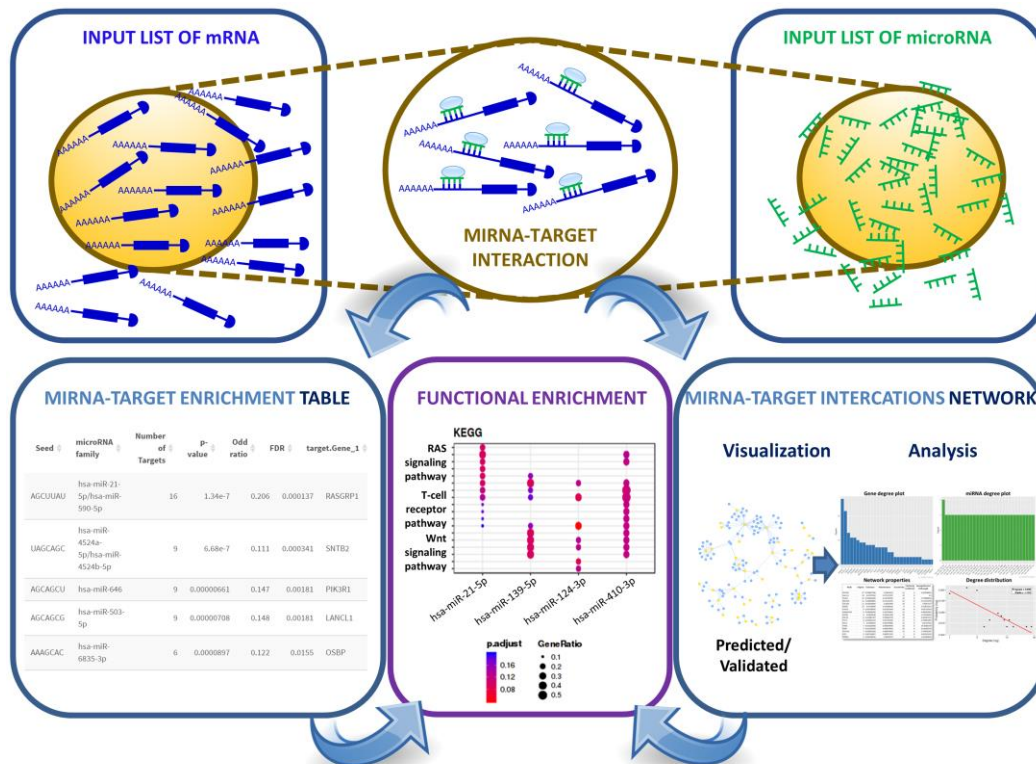
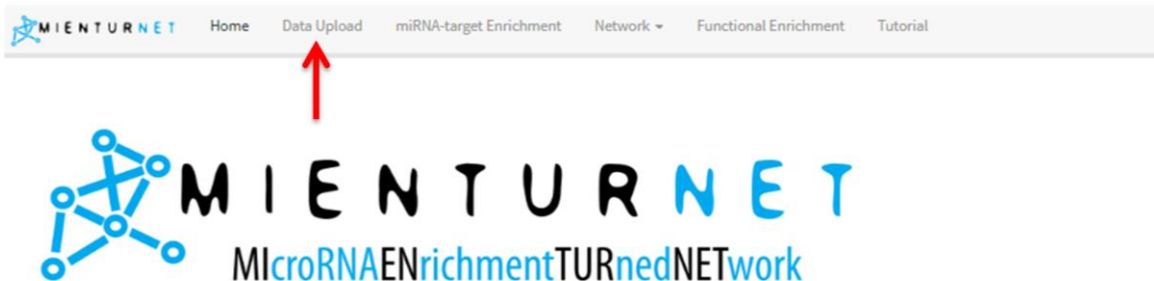


Figure 2. Sketch of MIENTURNET input/output files. MIENTURNET looks for miRNAs targeting an input list of mRNAs (top-left) or mRNAs targeted by an input list of miRNAs (top-right) and it performs a statistical analysis for over-representation of miRNA-target interactions (bottom-left) together with a network-based visualization and analysis of the resulting enriched interactions (bottom-right).

# Tutorial

---

From the **Home** page of MIENTURNET, click on **Data Upload** link on the top navigation bar to start the analysis.



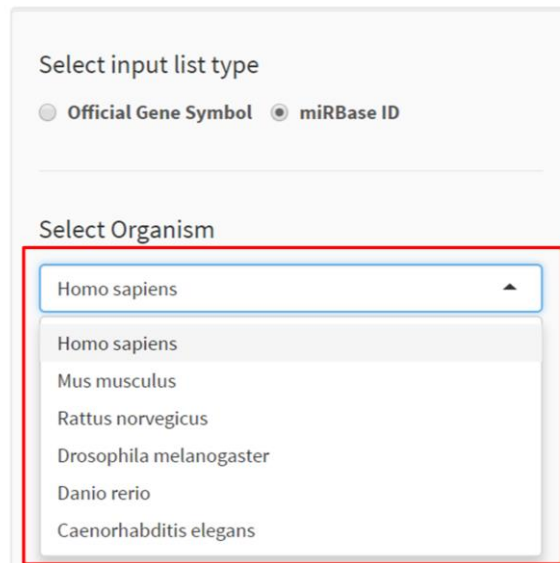
## Entering a list of miRNAs

### Data Upload link

If the user is interested to find targets of a given list of miRNAs, from the **Data Upload** page, select **miRBase ID** as type of the input list.

The image shows the 'Data Upload' form on the MIENTURNET website. The form is titled 'Select input list type' and has two radio buttons: 'Official Gene Symbol' and 'miRBase ID'. The 'miRBase ID' option is selected. Below this is a 'Select Organism' dropdown menu with 'Homo sapiens' selected. Underneath is the 'Enter input list' section with three radio buttons: 'Upload a file', 'Paste your list', and 'Load an example list'. The 'Upload a file' option is selected. Below this is the 'Upload a text list' section with a 'BROWSE...' button and the text 'No file selected'.

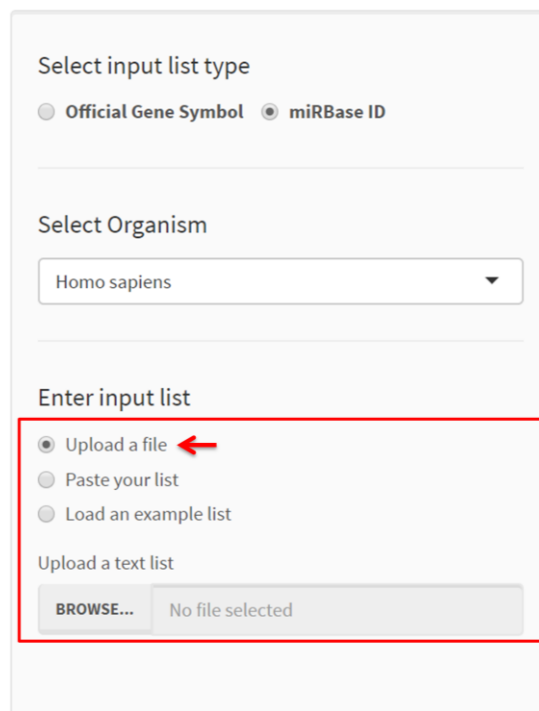
Select the organism from the drop-down menu.



The screenshot shows a web interface with two sections. The first section, 'Select input list type', has two radio buttons: 'Official Gene Symbol' (unselected) and 'miRBase ID' (selected). Below this is a horizontal line. The second section, 'Select Organism', features a dropdown menu with 'Homo sapiens' selected. A red rectangular box highlights the dropdown menu and its list of options: 'Homo sapiens', 'Mus musculus', 'Rattus norvegicus', 'Drosophila melanogaster', 'Danio rerio', and 'Caenorhabditis elegans'.

Enter the input list by using one of the following options:

- 1) **Upload a file** if the list is saved in a text file on the own computer and then click BROWSE to quickly access to the file



The screenshot shows the same web interface as above, but with the 'Enter input list' section highlighted by a red rectangular box. This section contains three radio buttons: 'Upload a file' (selected, with a red arrow pointing to it), 'Paste your list', and 'Load an example list'. Below these is the text 'Upload a text list' and a button labeled 'BROWSE...' next to the text 'No file selected'.

2) **Paste your list** in the specific text area and then click SUBMIT

Select input list type  
 Official Gene Symbol  miRBase ID

Select Organism  
Homo sapiens

Enter input list  
 Upload a file  
 Paste your list  
 Load an example list

Paste your list below:  
PASTE HERE

CLEAR DATA SUBMIT

3) **Load an example list** for playing with data. With this choice, the user has the possibility to explore the functionality of MIENTURNET by exploiting a usage example.

Select input list type  
 Official Gene Symbol  miRBase ID

Select Organism  
Homo sapiens

Enter input list  
 Upload a file  
 Paste your list  
 Load an example list

IDs found: 7 of 10

Copy CSV Excel

Showing 1 to 7 of 7 entries

| microRNA name   | miRBase accession            | microRNA mature sequence |
|-----------------|------------------------------|--------------------------|
| hsa-miR-125b-5p | <a href="#">MIMAT0000423</a> | UCCUGAGACCCUAACUUGUGA    |
| hsa-miR-155-3p  | <a href="#">MIMAT0004658</a> | CUCCUACAUAUUGAGCAUUAACA  |
| hsa-miR-16-5p   | <a href="#">MIMAT0000069</a> | UAGCAGCACGUAAAUAUUGGCG   |
| hsa-miR-17-5p   | <a href="#">MIMAT0000070</a> | CAAAGUGCUUACAGUGCAGGUAG  |
| hsa-miR-19a-5p  | <a href="#">MIMAT0004490</a> | AGUUUUGCAUAGUUGCACUACA   |
| hsa-miR-19b-3p  | <a href="#">MIMAT0000074</a> | UGUGCAAUCCAUGCAAAACUGA   |
| hsa-miR-21-5p   | <a href="#">MIMAT0000076</a> | UAGCUUAUCAGACUGAUUGUGA   |

Show 10 entries

Previous 1 Next

IDs not found: 3

Hereafter, we will show how MIENTURNET works by loading the example input list of miRNAs selected by the authors. Note that this list represents a subset of known oncomiR.

## Input list identifiers

The input list must include identifiers of the mature miRNAs as miRBase ID (e.g., hsa-miR-15a-5p). The tool processes the input file and immediately provides in the **IDs found** collapsible panel the total number of miRNAs recognized by miRBase database together with a table that includes: microRNA names, miRBase accession IDs as hyperlinks to miRBase, and microRNA mature sequences.

The screenshot shows the 'IDs found' panel of the tool. On the left, there are controls for 'Select input list type' (Official Gene Symbol, miRBase ID), 'Select Organism' (Homo sapiens), and 'Enter input list' (Upload a file, Paste your list, Load an example list). The main panel displays 'IDs found: 7 of 10' with a red arrow pointing to the count. Below this are 'Copy', 'CSV', and 'Excel' buttons. A search bar is on the right. The table shows 7 entries with columns for 'microRNA name', 'miRBase accession', and 'microRNA mature sequence'. At the bottom, there is a 'Show 10 entries' dropdown and 'Previous', '1', 'Next' navigation buttons. An orange bar at the bottom indicates 'IDs not found: 3'.

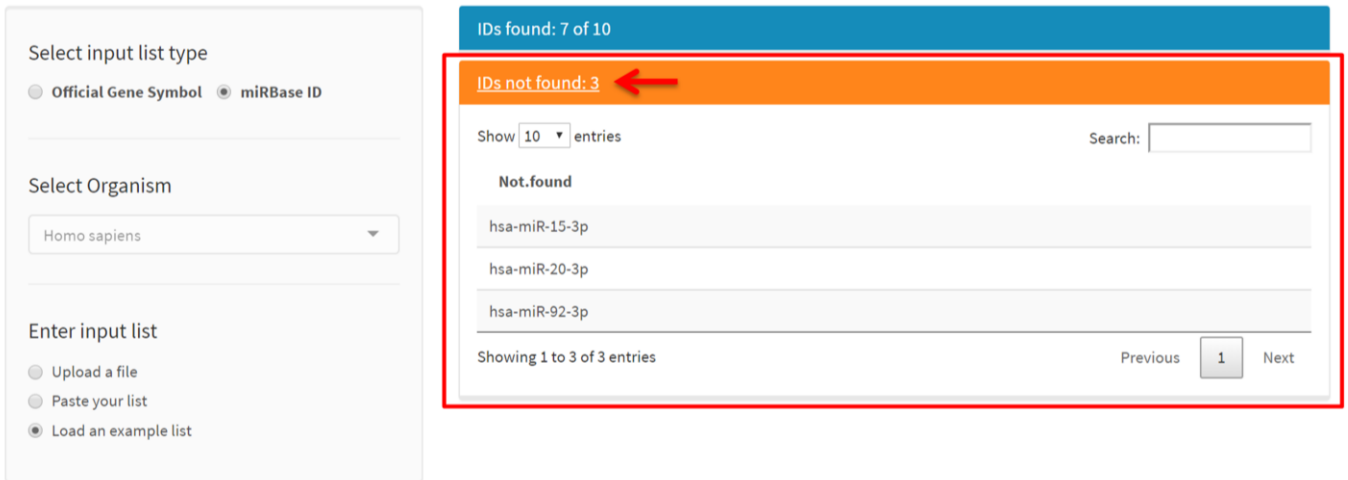
| microRNA name   | miRBase accession            | microRNA mature sequence |
|-----------------|------------------------------|--------------------------|
| hsa-miR-125b-5p | <a href="#">MIMAT0000423</a> | UCCUGAGACCCUAAUCUUGUGA   |
| hsa-miR-155-3p  | <a href="#">MIMAT0004658</a> | CUCCUACAUAUAGCAUUAACA    |
| hsa-miR-16-5p   | <a href="#">MIMAT0000069</a> | UAGCAGCAGCUAAUAUUGGGCG   |
| hsa-miR-17-5p   | <a href="#">MIMAT0000070</a> | CAAAGUGCUUACAGUGCAGGUAG  |
| hsa-miR-19a-5p  | <a href="#">MIMAT0004490</a> | AGUUUUGCAUAGUUGCACUACA   |
| hsa-miR-19b-3p  | <a href="#">MIMAT0000074</a> | UGUGCAAUCCAUGCAAACUGA    |
| hsa-miR-21-5p   | <a href="#">MIMAT0000076</a> | UAGCUUAUCAGACUGAUGUUGA   |

In the input list table, the user has also the possibility to:

- visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the bottom-left
- navigate the table through the navigation controller buttons at the bottom-right
- search for a specific miRNA in the input list by typing the name in the search bar at the top-right.
- copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left.

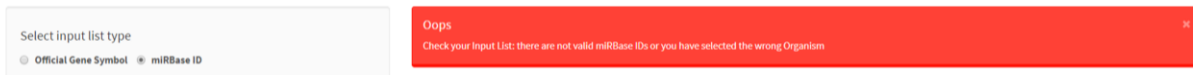
This screenshot is similar to the previous one but includes red boxes and labels 'i.', 'ii.', 'iii.', and 'iv.' pointing to specific UI elements. 'iv.' points to the 'Copy', 'CSV', and 'Excel' buttons. 'iii.' points to the search bar. 'ii.' points to the 'Previous', '1', 'Next' navigation buttons. 'i.' points to the 'Show 10 entries' dropdown menu.

The list of not recognized miRNAs (if any) can be viewed by clicking on the **IDs not found** collapsible panel.



The screenshot shows a search interface with two main panels. The left panel, titled "Select input list type", has two radio buttons: "Official Gene Symbol" (unselected) and "miRBase ID" (selected). Below this is a "Select Organism" dropdown menu set to "Homo sapiens". The "Enter input list" section has three radio buttons: "Upload a file" (unselected), "Paste your list" (unselected), and "Load an example list" (selected). The right panel has a blue header "IDs found: 7 of 10". Below it is an orange header "IDs not found: 3" with a red arrow pointing to it. Underneath is a search bar with "Show 10 entries" and "Search:". A section titled "Not.found" lists three miRNAs: "hsa-miR-15-3p", "hsa-miR-20-3p", and "hsa-miR-92-3p". At the bottom, it says "Showing 1 to 3 of 3 entries" with "Previous", "1", and "Next" buttons.

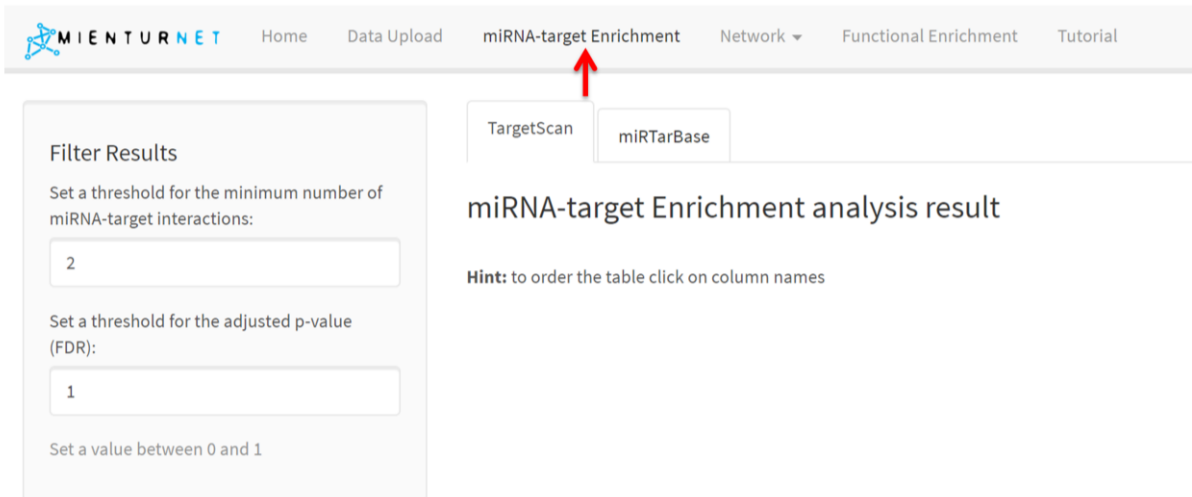
An error message appears either if the input list contains all miRBase IDs that are not valid or if the organism is incorrectly selected.



The screenshot shows the same search interface as above, but with a red error message banner at the top right. The banner says "Oops" and "Check your Input List: there are not valid miRBase IDs or you have selected the wrong Organism".

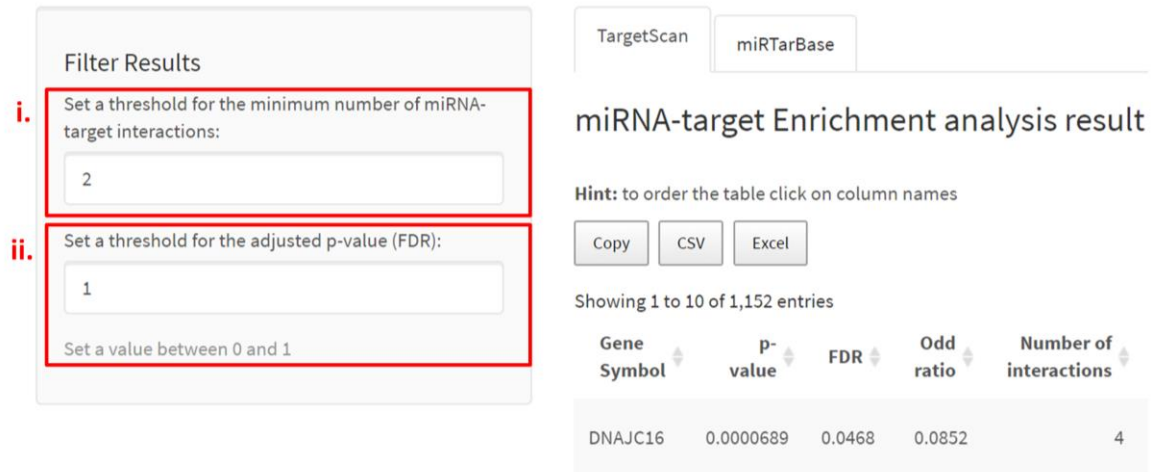
## miRNA-target Enrichment link

By clicking on **miRNA-target Enrichment** link, MIENTURNET will perform a statistical analysis for over-representation of miRNA-target interactions in the input miRNAs list.



The user has the possibility to perform the enrichment analysis by using a threshold on:


- i. the minimum number of miRNA-target interactions (default is 2)
- ii. the adjusted p-values (default is 1)



Showing 1 to 10 of 1,152 entries

| Gene Symbol | P-value   | FDR    | Odd ratio | Number of interactions |
|-------------|-----------|--------|-----------|------------------------|
| DNAJC16     | 0.0000689 | 0.0468 | 0.0852    | 4                      |

From **miRNA-target Enrichment** page, the user can choose the reference database for the miRNA-target interactions (i.e., TargetScan and/or miRTarBase) to be used to perform the enrichment analysis.

 **Caveat:** regardless of the database choice, MIENTURNET performs the miRNA-target enrichment analysis searching for both predicted and validated interactions. A progress bar in action will appear at the bottom-right of the page as long as both analyses are not completed.





By clicking on **TargetScan** tab, the computationally predicted miRNA-target interactions will be considered.

**Filter Results**  
Set a threshold for the minimum number of miRNA-target interactions:  
  
Set a threshold for the adjusted p-value (FDR):  
  
Set a value between 0 and 1


miRNA-target Enrichment analysis result

Hint: to order the table click on column names

Copy CSV Excel

Showing 1 to 10 of 1,152 entries

| Gene Symbol | p-value   | FDR    | Odd ratio | Number of interactions | microRNA 1      | microRNA 2    | microRNA 3     | microRNA 4     |
|-------------|-----------|--------|-----------|------------------------|-----------------|---------------|----------------|----------------|
| DNAJC16     | 0.0000689 | 0.0468 | 0.0852    | 4                      | hsa-miR-16-5p   | hsa-miR-17-5p | hsa-miR-19b-3p | hsa-miR-21-5p  |
| FBXO10      | 0.0000253 | 0.0468 | 0.0682    | 4                      | hsa-miR-125b-5p | hsa-miR-16-5p | hsa-miR-17-5p  | hsa-miR-19b-3p |
| NEK10       | 0.0000169 | 0.0468 | 0.0625    | 4                      | hsa-miR-125b-5p | hsa-miR-16-5p | hsa-miR-17-5p  | hsa-miR-19b-3p |

 **Caveat:** TargetScan predicts biological targets of miRNAs by searching for the presence of conserved sites that match the seed region (i.e., the region comprising nucleotides 2-7 at the 5'-end of the mature miRNA sequence) of each miRNA [3]. Note that, a miRNA family is comprised of miRNAs with the same seed sequence. This implies that members of the same miRNA family all share the same predicted targets. As a consequence, in this case, the enrichment analysis was performed considering miRNA families instead of single miRNAs.

By clicking on **miRTarBase** tab, the experimentally validated miRNA-target interactions will be considered.

**Filter Results**  
Set a threshold for the minimum number of miRNA-target interactions:  
  
Set a threshold for the adjusted p-value (FDR):  
  
Set a value between 0 and 1


miRNA-target Enrichment analysis result

Hint: to order the table click on column names

Copy CSV Excel

Showing 1 to 10 of 739 entries

| Gene Symbol | p-value | FDR     | Odd ratio | Number of interactions | microRNA 1    | microRNA 2     | microRNA 3     | microRNA 4    |
|-------------|---------|---------|-----------|------------------------|---------------|----------------|----------------|---------------|
| CNOT7       | 9.76e-7 | 0.00118 | 0.00802   | 3                      | hsa-miR-16-5p | hsa-miR-19b-3p | hsa-miR-17-5p  |               |
| GPAM        | 6.27e-7 | 0.00118 | 0.0214    | 4                      | hsa-miR-16-5p | hsa-miR-21-5p  | hsa-miR-19b-3p | hsa-miR-17-5p |

 **Caveat:** miRTarBase collects miRNA-target interactions experimentally validated by reporter assay, western blot, microarrays, and next-generation sequencing experiments. As a consequence, in this case, the enrichment analysis was performed considering miRNAs instead of miRNA families.

For each gene in the chosen database (TargetScan or miRTarBase), the hypergeometric test was used to calculate the significance (p-value <0.05) of the enrichment of the input list in miRNAs targeting that gene.

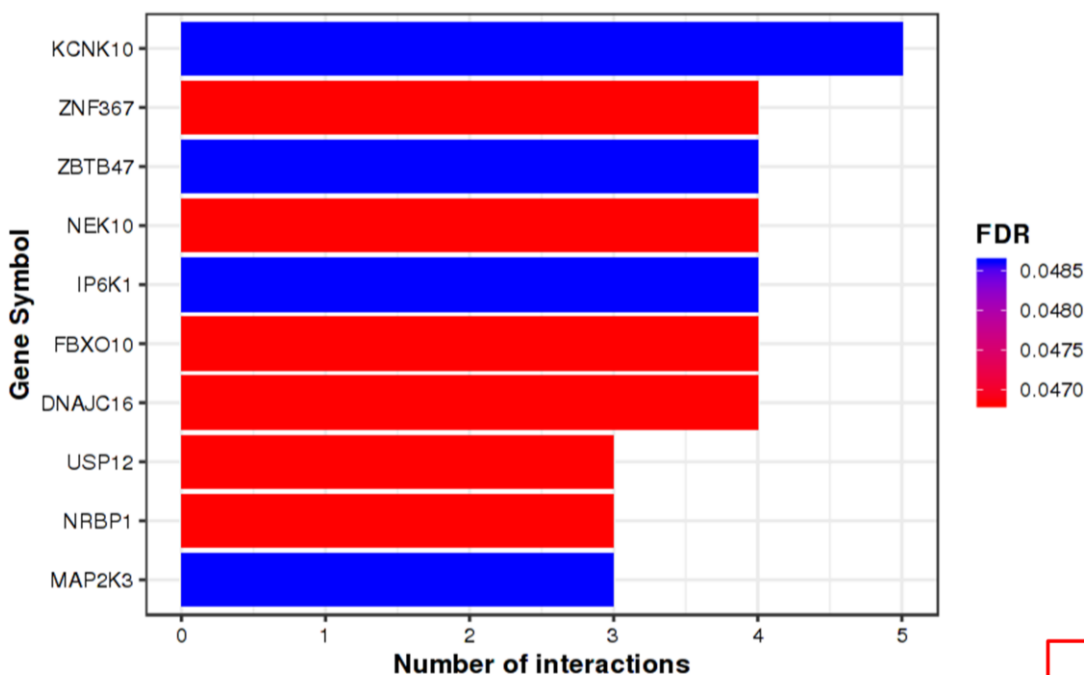
The p-value is computed as

$$p = 1 - \sum_{i=0}^{X-1} \frac{\binom{K}{i} \binom{M-K}{N-i}}{\binom{M}{N}}$$

where M is the dimension of the universe, that is the number of all predicted (validated) miRNA-target interactions encompassed in TargetScan (miRTarBase); K is the number of predicted (validated) miRNA-target interactions encompassed in TargetScan (miRTarBase) for the selected target gene; N is the number of miRNA families (number of miRNAs) of the input list recognized by TargetScan (miRTarBase); X is the number of miRNA families (number of miRNAs) for which predicted (validated) miRNA-target interactions, for the selected gene, exist.

The results of the enrichment analysis are presented as<sup>1</sup>:

- i. a bar plot where the Y-axis refers to the top ten target genes resulted from the enrichment analysis, while the X-axis represents the number of miRNAs targeting them. The color code reflects the FDR value increasing from red to blue. This plot is resizable by grabbing and dragging an active corner at the bottom right of the page.



<sup>1</sup> Hereafter, all snapshots refer to TargetScan visualization.

- ii. an interactive table where the user has also always the possibility to:
1. visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the bottom-left
  2. navigate the table through the navigation controller buttons at the bottom-right
  3. search for a miRNA or a Target Gene of interest by typing the name in the search bar at the top-right
  4. copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left

TargetScan
miRTarBase

### miRNA-target Enrichment analysis result

Hint: to order the table click on column names

4.
Copy
CSV
Excel

3.
Search:

Showing 1 to 10 of 1,152 entries

| Gene Symbol | p-value   | FDR    | Odd ratio | Number of interactions | microRNA 1      | microRNA 2     | microRNA 3     | microRNA 4     | microRNA 5    |
|-------------|-----------|--------|-----------|------------------------|-----------------|----------------|----------------|----------------|---------------|
| DNAJC16     | 0.0000689 | 0.0468 | 0.0852    | 4                      | hsa-miR-16-5p   | hsa-miR-17-5p  | hsa-miR-19b-3p | hsa-miR-21-5p  |               |
| FBXO10      | 0.0000253 | 0.0468 | 0.0682    | 4                      | hsa-miR-125b-5p | hsa-miR-16-5p  | hsa-miR-17-5p  | hsa-miR-19b-3p |               |
| NEK10       | 0.0000169 | 0.0468 | 0.0625    | 4                      | hsa-miR-125b-5p | hsa-miR-16-5p  | hsa-miR-17-5p  | hsa-miR-19b-3p |               |
| NRBP1       | 0.0000563 | 0.0468 | 0.0379    | 3                      | hsa-miR-16-5p   | hsa-miR-17-5p  | hsa-miR-19b-3p |                |               |
| USP12       | 0.0000563 | 0.0468 | 0.0379    | 3                      | hsa-miR-125b-5p | hsa-miR-16-5p  | hsa-miR-19b-3p |                |               |
| ZNF367      | 0.0000689 | 0.0468 | 0.0852    | 4                      | hsa-miR-16-5p   | hsa-miR-17-5p  | hsa-miR-19b-3p | hsa-miR-21-5p  |               |
| IP6K1       | 0.000119  | 0.0486 | 0.0966    | 4                      | hsa-miR-125b-5p | hsa-miR-16-5p  | hsa-miR-17-5p  | hsa-miR-19b-3p |               |
| KCNK10      | 0.000106  | 0.0486 | 0.168     | 5                      | hsa-miR-125b-5p | hsa-miR-16-5p  | hsa-miR-17-5p  | hsa-miR-19b-3p | hsa-miR-21-5p |
| MAP2K3      | 0.000112  | 0.0486 | 0.0455    | 3                      | hsa-miR-16-5p   | hsa-miR-19b-3p | hsa-miR-21-5p  |                |               |
| ZBTB47      | 0.0000916 | 0.0486 | 0.0909    | 4                      | hsa-miR-125b-5p | hsa-miR-17-5p  | hsa-miR-19b-3p | hsa-miR-21-5p  |               |

1.
Show 10 entries

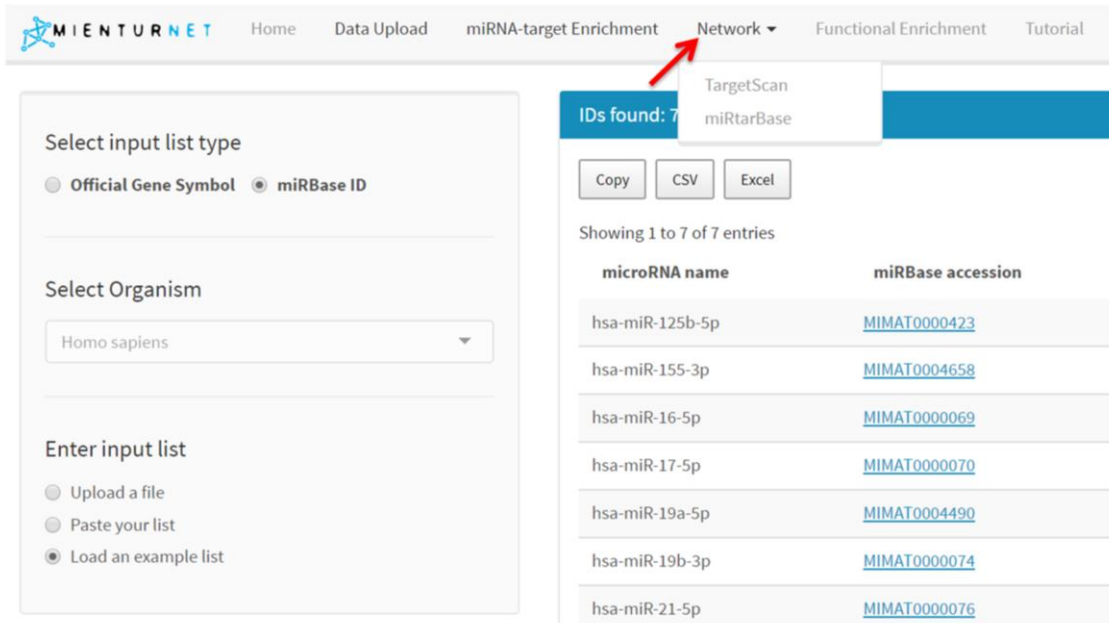
2.
Previous
1
2
3
4
5
...
116
Next

The columns of the table report:

- *Gene Symbol*: names of target genes
- *p-value*: p-values of the enrichment analysis
- *FDR*: the adjusted p-values obtained by using the Benjamini-Hochberg (False Discovery Rate, FDR) procedure for multiple testing
- *Odd ratio*: ratios calculated as:  $\frac{K/M}{X/N}$
- *Number of interactions*: total numbers of interactions for each gene
- Additional extra columns reporting the names of miRNA of the input list for each gene, named with increasing numerical values

## Network menu

In order to build the network of miRNA-target interactions identified by the enrichment analysis, click on **Network** menu and choose the reference database (i.e., TargetScan and/or miRTarBase) from the drop-down menu.




The screenshot shows the MENTURNET web interface. The top navigation bar includes 'Home', 'Data Upload', 'miRNA-target Enrichment', 'Network', 'Functional Enrichment', and 'Tutorial'. The 'Network' menu is open, showing options for 'TargetScan' and 'miRtarBase'. Below the menu, there are buttons for 'Copy', 'CSV', and 'Excel'. A table displays 7 entries of miRNA accessions:

| microRNA name   | miRBase accession            |
|-----------------|------------------------------|
| hsa-miR-125b-5p | <a href="#">MIMAT0000423</a> |
| hsa-miR-155-3p  | <a href="#">MIMAT0004658</a> |
| hsa-miR-16-5p   | <a href="#">MIMAT0000069</a> |
| hsa-miR-17-5p   | <a href="#">MIMAT0000070</a> |
| hsa-miR-19a-5p  | <a href="#">MIMAT0004490</a> |
| hsa-miR-19b-3p  | <a href="#">MIMAT0000074</a> |
| hsa-miR-21-5p   | <a href="#">MIMAT0000076</a> |

## Selecting miRNA-target interactions database and filtering

By choosing **TargetScan**, the results can be filtered by using a threshold on:

- the minimum number of miRNA-target interactions
- the adjusted p-values obtained from the miRNA-target enrichment analysis
- the *cumulative weighted context++ score* (CWCS) parameter [4] provided by TargetScan<sup>2</sup>
- the *probability of conserved targeting* ( $P_{CT}$ ) parameter [5] provided by TargetScan



The screenshot shows the 'Network microRNA/Genes - TargetScan' interface. The 'Filter Network' section is highlighted with a red box and contains four filter options:

- Set a threshold for the minimum number of miRNA-target interactions:
- Set a threshold for the adjusted p-value (FDR):
- Set a threshold for the cumulative weighted context++ score (CWCS) [Click here for CWCS help page](#)  
Range: 0 to 1
- Set a threshold for the probability of conserved targeting (PCT) [Click here for PCT help page](#)  
Range: 0 to 1

Other interface elements include 'Network', 'Network Properties', 'miRNA-target Table', 'SAVE NETWORK', 'DOWNLOAD NETWORK', and a 'Select by id' dropdown menu. A network graph visualization is shown on the right side of the interface.

<sup>2</sup> This threshold refers to a percentile of the CWCS distribution.

The user can choose to apply one of these filters, more than one or none. By default, the first two thresholds are set to the value chosen in the **miRNA-target Enrichment** page. The other two parameters, CWCS and  $P_{CT}$ , are set by default to 0.9 and 0.5, respectively.

By choosing **miRTarBase**, the results can be filtered according to

- i. the minimum number of miRNA-target interactions
- ii. the adjusted p-values obtained from the miRNA-target enrichment analysis
- iii. the type of *Evidence categories* used by miRTarBase to validate the miRNA-target interactions:
  - a. ‘Strong’ for considering strong experimental methods (e.g., Luciferase assay, Western)
  - b. ‘Weak’ for considering weaker experimental evidence (e.g., CLIP)
  - c. ‘Strong and Weak’ for considering both strong and weak experimental methods

### Network microRNA/Genes - miRTarBase

The screenshot shows the 'miRTarBase' network filtering interface. The 'Filter Network' panel on the left is highlighted with a red box. It contains three sections: i. 'Set a threshold for the minimum number of miRNA-target interactions:' with a text input field containing '2'; ii. 'Set a threshold for the adjusted p-value (FDR):' with a text input field containing '1'; iii. 'Filter by Evidence categories:' with three radio buttons: 'Strong' (selected), 'Weak', and 'Strong and Weak'. Below this is a 'Customize Network Image' section with a 'Choice network layout' dropdown set to 'Auto' and a 'Choice source (microRNA) node size' label. On the right, there are tabs for 'Network', 'Network Properties', and 'miRNA-target Table'. Below the tabs are buttons for 'SAVE NETWORK' and 'DOWNLOAD NETWORK', and a dropdown menu labeled 'Select by id'. The main area displays a network graph with blue nodes and edges, and several clusters of yellow nodes.

As before, by default the first two thresholds are set to the value chosen in the **miRNA-target Enrichment** page. Instead, the *Evidence categories* filter is configured as ‘Strong’.



**Caveat:** regardless of the database choice, the user that is not interested in the enrichment analysis can skip the previous step (i.e., the user can avoid to click on **miRNA-target Enrichment** link) and jump to the next step of **Network** menu. In this case, MIENTURNET will still perform the enrichment analysis, but will set the default value for the adjusted p-values to 1.

## Network visualization and analysis

Both for **TargetScan** and **miRTarBase**, the user can interactively play with the miRNA-interaction network by pressing the following tabs:

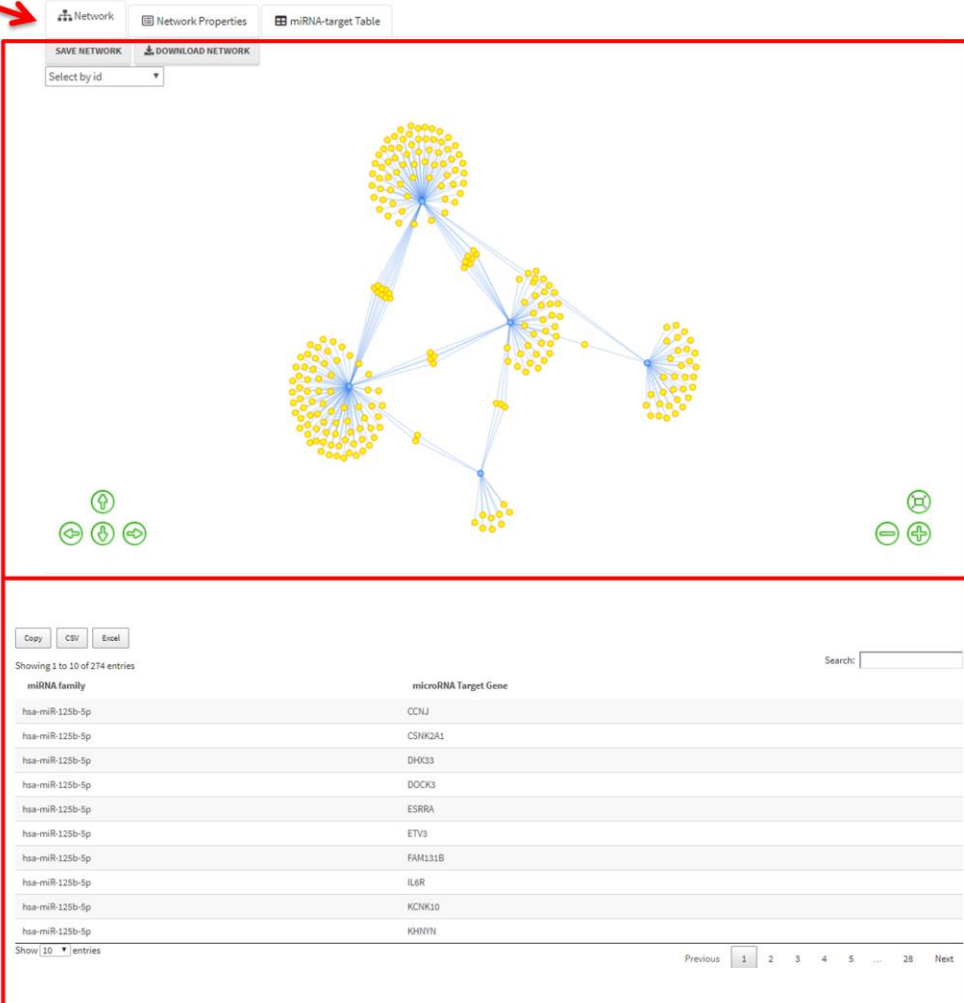
1. **Network** tab: it allows to visualize, customize, and download the network in the form of an edgelist (i.e., a 2-column table where each row represents a single edge)
2. **Network properties** tab: it allows to perform a network analysis
3. **miRNA-target Table** tab: it allows to view and download a table where the columns are the miRNAs with the corresponding own targets

In the following, there is a detailed description for each of these tabs<sup>3</sup>.

### 1. Network tab

From **Network** tab, the user can visualize the miRNA-target interaction network:

- i. as an image that can be interactively explored and customized
- ii. as a 2-column table in the form of an edgelist (i.e., each row represents a miRNA-target interaction).



The screenshot shows the 'Network' tab interface. At the top, there are three tabs: 'Network', 'Network Properties', and 'miRNA-target Table'. Below the tabs are buttons for 'SAVE NETWORK' and 'DOWNLOAD NETWORK', and a 'Select by id' dropdown menu. The main area is divided into two sections:

**i.** A network visualization showing a complex graph of nodes (yellow circles) and edges (blue lines). The nodes are clustered into several groups, with some larger clusters and some smaller ones. The edges connect these clusters, forming a network structure.

**ii.** A table view showing the first 10 entries of the network. The table has two columns: 'miRNA family' and 'microRNA Target Gene'. The table is paginated, showing 'Showing 1 to 10 of 274 entries' and a search bar. The table content is as follows:

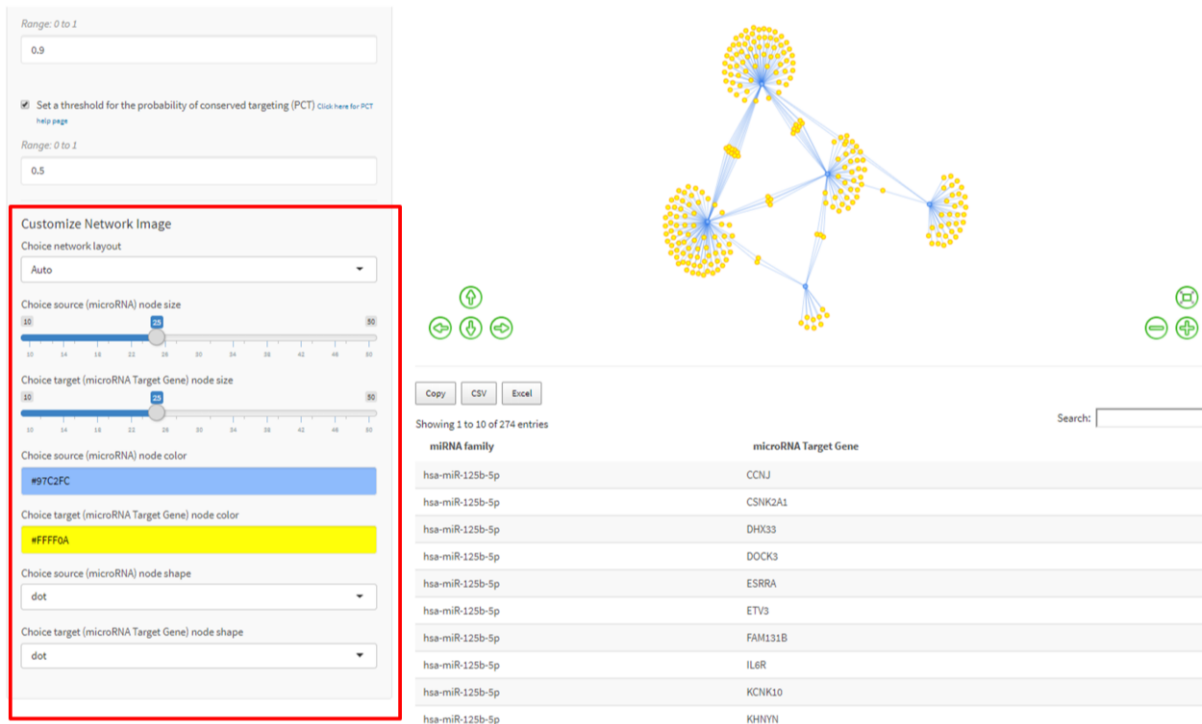
| miRNA family    | microRNA Target Gene |
|-----------------|----------------------|
| hsa-miR-125b-5p | CCLJ                 |
| hsa-miR-125b-5p | CSNK2A1              |
| hsa-miR-125b-5p | DHK33                |
| hsa-miR-125b-5p | DOCK3                |
| hsa-miR-125b-5p | ESRRA                |
| hsa-miR-125b-5p | ETV3                 |
| hsa-miR-125b-5p | FAM131B              |
| hsa-miR-125b-5p | IL6R                 |
| hsa-miR-125b-5p | KCNK10               |
| hsa-miR-125b-5p | KHNYN                |

At the bottom of the table view, there are buttons for 'Copy', 'CSV', and 'Excel', and a 'Show | 10 | entries' dropdown menu. The pagination shows 'Previous 1 2 3 4 5 ... 28 Next'.

<sup>3</sup> All snapshots refer to TargetScan visualization, where source nodes are the miRNA families to which each miRNA of the input list belongs. Otherwise, in the miRTarBase visualization, source nodes are the miRNAs of the input list.

By using the *Customize network image* menu on the left, the user may customize:

- the network layout (details about the layout algorithms are available at <http://igraph.org>)
- the size of source/target nodes
- the shape of source/target nodes
- the color of source/target nodes. By default, source nodes (miRNA families or single miRNAs) are blue circles, while target nodes (mRNAs) are yellow circles.



The screenshot shows a network visualization interface. On the left, there is a 'Customize Network Image' panel with various settings:

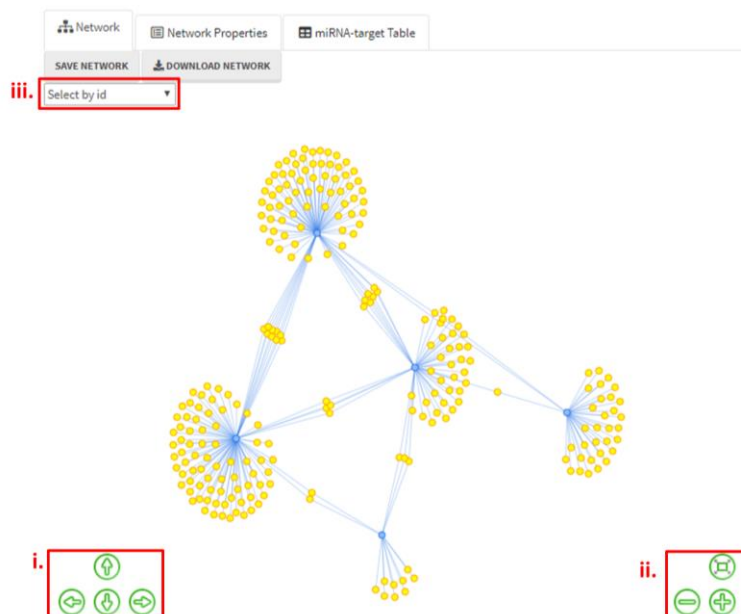
- Range: 0 to 1, value: 0.9
- Set a threshold for the probability of conserved targeting (PCT) [Click here for PCT help page](#)
- Range: 0 to 1, value: 0.5
- Choice network layout: Auto
- Choice source (microRNA) node size: slider from 10 to 30, value at 25
- Choice target (microRNA Target Gene) node size: slider from 10 to 30, value at 25
- Choice source (microRNA) node color: #97C2FC
- Choice target (microRNA Target Gene) node color: #FFFF0A
- Choice source (microRNA) node shape: dot
- Choice target (microRNA Target Gene) node shape: dot

The network graph shows source nodes (blue circles) connected to target nodes (yellow circles). Below the graph are navigation controls (up, down, left, right arrows) and zoom controls (minus, plus buttons). A table below the graph shows the first 10 entries of 274 entries:

| miRNA family    | miRNA Target Gene |
|-----------------|-------------------|
| hsa-miR-125b-5p | CCNJ              |
| hsa-miR-125b-5p | CSNK2A1           |
| hsa-miR-125b-5p | DHX33             |
| hsa-miR-125b-5p | DOCK3             |
| hsa-miR-125b-5p | ESRRA             |
| hsa-miR-125b-5p | ETV3              |
| hsa-miR-125b-5p | FAM131B           |
| hsa-miR-125b-5p | IL6R              |
| hsa-miR-125b-5p | KCNK10            |
| hsa-miR-125b-5p | KHNYN             |

On the network image, the user has also the possibility to:

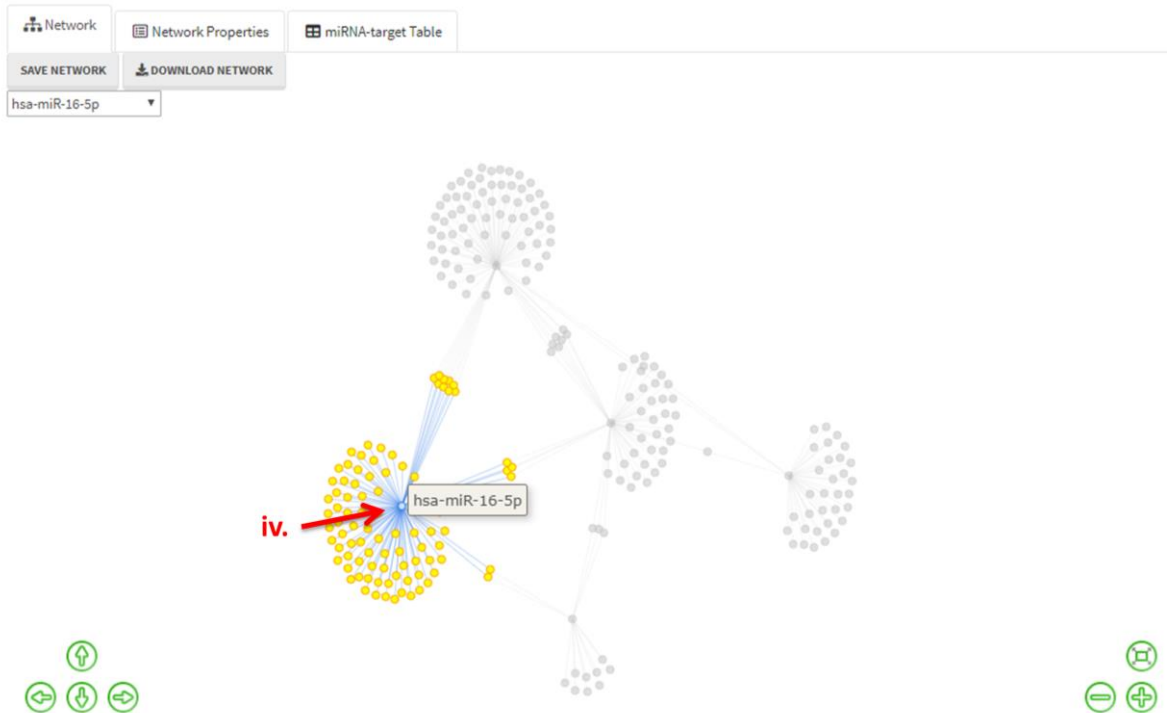
- move the network around by using the up-down left-right arrows
- zoom in/out the image by using the plus/minus buttons
- select a specific node by scrolling down the drop-down menu “Select by id” at the top-left



The screenshot highlights the navigation and selection controls:

- iii. Select by id (dropdown menu)
- i. Navigation arrows (up, down, left, right)
- ii. Zoom controls (minus, plus buttons)

- iv. select a specific node by placing the pointer over the node to be selected. Note, the selection will interest the node and its first neighbors. For example, if a miRNA is selected both miRNA and its targets will be highlighted.
- v. move one or more node by selecting and dragging them with mouse



The user can export the network picture as:

- i. a dynamic image in HTML format, by clicking first SAVE NETWORK button and then DOWNLOAD NETWORK button at the top-left
- ii. a static image in PNG format, by pressing down on the right mouse button (i.e., right-clicking)

#### Network microRNA/Genes - TargetScan





On the network table below the network image, the user has always the possibility to:

- i. visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the bottom-left
- ii. navigate the table through the navigation controller buttons at the bottom-right
- iii. search for a miRNA or a target gene of interest by typing the name in the search bar at the top-right
- iv. copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left.

iv. Copy CSV Excel

Showing 1 to 10 of 274 entries

iii. Search:

| miRNA family    | microRNA Target Gene |
|-----------------|----------------------|
| hsa-miR-125b-5p | CCNJ                 |
| hsa-miR-125b-5p | CSNK2A1              |
| hsa-miR-125b-5p | DHX33                |
| hsa-miR-125b-5p | DOCK3                |
| hsa-miR-125b-5p | ESRRA                |
| hsa-miR-125b-5p | ETV3                 |
| hsa-miR-125b-5p | FAM131B              |
| hsa-miR-125b-5p | IL6R                 |
| hsa-miR-125b-5p | KCNK10               |
| hsa-miR-125b-5p | KHNYN                |

i. Show 10 entries

ii. Previous 1 2 3 4 5 ... 28 Next

One or more miRNAs in the input list, although recognized in miRBase, could not be in the chosen database (TargetScan or miRTarBase). In this case, the miRNAs not found are listed in the **IDs not found** collapsible panel.

→ IDs not found in TargetScan

There are 5 microRNAs not present in TargetScan database:

Show 10 entries

Search:

Excluded microRNAs

- hsa-miR-15-3p
- hsa-miR-155-3p
- hsa-miR-19a-5p
- hsa-miR-20-3p
- hsa-miR-92-3p

Showing 1 to 5 of 5 entries

Previous 1 Next

## 2. Network Properties tab

From **Network Properties** tab, the user can:

- i. visualize a table with the topological properties for each node<sup>4</sup>, where it is possible to:
  1. sort each column in decreasing or increasing order by clicking on the up-down arrows close to the column names
  2. visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the bottom-left
  3. navigate the table through the navigation controller buttons at the bottom-right
  4. search for a node of interest by typing the name in the search bar at the top-right
  5. copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left

5. Copy CSV Excel

4. Search:

Showing 1 to 10 of 251 entries

| Node            | Degree | Closeness | Betweenness | Eccentricity | ClusteringCoefficient | AverageShortestPathLength |
|-----------------|--------|-----------|-------------|--------------|-----------------------|---------------------------|
| hsa-miR-16-5p   | 90     | 0.396     | 0.545       | 5            | 0                     | 2.52                      |
| hsa-miR-19b-3p  | 86     | 0.421     | 0.584       | 5            | 0                     | 2.37                      |
| hsa-miR-17-5p   | 49     | 0.386     | 0.335       | 3            | 0                     | 2.58                      |
| hsa-miR-125b-5p | 35     | 0.293     | 0.241       | 5            | 0                     | 3.39                      |
| hsa-miR-21-5p   | 14     | 0.270     | 0.0750      | 5            | 0                     | 3.69                      |
| ENPP5           | 2      | 0.339     | 0.0159      | 4            | 0                     | 2.94                      |
| BMP2            | 2      | 0.339     | 0.0159      | 4            | 0                     | 2.94                      |
| F3              | 2      | 0.339     | 0.0159      | 4            | 0                     | 2.94                      |
| CDC37L1         | 2      | 0.346     | 0.0296      | 4            | 0                     | 2.88                      |
| KCNK10          | 2      | 0.302     | 0.0732      | 4            | 0                     | 3.29                      |

2. Show 10 entries

3. Previous 1 2 3 4 5 ... 26 Next

In particular, the columns of the table report:

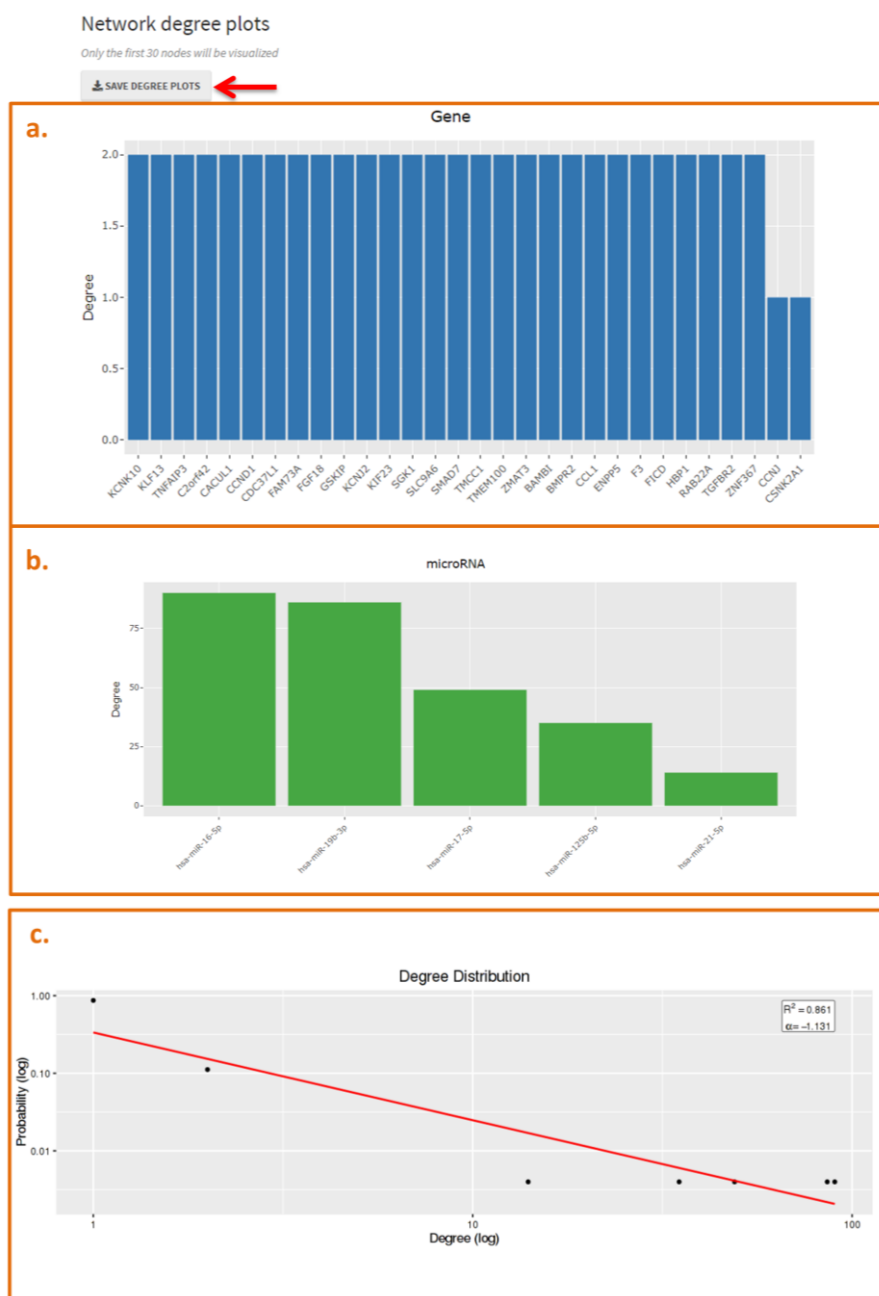
- *degree* - the number of incoming and outgoing edges of each node
  - *betweenness* – the number of shortest paths<sup>5</sup> going through the node
  - *average shortest path length* – the average length of the shortest paths between a node and all other nodes in the network
  - *eccentricity* – the shortest path distance of a node from the farthest other node in the network
  - *clustering coefficient* – measurement the probability that the adjacent nodes of a node are connected. The clustering coefficient is set to 0 for nodes with degree zero and one.
  - *closeness* – the inverse of the average length of the shortest paths between a node and all other nodes in the network. Note that the closeness centrality measure is not well-defined for disconnected graphs. If there is no (directed) path between node  $i$  and  $j$ , then the total number of nodes is used in the formula instead of the path length.
- ii. visualize the following network plots:

<sup>4</sup> Details about the network topological properties are available at <http://igraph.org>

<sup>5</sup> the shortest path between two nodes is the minimum number of consecutive edges connecting them

- a bar plot of the mRNA degree, where X-axis refers to the first 30 target genes (sorted in a decreasing order according to the degree) and Y-axis refers to their degree (i.e., number of miRNA targeting them)
- a bar plot of the miRNA degree, where X-axis refers to the first 30 miRNAs (sorted in a decreasing order according to the degree) and Y-axis refers to their degree (i.e., number of their targets)
- the nodes degree distribution  $P(k)$ , with  $k$  being the degree, by plotting  $\log k$  on the X-axis and  $\log P(k)$  on the Y-axis. Then, the method of least squares is used to find the best-fitting curve  $\log P \sim \alpha \log k$ , where  $\alpha$  is the constant to estimate. The sum of the squared residuals  $R^2$  (i.e., the sum of the vertical deviations from the best-fitting line) is reported together with the estimation of the coefficient  $\alpha$ .

All plots can be exported as a compressed .zip file by clicking on SAVE DEGREE PLOTS button.



### 3. miRNA-target Table tab

From **miRNA-target Table** tab, the user can visualize an interactive table where the columns are the miRNAs of the input list found in the selected database (TargetScan or miRTarBase) with the corresponding own targets. The columns of the table are sorted in a decreasing order according to the number of target genes of each mRNA. The user has always the possibility to:

- i. visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the top-left
- ii. navigate the table through the navigation controller buttons at bottom-right
- iii. search for a miRNA or a Target Gene of interest by typing the name in the search bar at the top-right
- iv. copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left.

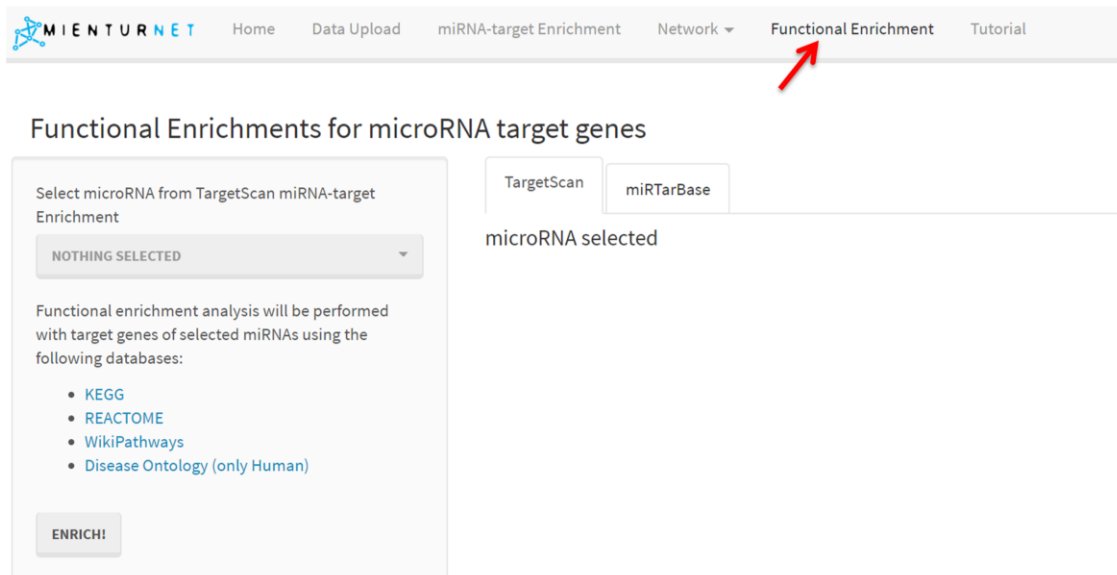
The screenshot shows the 'miRNA-target Table' interface. At the top, there are three tabs: 'Network', 'Network Properties', and 'miRNA-target Table'. Below the tabs are three buttons: 'Copy', 'CSV', and 'Excel', which are highlighted with a red box and labeled 'iv.'. To the right of these buttons is a search bar labeled 'Search:' with a text input field, highlighted with a red box and labeled 'iii.'. Below the search bar, it says 'Showing 1 to 10 of 90 entries'. The main part of the interface is a table with five columns representing different miRNAs: hsa-miR-16-5p, hsa-miR-19b-3p, hsa-miR-17-5p, hsa-miR-125b-5p, and hsa-miR-21-5p. The table contains 10 rows of target genes. At the bottom left, there is a dropdown menu labeled 'Show 10 entries', highlighted with a red box and labeled 'i.'. At the bottom right, there is a navigation controller with buttons for 'Previous', '1', '2', '3', '4', '5', '...', '9', and 'Next', highlighted with a red box and labeled 'ii.'.

| hsa-miR-16-5p | hsa-miR-19b-3p | hsa-miR-17-5p | hsa-miR-125b-5p | hsa-miR-21-5p |
|---------------|----------------|---------------|-----------------|---------------|
| PLAG1         | ATL2           | MAP3K2        | LIN28B          | RAB22A        |
| CCNT2         | PTEN           | MASTL         | SBNO1           | CCL1          |
| TMEM100       | NFIA           | SLC17A7       | ST8SIA4         | YOD1          |
| IPPK          | RAP2C          | ZNF800        | TRIM71          | SMAD7         |
| ZMAT3         | QKI            | PFN2          | LIN28A          | PDCD4         |
| RNF24         | CHIC1          | C11orf30      | SSTR3           | PLEKHA1       |
| LUZP1         | ATXN1          | FCHO2         | FAM131B         | ZNF367        |
| FGF7          | EFNB2          | ENPP5         | TET2            | PPP1R3B       |
| SCN8A         | RORA           | ARID4B        | SLC39A9         | SOX5          |
| USP3          | CLOCK          | BMP2R2        | PODXL           | SATB1         |

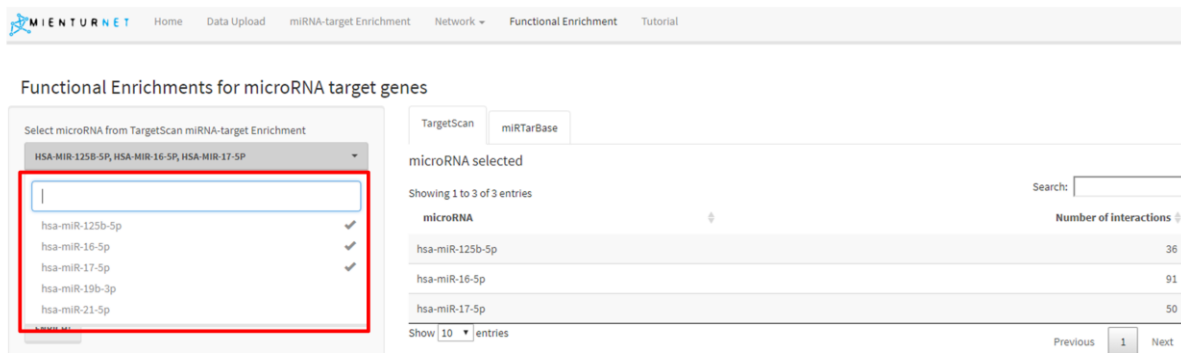
## Functional Enrichment link

In order to perform the functional enrichment analysis of target genes of selected miRNAs, click on **Functional Enrichment** link and choose TargetScan or miRTarBase <sup>6</sup>.

Currently, MIENTURNET allows to query the following annotation databases: KEGG, REACTOME, WikiPathways, Disease Ontology. Note that the last one will be available only when *Homo sapiens* is the selected organism.



The user can select one or more miRNA families (or single miRNA in the case of miRTarBase) by clicking on each item or by typing the name in the search box. In this example, the first three miRNA families have been selected.



<sup>6</sup> All snapshots refer to TargetScan visualization, where for each selected miRNA the family to which it belongs will be reported. Otherwise, in the miRTarBase visualization, the selected miRNAs will be considered.

The selected miRNA families will appear in a table on the right along with the number of their interactions.

Functional Enrichments for microRNA target genes

Select microRNA from TargetScan miRNA-target Enrichment

HSA-MIR-125B-5P, HSA-MIR-16-5P, HSA-MIR-17-5P

TargetScan miRTarBase

microRNA selected

Showing 1 to 3 of 3 entries

| microRNA        | Number of interactions |
|-----------------|------------------------|
| hsa-miR-125b-5p | 36                     |
| hsa-miR-16-5p   | 91                     |
| hsa-miR-17-5p   | 50                     |

Show 10 entries

Previous 1 Next

To start the analysis, click the ENRICH button.

Functional Enrichments for microRNA target genes

Select microRNA from TargetScan miRNA-target Enrichment

HSA-MIR-125B-5P, HSA-MIR-16-5P, HSA-...

Functional enrichment analysis will be performed with target genes of selected miRNAs using the following databases:

- KEGG
- REACTOME
- WikiPathways
- Disease Ontology (only Human)

ENRICH!

TargetScan miRTarBase


microRNA selected

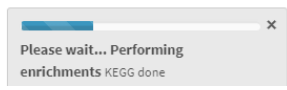
Showing 1 to 3 of 3 entries

| microRNA        | Number of interactions |
|-----------------|------------------------|
| hsa-miR-125b-5p | 36                     |
| hsa-miR-16-5p   | 91                     |
| hsa-miR-17-5p   | 50                     |

Show 10 entries

Previous 1 Next

 **Caveat:** The function enrichment analysis will regard the targets of each selected miRNA, separately. The user could see a progress bar in action at the bottom-right of the page.



For each annotation database, the results of the functional enrichment analysis are presented as<sup>7</sup>:

1. an interactive table reporting: the miRNA name, the category name of the annotation database (e.g., KEGG ID), the category description, the p-value and the adjusted p-value (FDR), the list of target genes, the number of targets genes.
2. a dot plot, where the Y-axis reports the annotation categories (e.g., KEGG pathways) and the X-axis reports the miRNA family with the number of recognized targets in round brackets. The colors of the dots represent the adjusted p-values (FDR), whereas the size of the dots represents gene ratio (i.e., the number of miRNA targets found annotated in each category over the number of total genes annotated in that category). All the plots are resizable by grabbing and dragging an active corner at the bottom right of the page.

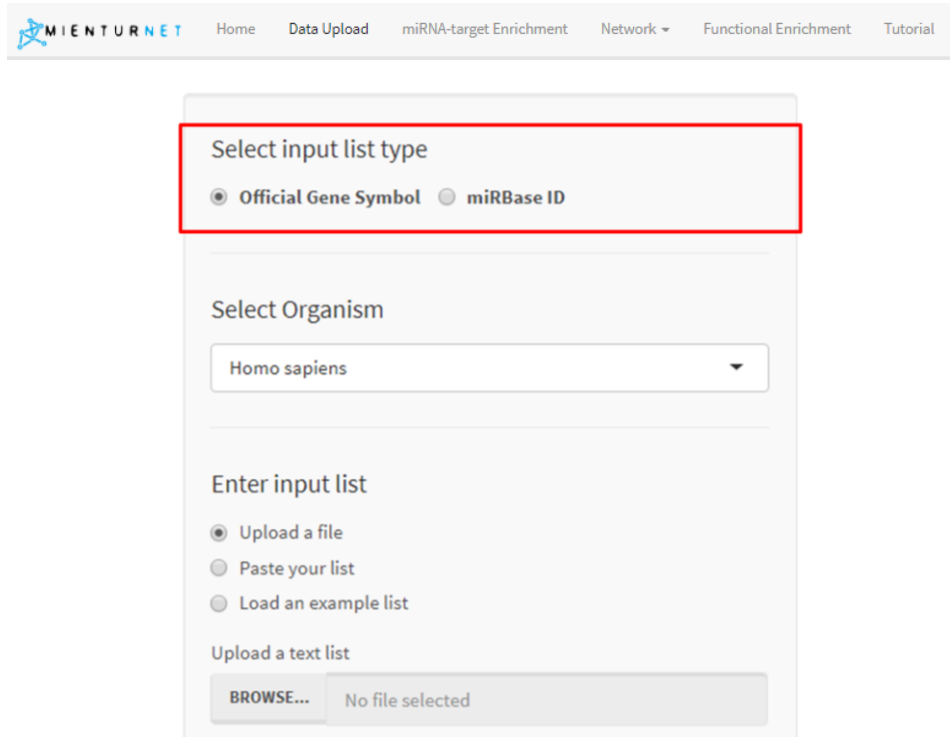


<sup>7</sup> The results presented here refer to the KEGG enrichment analysis.

## Entering a list of mRNAs

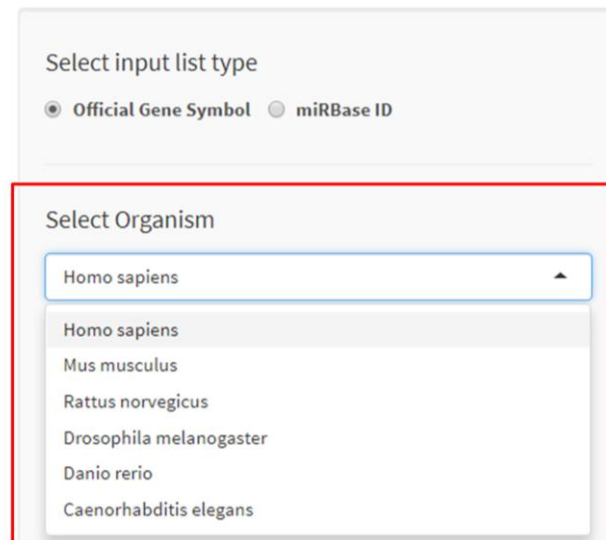
### Data Upload link

If the user is interested to find miRNAs targeting a given list of mRNAs, from the **Data Upload** page, select **Official Gene Symbol** as type of the input list.



The screenshot shows the MENTURNET Data Upload page. The navigation bar includes the MENTURNET logo and links for Home, Data Upload, miRNA-target Enrichment, Network, Functional Enrichment, and Tutorial. The main content area is titled 'Select input list type' and is highlighted with a red box. It contains two radio buttons: 'Official Gene Symbol' (selected) and 'miRBase ID'. Below this is a 'Select Organism' section with a dropdown menu currently showing 'Homo sapiens'. Further down is an 'Enter input list' section with three radio buttons: 'Upload a file' (selected), 'Paste your list', and 'Load an example list'. At the bottom is an 'Upload a text list' section with a 'BROWSE...' button and the text 'No file selected'.

Select the organism from the drop-down menu.

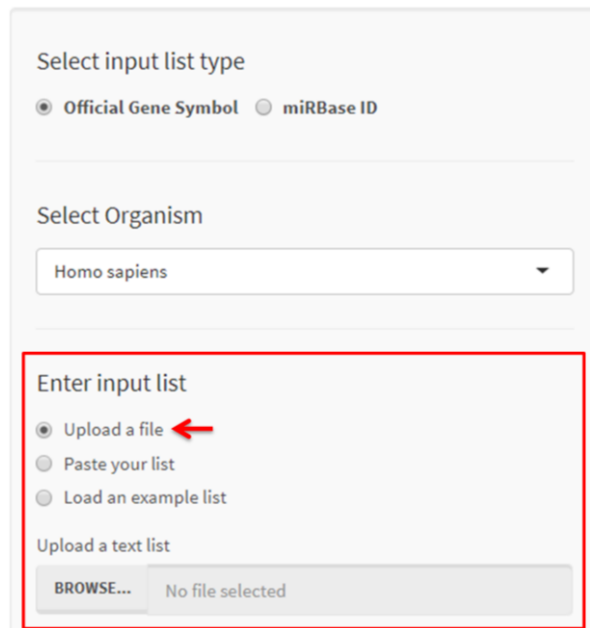


This screenshot shows the 'Select Organism' dropdown menu open, highlighted with a red box. The dropdown menu lists several organisms: 'Homo sapiens', 'Mus musculus', 'Rattus norvegicus', 'Drosophila melanogaster', 'Danio rerio', and 'Caenorhabditis elegans'. The 'Official Gene Symbol' radio button is still selected in the section above.



Enter the input list by using one of the following options:

- 1) **Upload a file** if the list is saved in a text file on the own computer and then click BROWSE to quickly access to the file




Select input list type

Official Gene Symbol  miRBase ID

Select Organism

Homo sapiens

Enter input list

Upload a file 

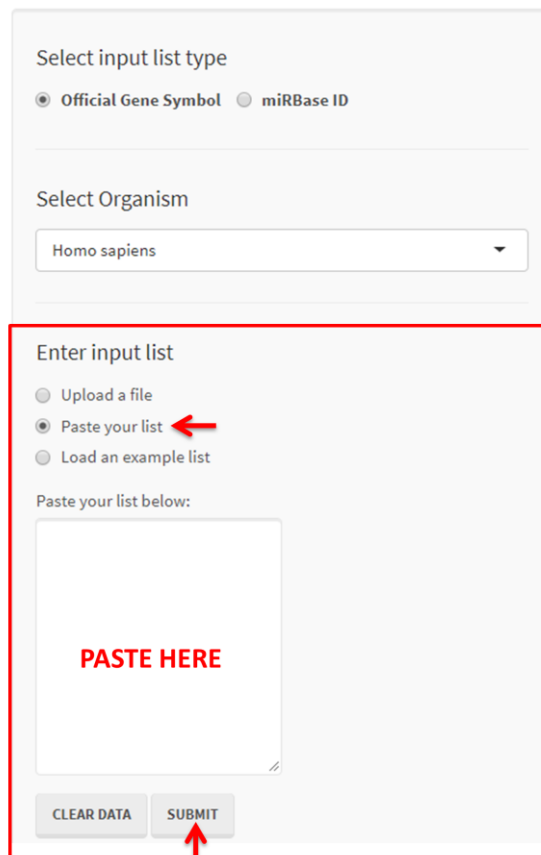
Paste your list

Load an example list

Upload a text list

BROWSE... No file selected

- 2) **Paste your list** in the specific text area and then click SUBMIT



Select input list type


Official Gene Symbol  miRBase ID

Select Organism

Homo sapiens

Enter input list


Upload a file

Paste your list 

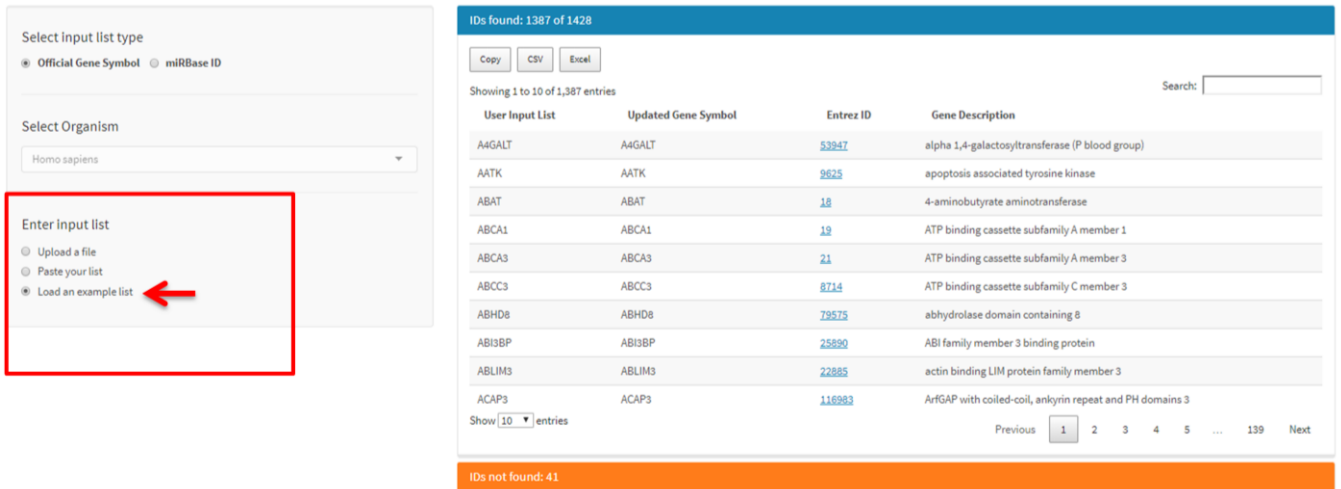
Load an example list

Paste your list below:

**PASTE HERE**

CLEAR DATA SUBMIT 

3) **Load an example list** for playing with data. With this choice, the user has the possibility to explore the functionality of MIENTURNET by exploiting a usage example.



The screenshot shows the MIENTURNET web interface. On the left, the 'Enter Input list' section is highlighted with a red box, and the 'Load an example list' option is selected with a red arrow. The main area displays a table of 1,387 genes found out of 1,428 total. The table has four columns: User Input List, Updated Gene Symbol, Entrez ID, and Gene Description. The first 10 rows are visible, showing genes like AAGALT, AATK, ABAT, ABCA1, ABCA3, ABCC3, ABHD8, ABI3BP, ABLIM3, and ACAP3. The table is paginated, showing page 1 of 139.

| User Input List | Updated Gene Symbol | Entrez ID              | Gene Description   |
|-----------------|---------------------|------------------------|--|
| AAGALT          | AAGALT              | <a href="#">53247</a>  | alpha 1,4-galactosyltransferase (P blood group)          |
| AATK            | AATK                | <a href="#">9525</a>   | apoptosis associated tyrosine kinase                     |
| ABAT            | ABAT                | <a href="#">18</a>     | 4-aminobutyrate aminotransferase                         |
| ABCA1           | ABCA1               | <a href="#">19</a>     | ATP binding cassette subfamily A member 1                |
| ABCA3           | ABCA3               | <a href="#">21</a>     | ATP binding cassette subfamily A member 3                |
| ABCC3           | ABCC3               | <a href="#">8714</a>   | ATP binding cassette subfamily C member 3                |
| ABHD8           | ABHD8               | <a href="#">79575</a>  | abhydrolase domain containing 8                          |
| ABI3BP          | ABI3BP              | <a href="#">25890</a>  | ABI family member 3 binding protein                      |
| ABLIM3          | ABLIM3              | <a href="#">22885</a>  | actin binding LIM protein family member 3                |
| ACAP3           | ACAP3               | <a href="#">116983</a> | ArfGAP with coiled-coil, ankyrin repeat and PH domains 3 |

Hereafter, we will show how MIENTURNET works by loading the example input list of mRNAs selected by the authors. Note that this list represents the differentially expressed genes resulting from a recent computational study of glioblastoma cells that was aimed to identify genes playing a key role in the transition from stem-like tumor propagating cells towards differentiated glioblastoma cells [6].

## Input list identifiers

The input list must include identifiers of mRNAs as Official Gene Symbol (e.g., PTEN for *human* species, Pten for *mouse* species). The tool processes the input file and immediately provides in the **IDs found** collapsible panel the total number of mRNAs recognized by NCBI database together with a table that includes: gene symbols of the input list, the corresponding updated gene symbols (i.e., the latest version of the gene name according to NCBI database), Entrez IDs as hyperlinks to NCBI Gene, and gene descriptions.

Select input list type  
● Official Gene Symbol ● miRBase ID

Select Organism  
Homo sapiens

Enter input list  
● Upload a file  
● Paste your list  
● Load an example list

IDs found: 1387 of 1428


Copy CSV Excel

Showing 1 to 10 of 1,387 entries Search:

| User Input List | Updated Gene Symbol | Entrez ID              | Gene Description   |
|-----------------|---------------------|------------------------|--|
| A4GALT          | A4GALT              | <a href="#">53947</a>  | alpha 1,4-galactosyltransferase (P blood group)          |
| AATK            | AATK                | <a href="#">9625</a>   | apoptosis associated tyrosine kinase                     |
| ABAT            | ABAT                | <a href="#">18</a>     | 4-aminobutyrate aminotransferase                         |
| ABCA1           | ABCA1               | <a href="#">19</a>     | ATP binding cassette subfamily A member 1                |
| ABCA3           | ABCA3               | <a href="#">21</a>     | ATP binding cassette subfamily A member 3                |
| ABCC3           | ABCC3               | <a href="#">8714</a>   | ATP binding cassette subfamily C member 3                |
| ABHD8           | ABHD8               | <a href="#">79575</a>  | abhydrolase domain containing 8                          |
| ABI3BP          | ABI3BP              | <a href="#">25890</a>  | ABI family member 3 binding protein                      |
| ABLIM3          | ABLIM3              | <a href="#">22885</a>  | actin binding LIM protein family member 3                |
| ACAP3           | ACAP3               | <a href="#">116983</a> | ArfGAP with coiled-coil, ankyrin repeat and PH domains 3 |

Show 10 entries Previous 1 2 3 4 5 ... 139 Next

IDs not found: 41

 **Caveat:** the analysis will proceed by considering the updated gene symbols.

In the input list table, the user has also the possibility to:

- visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the bottom-left
- navigate the table through the navigation controller buttons at the bottom-right
- search for a specific miRNA in the input list by typing the name in the search bar at the top-right.
- copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left.

IDs found: 1387 of 1428

iv. Copy CSV Excel

iii. Search:

Showing 1 to 10 of 1,387 entries

| User Input List | Updated Gene Symbol | Entrez ID              | Gene Description   |
|-----------------|---------------------|------------------------|--|
| A4GALT          | A4GALT              | <a href="#">53947</a>  | alpha 1,4-galactosyltransferase (P blood group)          |
| AATK            | AATK                | <a href="#">9625</a>   | apoptosis associated tyrosine kinase                     |
| ABAT            | ABAT                | <a href="#">18</a>     | 4-aminobutyrate aminotransferase                         |
| ABCA1           | ABCA1               | <a href="#">19</a>     | ATP binding cassette subfamily A member 1                |
| ABCA3           | ABCA3               | <a href="#">21</a>     | ATP binding cassette subfamily A member 3                |
| ABCC3           | ABCC3               | <a href="#">8714</a>   | ATP binding cassette subfamily C member 3                |
| ABHD8           | ABHD8               | <a href="#">79575</a>  | abhydrolase domain containing 8                          |
| ABI3BP          | ABI3BP              | <a href="#">25890</a>  | ABI family member 3 binding protein                      |
| ABLIM3          | ABLIM3              | <a href="#">22885</a>  | actin binding LIM protein family member 3                |
| ACAP3           | ACAP3               | <a href="#">116983</a> | ArfGAP with coiled-coil, ankyrin repeat and PH domains 3 |

i. Show 10 entries ii. Previous 1 2 3 4 5 ... 139 Next

The list of not recognized mRNAs (if any) can be viewed by clicking on the **IDs not found** collapsible panel.

Select input list type  
 Official Gene Symbol  miRBase ID

Select Organism  
Homo sapiens

Enter input list  
 Upload a file  
 Paste your list  
 Load an example list

IDs found: 1387 of 1428

IDs not found: 41

Showing 1 to 10 of 41 entries Search:

**Not found**

- FLJ22184
- FLJ27352
- FLJ32063
- FLJ43860
- LOC100127888
- LOC100128822
- LOC100130000
- LOC100132707
- LOC100132891
- LOC100133985

Show 10 entries Previous 1 2 3 4 5 Next

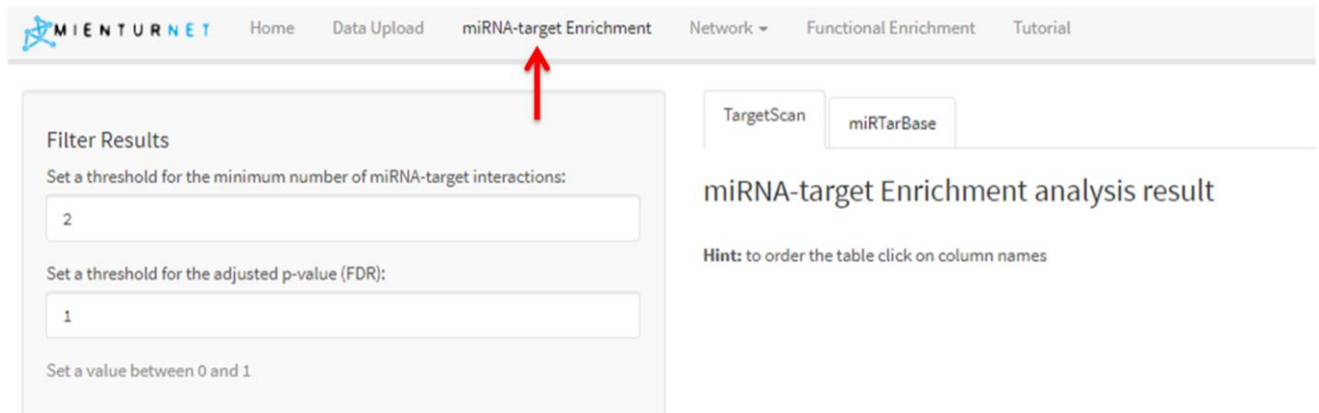
An error message appears either if the input list contains Official Gene Symbols that are not valid, or if the organism is incorrectly selected.

Select input list type  
 Official Gene Symbol  miRBase ID

Oops  
Check your Input List: there are not valid Gene Symbol IDs or you have selected the wrong Organism

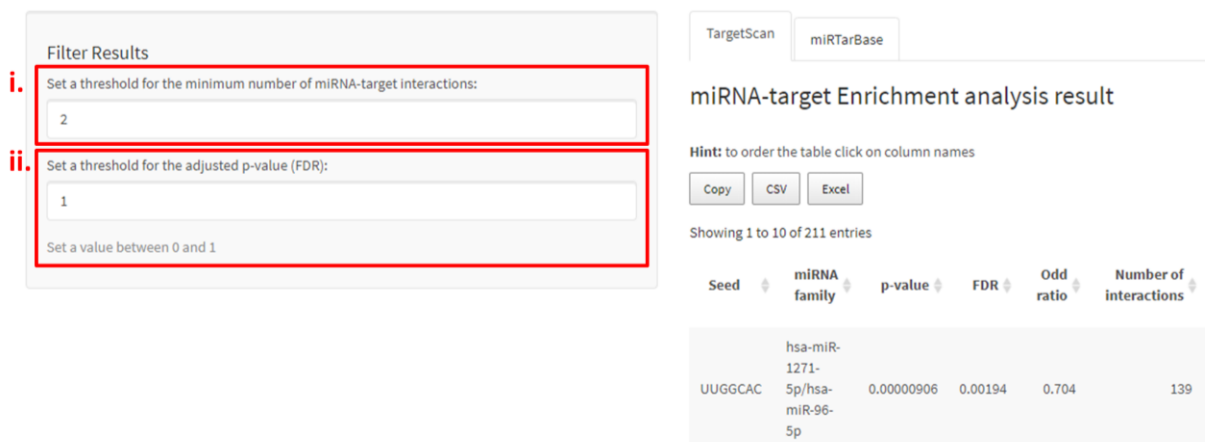
## miRNA-target Enrichment link

By clicking on **miRNA-target Enrichment** link, MIENTURNET will perform a statistical analysis for over-representation of miRNA-target interactions in the input gene list.




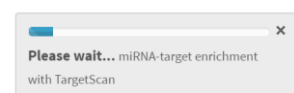
The user has the possibility to perform the enrichment analysis by using a threshold on:

- i. the minimum number of miRNA-target interactions (default is 2)
- ii. the adjusted p-values (default is 1)



From **miRNA-target Enrichment** page, the user can choose the reference database for the miRNA-target interactions (i.e., TargetScan and/or miRTarBase) to be used to perform the enrichment analysis.

 **Caveat:** regardless of the database choice, MIENTURNET performs the miRNA-target enrichment analysis searching for both predicted and validated interactions. A progress bar in action will appear at the bottom-right of the page as long as both analyses are not completed.



By clicking on **TargetScan** tab, the computationally predicted miRNA-target interactions will be considered.


miRNA-target Enrichment analysis result

Hint: to order the table click on column names

Copy CSV Excel

Showing 1 to 10 of 211 entries

| Seed    | miRNA family                  | p-value    | FDR     | Odd ratio | Number of interactions |
|---------|-------------------------------|------------|---------|-----------|------------------------|
| UUGGCAC | hsa-miR-1271-5p/hsa-miR-96-5p | 0.00000906 | 0.00194 | 0.704     | 139                    |

 **Caveat:** TargetScan predicts biological targets of miRNAs by searching for the presence of conserved sites that match the seed region (i.e., the region comprising nucleotides 2-7 at the 5'-end of the mature miRNA sequence) of each miRNA [3]. Note that, a miRNA family is comprised of miRNAs with the same seed sequence. This implies that members of the same miRNA family all share the same predicted targets. As a consequence, in this case, the enrichment analysis was performed considering miRNA families instead of single miRNAs.

By clicking on **miRTarBase** tab, the experimentally validated miRNA-target interactions will be considered.


miRNA-target Enrichment analysis result

Hint: to order the table click on column names

Copy CSV Excel

Showing 1 to 10 of 2,264 entries

| microRNA       | p-value  | FDR      | Odd ratio | Number of interactions |
|----------------|----------|----------|-----------|------------------------|
| hsa-miR-124-3p | 2.34e-14 | 5.80e-11 | 0.578     | 183                    |

 **Caveat:** miRTarBase collects miRNA-target interactions experimentally validated by reporter assay, western blot, microarrays, and next-generation sequencing experiments. As a consequence, in this case, the enrichment analysis was performed considering miRNAs instead of miRNA families.

For each miRNA in the chosen database (TargetScan or miRTarBase), the hypergeometric test was used to calculate the significance (p-value <0.05) of the enrichment of the input list in targets of that miRNA.

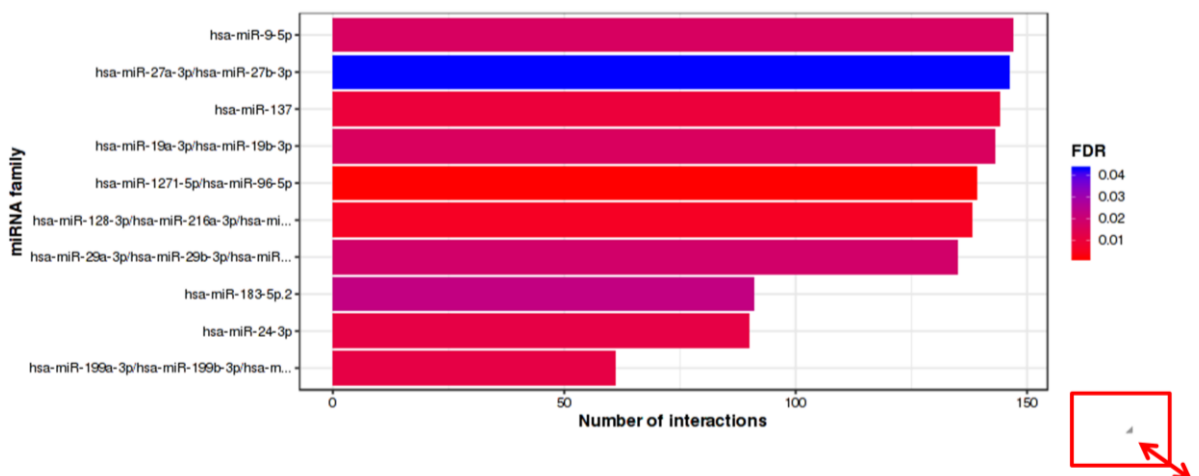
The p-value is computed as

$$p = 1 - \sum_{i=0}^{X-1} \frac{\binom{K}{i} \binom{M-K}{N-i}}{\binom{M}{N}}$$

where M is the dimension of the universe, that is the number of all predicted (validated) miRNA-target interactions encompassed in TargetScan (miRTarBase); K is the number of predicted (validated) miRNA-target interactions encompassed in TargetScan (miRTarBase) for the selected miRNA family (selected miRNA); N is the number of genes of the input gene list recognized by TargetScan (miRTarBase); X is the number of genes for which predicted (validated) miRNA-target interactions, for the selected miRNA family (selected miRNA), exist.

The results of the enrichment analysis are presented as<sup>8</sup>:

- i. a bar plot where the Y-axis reports the top ten miRNA families resulted from the enrichment analysis, while the X-axis reports the number of their targets. The color code reflects the FDR value increasing from red to blue. This plot is resizable by grabbing and dragging an active corner at the bottom right of the page.



<sup>8</sup> Hereafter, all snapshots refer to TargetScan visualization.

- ii. an interactive table where the user has also always the possibility to:
  1. visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the bottom-left
  2. navigate the table through the navigation controller buttons at the bottom-right
  3. search for a miRNA or a Target Gene of interest by typing the name in the search bar at the top-right
  4. copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left

TargetScan miRTarBase

miRNA-target Enrichment analysis result

Hint: to order the table click on column names

4. Copy CSV Excel

Showing 1 to 10 of 211 entries

3. Search:

| Seed    | miRNA family                                    | p-value    | FDR     | Odd ratio | Number of interactions | Target Gene 1 | Target Gene 2 | Target Gene 3 | Target Gene 4 | Target Gene 5 | Target Gene 6 | Target Gene 7 | Target Gene 8 | Target Gene 9 | Target Gene 10 | Target Gene 11 | Target Gene 12 |
|---------|---|------------|---------|-----------|------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|----------------|----------------|
| UUGGCAC | hsa-miR-1271-5p/hsa-miR-96-5p                   | 0.00000906 | 0.00194 | 0.704     | 139                    | SLC1A1        | ZNF704        | SOX5          | SOX6          | ATXN1         | FZD3          | NOVA1         | MTSS1         | SH3BP5        | INSIG2         | TNS3           | VAT1           |
| CACAGUG | hsa-miR-128-3p/hsa-miR-216a-3p/hsa-miR-3681-3p  | 0.0000525  | 0.00562 | 0.729     | 138                    | GRIK3         | AFF3          | SNAP25        | GREM1         | DCX           | SOCSS         | SORL1         | VEGFC         | RNF144A       | IRS1           | APBA2          | SAMD.          |
| UAUUGCU | hsa-miR-137                                     | 0.000135   | 0.00963 | 0.749     | 144                    | PDE3A         | AKAP2         | PALM2-AKAP2   | KCNAB3        | PLXDC2        | DIRAS1        | SBK1          | MSRB3         | COL19A1       | MYO1C          | SLC30A4        | SLC43          |
| CAGUAGU | hsa-miR-199a-3p/hsa-miR-199b-3p/hsa-miR-3129-5p | 0.000272   | 0.0116  | 0.638     | 61                     | ITGA1         | NOVA1         | CFL2          | FGF7          | DCBLD2        | CHSY3         | CD2AP         | C1orf21       | CELF2         | CD44           | FAM49B         | TWIST          |
| UCACAGU | hsa-miR-27a-3p/hsa-miR-27b-3p                   | 0.00200    | 0.0427  | 0.799     | 146                    | HIVEP3        | NABP1         | LIFR          | SORL1         | SNAP25        | AFAP1         | SSH1          | SEMA6A        | SLC7A11       | CDH11          | NOVA1          | TMEM           |

1. Show 10 entries

2. Previous 1 2 3 4 5 ... 22 Next

The columns of the table report:

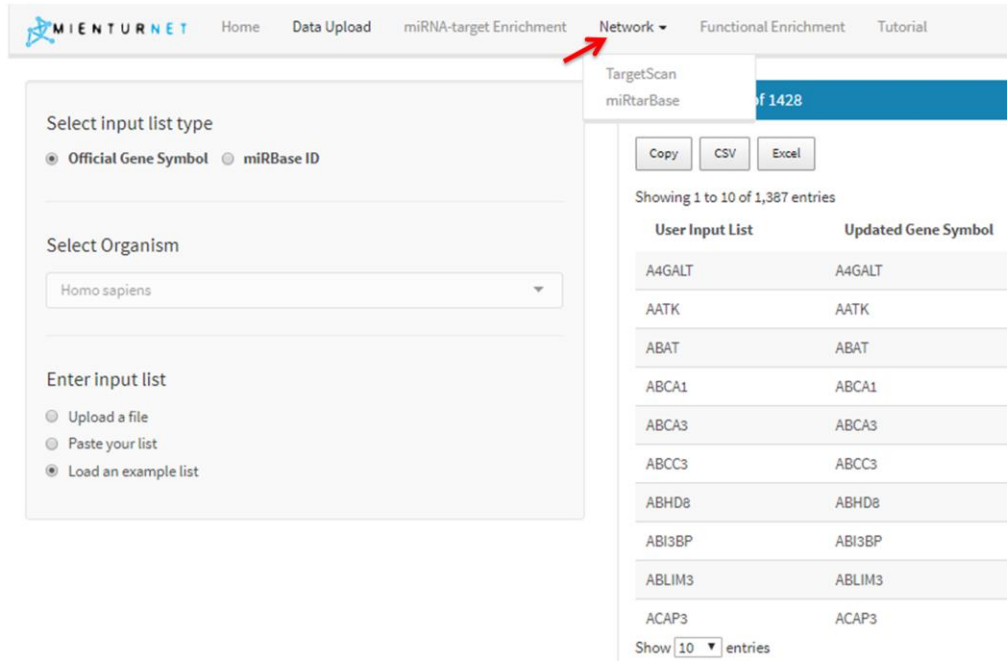
- *Seed*: seeds for each miRNA family
- *miRNA family*: names of all members for each miRNA family
- *p-value*: p-values of the enrichment analysis
- *FDR*: the adjusted p-values obtained by using the Benjamini-Hochberg (False Discovery Rate, FDR) procedure for multiple testing
- *Odd ratio*: ratios calculated as:  $\frac{K/M}{X/N}$
- *Number of interactions*: total numbers of interactions for each miRNA family
- Additional extra columns reporting the names of targets included in the gene input list for each miRNA family, named with increasing numerical values

Note that, selecting miRTarBase, the first two columns of the above table will be replaced with a single column with the miRNA names (i.e., miRbase ID).



## Network menu

In order to build the network of miRNA-target interactions identified by the enrichment analysis, click on **Network** menu and choose the reference database (i.e., TargetScan and/or miRTarBase) from the drop-down menu.



The screenshot shows the MIENTURNET web interface. The top navigation bar includes 'Home', 'Data Upload', 'miRNA-target Enrichment', 'Network', 'Functional Enrichment', and 'Tutorial'. The 'Network' menu is open, showing options for 'TargetScan' and 'miRtarBase'. Below the menu, there are buttons for 'Copy', 'CSV', and 'Excel'. A table displays a list of genes with columns for 'User Input List' and 'Updated Gene Symbol'. The table shows the following entries:

| User Input List | Updated Gene Symbol |
|-----------------|---------------------|
| A4GALT          | A4GALT              |
| AATK            | AATK                |
| ABAT            | ABAT                |
| ABCA1           | ABCA1               |
| ABCA3           | ABCA3               |
| ABCC3           | ABCC3               |
| ABHD8           | ABHD8               |
| ABI3BP          | ABI3BP              |
| ABLIM3          | ABLIM3              |
| ACAP3           | ACAP3               |

## Selecting miRNA-target interactions database and filtering

By choosing **TargetScan**, the results can be filtered by using a threshold on:

- the minimum number of miRNA-target interactions
- the adjusted p-values obtained from the miRNA-target enrichment analysis
- the *cumulative weighted context++ score* (CWCS) parameter [4] provided by TargetScan<sup>9</sup>
- the *probability of conserved targeting* (P<sub>CT</sub>) parameter [5] provided by TargetScan



The screenshot shows the 'Network microRNA/Genes - TargetScan' interface. On the left, there is a 'Filter Network' section with four red-bordered boxes corresponding to the filtering criteria listed in the previous section:

- Set a threshold for the minimum number of miRNA-target interactions: 2
- Set a threshold for the adjusted p-value (FDR): 1
- Set a threshold for the cumulative weighted context++ score (CWCS) (Click here for CWCS help page): 0.9
- Set a threshold for the probability of conserved targeting (PCT) (Click here for PCT help page): 0.5

Below the filtering section, there are options to 'Customize Network Image', including 'Choice network layout' (set to 'Auto') and 'Choice source (microRNA) node size' (set to '25'). On the right, there is a network visualization showing a dense cluster of nodes (yellow and blue) connected by edges. The interface also includes a 'Network Properties' tab and a 'miRNA-target Table' tab. There are buttons for 'SAVE NETWORK' and 'DOWNLOAD NETWORK', and a 'Select by id' dropdown menu.

<sup>9</sup> This threshold refers to a percentile of the CWCS distribution.

The user can choose to apply one of these filters, more than one or none. By default, the first two thresholds are set to the values chosen in the **miRNA-target Enrichment** page. The last two parameters, CWCS and  $P_{CT}$ , are set by default to 0.9 and 0.5, respectively.

By choosing **miRTarBase**, the results can be filtered according to:

- i. a threshold on the minimum number of miRNA-target interactions
- ii. a threshold on the adjusted p-values obtained from the miRNA-target enrichment analysis
- iii. the type of *Evidence categories* used by miRTarBase to validate the miRNA-target interactions:
  - a. ‘Strong’ for considering strong experimental methods (e.g., Luciferase assay, Western)
  - b. ‘Weak’ for considering weaker experimental evidence (e.g., CLIP)
  - c. ‘Strong and Weak’ for considering both strong and weak experimental methods

The user can choose to apply one of these filters, more than one or none. As before, by default the first two thresholds are set to the values chosen in the **miRNA-target Enrichment** page. Instead, the *Evidence categories* filter is configured as ‘Strong’.

**! Caveat:** regardless of the database choice, the user that is not interested in the enrichment analysis can skip the previous step (i.e., the user can avoid to click on **miRNA-target Enrichment** link) and jump to the next step of **Network** menu. In this case, MIENURNET will still perform the enrichment analysis, but will set the default value for the adjusted p-values to 1.

## Network visualization and analysis

Both for **TargetScan** and **miRTarBase**, the user can interactively play with the miRNA-interaction network by pressing the following tabs:

1. **Network** tab: it allows to visualize, customize, and download the network in the form of an edgelist (i.e., a 2-column table where each row represents a single edge)
2. **Network properties** tab: it allows to perform a network analysis
3. **miRNA-target Table** tab: it allows to view and download a table where the columns are the miRNAs with the corresponding own targets

In the following, there is a detailed description for each of these tabs<sup>10</sup>.

### 1. Network tab

From **Network** tab, the user can visualize the miRNA-target interactions network:

- i. as an image that can be interactively explored and customized
- ii. as a 2-column table in the form of an edgelist (i.e., each row represents a miRNA-target interaction).

**i.**

Network Network Properties miRNA-target Table

SAVE NETWORK DOWNLOAD NETWORK

Select by id

**ii.**

Copy CSV Excel

Showing 1 to 10 of 628 entries

Search:

| miRNA family  | microRNA Target Gene |
|---|----------------------|
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | ADRB2                |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | ARL4D                |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | CCND2                |

<sup>10</sup> All snapshots refer to TargetScan visualization, where source nodes are the miRNA families to which each miRNA belongs. Otherwise, in the miRTarBase visualization, source nodes are single miRNAs.

By using the *Customize network image* menu on the left, the user may customize:

- the network layout (details about the layout algorithms are available at <http://igraph.org>)
- the size of source/target nodes
- the shape of source/target nodes
- the color of source/target nodes. By default, source nodes (miRNA families or single miRNAs) are blue circles, while target nodes (mRNAs) are yellow circles.

The screenshot displays the 'Customize Network Image' panel on the left, which includes the following settings:

- Choice network layout: Auto
- Choice source (microRNA) node size: 25
- Choice target (microRNA Target Gene) node size: 25
- Choice source (microRNA) node color: #97C2FC
- Choice target (microRNA Target Gene) node color: #FFFF0A
- Choice source (microRNA) node shape: dot
- Choice target (microRNA Target Gene) node shape: dot

The network graph on the right shows a dense cluster of blue and yellow nodes connected by edges. Below the graph, there are buttons for 'Copy', 'CSV', and 'Excel', and a search bar. A table of entries is visible below the search bar.

| miRNA family  | microRNA Target Gene |
|---|----------------------|
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | ADRB2                |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | ARL4D                |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | CCND2                |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | COL1A2               |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | CPA4                 |

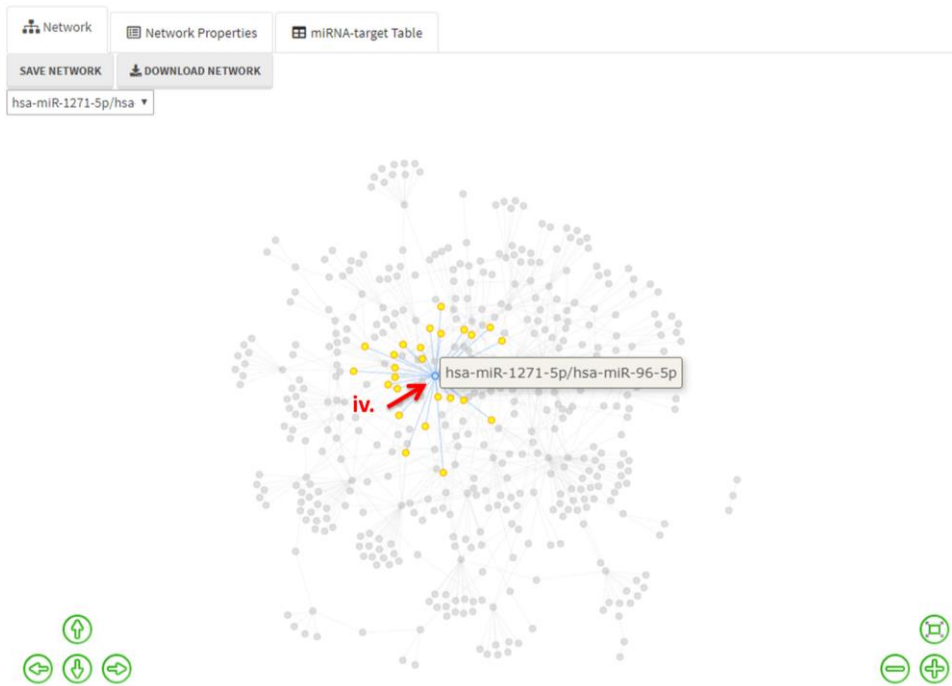
On the network image, the user has also the possibility to:

- move the network around by using the up-down left-right arrows
- zoom in/out the image by using the plus/minus buttons
- select a specific node by scrolling down the drop-down menu "Select by id" at the top-left

The screenshot displays the network visualization interface with the following elements:

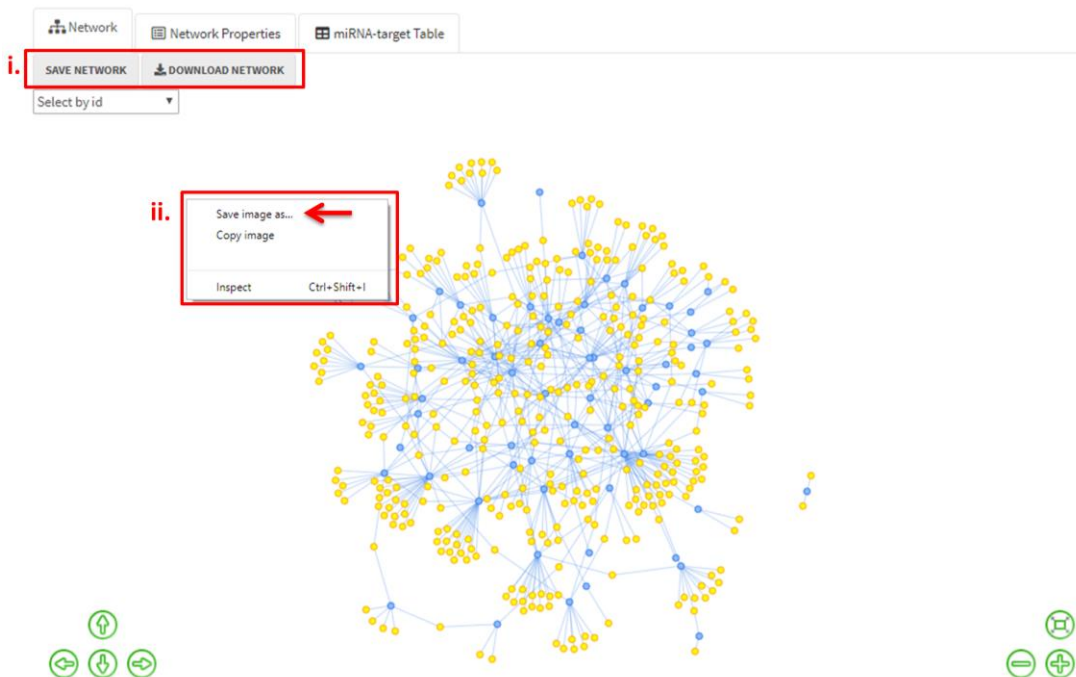
- Navigation buttons: Network, Network Properties, miRNA-target Table
- Actions: SAVE NETWORK, DOWNLOAD NETWORK
- Dropdown menu: Select by id (highlighted with a red box)
- Navigation icons: Up, Down, Left, Right arrows (highlighted with a red box) and Plus, Minus buttons (highlighted with a red box)
- Network graph: A dense cluster of blue and yellow nodes connected by edges.

- iv. select a specific node by placing the pointer over the node to be selected. Note, the selection will interest the node and its first neighbors.
- v. move one or more node by selecting and dragging them with mouse



The user can export the network picture as:

- i. a dynamic image in HTML format, by clicking first SAVE NETWORK button and then DOWNLOAD NETWORK button at the top-left
- ii. a static image in PNG format, by pressing down on the right mouse button (i.e., right-clicking)



On the network table below the network image, the user has always the possibility to:

- i. visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the bottom-left
- ii. navigate the table through the navigation controller buttons at the bottom-right
- iii. search for a miRNA or a target gene of interest by typing the name in the search bar at the top-right
- iv. copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left.

iv.

Showing 1 to 10 of 628 entries

| miRNA family  | microRNA Target Gene |
|---|----------------------|
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | ADRB2                |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | ARL4D                |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | CCND2                |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | COL1A2               |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | CPA4                 |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | EDN1                 |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | FAM214B              |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | FAS                  |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | FZD3                 |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | GALC                 |

i. Show 10 entries

ii. Previous 1 2 3 4 5 ... 63 Next

One or more mRNAs in the input list, although recognized in NCBI, could not be in the chosen database (TargetScan or miRTarBase). In this case, the mRNAs not found are listed in the **IDs not found** collapsible panel.

→ IDs not found in TargetScan

There are 317 genes not in TargetScan database:

Show 10 entries

Search:

Excluded genes

- A4GALT
- ABCC3
- ACSL5
- ADAMTS6
- ADTRP
- AIG1
- AKR1B10
- AKR1C1
- ANKRD20A1
- ANKRD20A2

Showing 1 to 10 of 317 entries

Previous 1 2 3

## 2. Network Properties tab

From **Network Properties** tab, the user can:

- i. visualize a table with the topological properties for each node<sup>11</sup>, where it is possible to:
  1. sort each column in decreasing or increasing order by clicking on the up-down arrows close to the column names
  2. visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the bottom-left
  3. navigate the table through the navigation controller buttons at the bottom-right
  4. search for a node of interest by typing the name in the search bar at the top-right
  5. copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left

The screenshot shows the 'Network Properties' tab with a table of miRNA nodes and their topological properties. The table is sorted by Degree in descending order. The interface includes a search bar at the top right, export buttons (Copy, CSV, Excel) at the top left, and a pagination controller at the bottom right. The table shows 10 entries, with a 'Showing 1 to 10 of 458 entries' indicator at the bottom left.

| Node  | Degree | Closeness | Betweenness | Eccentricity | ClusteringCoefficient | AverageShortestPathLength |
|---|--------|-----------|-------------|--------------|-----------------------|---------------------------|
| hsa-miR-29a-3p/hsa-miR-29b-3p/hsa-miR-29c-3p  | 37     | 0.153     | 0.171       | 7            | 0                     | 3.53                      |
| hsa-miR-124-3p.1  | 27     | 0.148     | 0.118       | 9            | 0                     | 3.77                      |
| hsa-miR-34a-5p/hsa-miR-34c-5p/hsa-miR-449a/hsa-miR-449b-5p  | 27     | 0.150     | 0.104       | 7            | 0                     | 3.69                      |
| hsa-miR-1271-5p/hsa-miR-96-5p   | 27     | 0.158     | 0.162       | 7            | 0                     | 3.32                      |
| hsa-miR-15a-5p/hsa-miR-15b-5p/hsa-miR-16-5p/hsa-miR-195-5p/hsa-miR-424-5p/hsa-miR-497-5p/hsa-miR-6838-5p  | 23     | 0.147     | 0.0950      | 7            | 0                     | 3.79                      |
| hsa-miR-19a-3p/hsa-miR-19b-3p   | 21     | 0.153     | 0.110       | 7            | 0                     | 3.54                      |
| hsa-miR-182-5p  | 20     | 0.149     | 0.0811      | 7            | 0                     | 3.74                      |
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | 19     | 0.140     | 0.0796      | 9            | 0                     | 4.14                      |
| hsa-miR-130a-3p/hsa-miR-130b-3p/hsa-miR-301a-3p/hsa-miR-301b-3p/hsa-miR-3666/hsa-miR-4295/hsa-miR-454-3p  | 19     | 0.151     | 0.119       | 7            | 0                     | 3.64                      |
| hsa-miR-218-5p  | 17     | 0.141     | 0.0579      | 9            | 0                     | 4.08                      |

The columns of the table report:

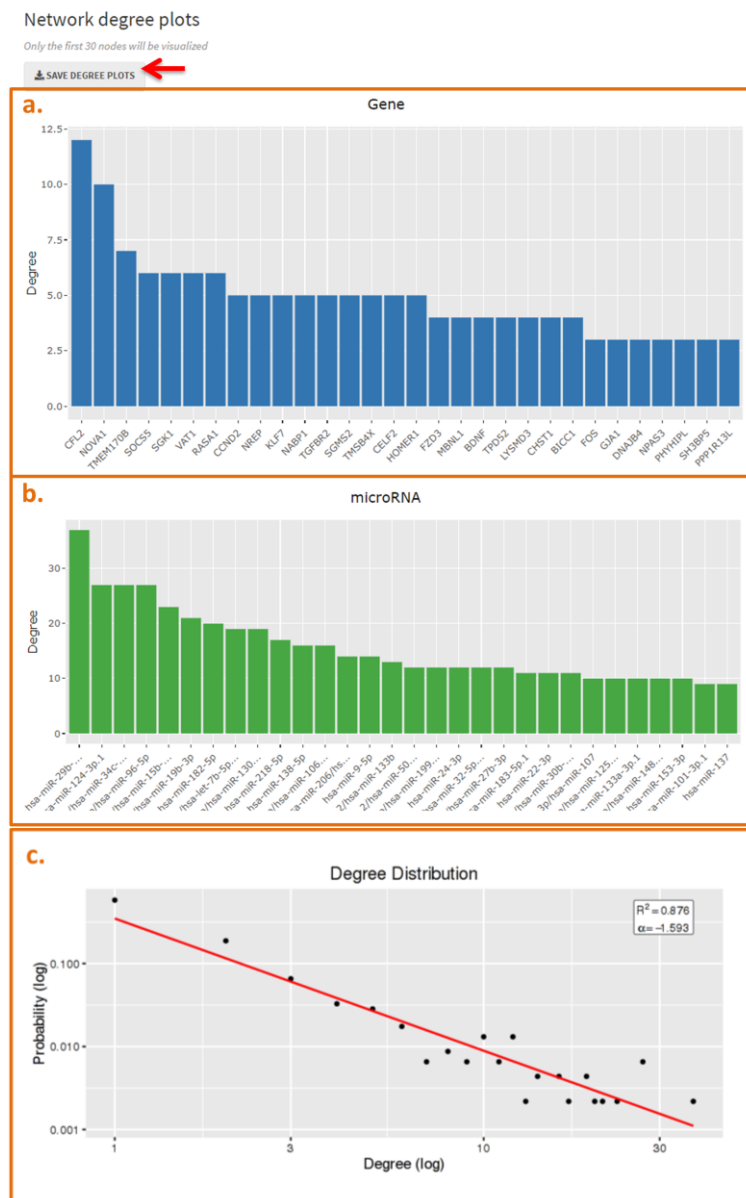
- *degree* - the number of incoming and outgoing edges of each node
- *betweenness* – the number of shortest paths<sup>12</sup> going through the node
- *average shortest path length* – the average length of the shortest paths between a node and all other nodes in the network
- *eccentricity* – the shortest path distance of a node from the farthest other node in the network
- *clustering coefficient* – measurement the probability that the adjacent nodes of a node are connected. The clustering coefficient is set to 0 for nodes with degree zero and one.
- *closeness* – the inverse of the average length of the shortest paths between a node and all other nodes in the network. Note that the closeness centrality measure is not well-defined for disconnected graphs. If there is no (directed) path between node  $i$  and  $j$ , then the total number of nodes is used in the formula instead of the path length.

<sup>11</sup> Details about the network topological properties are available at <http://igraph.org>

<sup>12</sup> The shortest path between two nodes is the minimum number of consecutive edges connecting them

- ii. visualize the following network degree plots:
- a bar plot of the mRNA degree, where X-axis refers to the first 30 target genes (sorted in a decreasing order according to the degree) and Y-axis refers to their degree (i.e., number of miRNA targeting them)
  - a bar plot of the miRNA degree, where X-axis refers to the first 30 miRNAs (sorted in a decreasing order according to the degree) and Y-axis refers to their degree (i.e., number of their targets)
  - the nodes degree distribution  $P(k)$ , with  $k$  being the degree, by plotting  $\log k$  on the X-axis and  $\log P(k)$  on the Y-axis. Then, the method of least squares is used to find the best-fitting curve  $\log P \sim \alpha \log k$ , where  $\alpha$  is the constant to estimate. The sum of the squared residuals  $R^2$  (i.e., the sum of the vertical deviations from the best-fitting line) is reported together with the estimation of the coefficient  $\alpha$ .

All plots can be exported as a compressed .zip file by clicking on SAVE DEGREE PLOTS button.





### 3. miRNA-target Table tab

From **miRNA-target Table** tab, the user can visualize an interactive table where the columns are the mRNAs of the input list found in the selected database (TargetScan or miRTarBase) with the corresponding miRNAs targeting them. The columns of the table are sorted in a decreasing order according to the number of miRNAs targeting each gene. The user has always the possibility to:

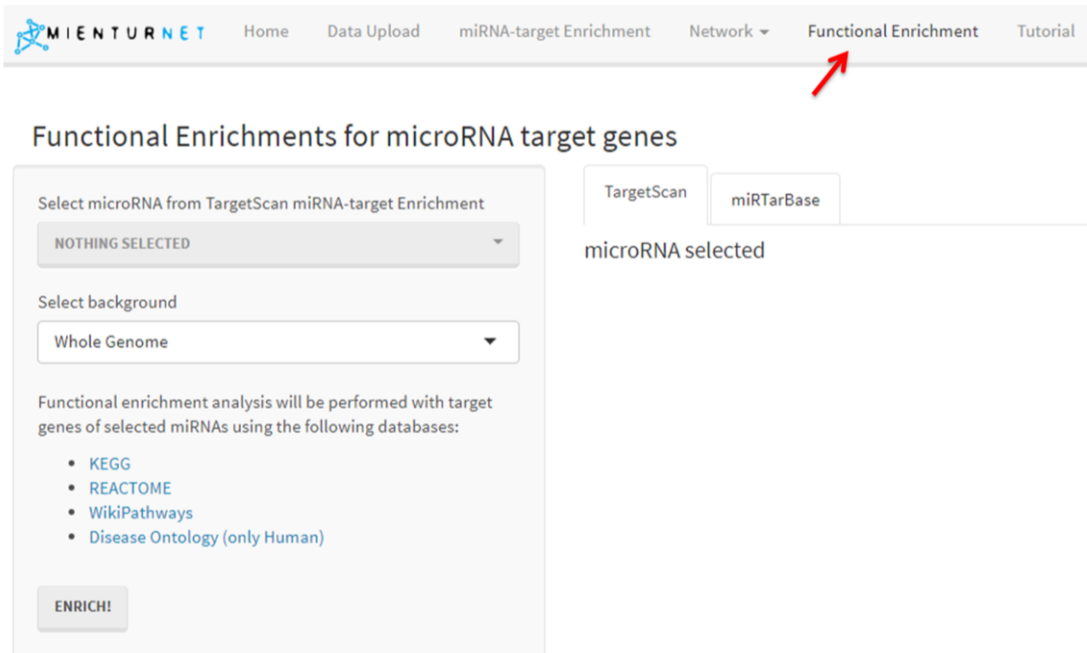
- i. visualize the first 10, 25, 50, 100 entries by clicking the drop-down menu at the top-left
- ii. navigate the table through the navigation controller buttons at bottom-right
- iii. search for a miRNA or a Target Gene of interest by typing the name in the search bar at the top-right
- iv. copy the table to the clipboard, export it in a comma separated format (CSV) or in EXCEL format through the dedicated buttons placed at the top-left.

The screenshot displays the 'miRNA-target Table' interface. At the top, there are three tabs: 'Network', 'Network Properties', and 'miRNA-target Table'. Below the tabs, there are three buttons: 'Copy', 'CSV', and 'Excel', which are highlighted with a red box and labeled 'iv.'. To the right of these buttons is a search bar labeled 'iii.' with the text 'Search:'. Below the search bar, there is a list of gene names: CFL2, NOVA1, TMEM170B, RASA1, SGK1, SOCS5, VAT1, CCND2, CELF2, HOMER1, and KLF7. The main area of the interface is a table with multiple columns, each containing a list of miRNA identifiers. The table is labeled 'Showing 1 to 10 of 12 entries'. At the bottom of the interface, there is a navigation bar with a dropdown menu labeled 'i.' showing 'Show 10 entries' and a set of navigation buttons labeled 'ii.' including 'Previous', '1', '2', '3', '4', and 'Next'.

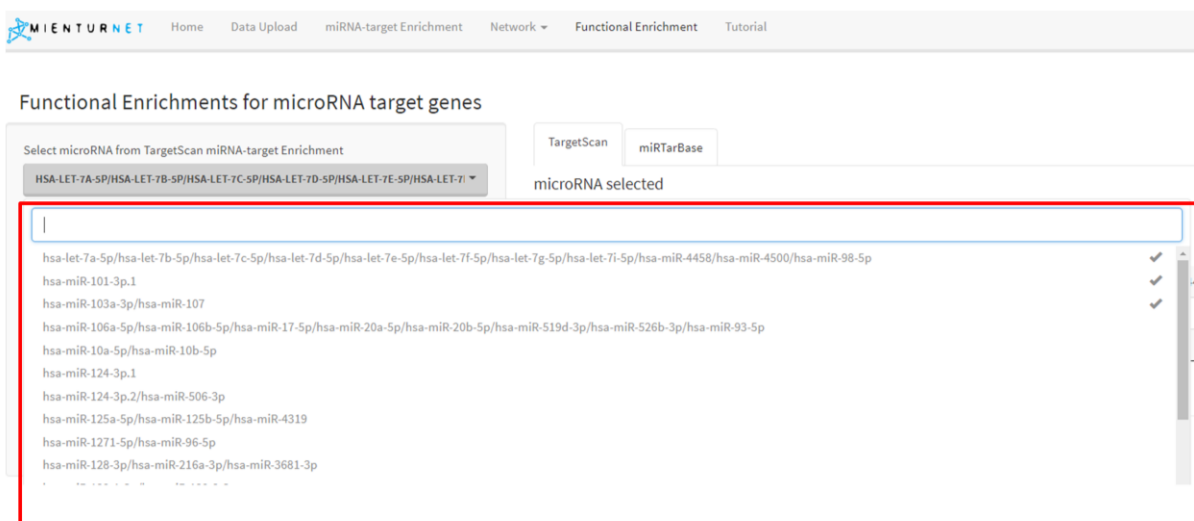
## Functional Enrichment link

In order to perform the functional enrichment analysis of target genes of selected miRNAs, click on **Functional Enrichment** link and choose TargetScan or miRTarBase <sup>13</sup>.

Currently, MIENTURNET allows to query the following annotation databases: KEGG, REACTOME, WikiPathways, Disease Ontology. Note that the last one will be available only when *Homo sapiens* is the selected organism.



Firstly, the user can select one or more miRNA families (or single miRNA in the case of miRTarBase) by clicking on each item or by typing the name in the search box. In this example, the first three miRNA families have been selected.



<sup>13</sup> All snapshots refer to TargetScan visualization, where for each selected miRNA the family to which it belongs will be reported. Otherwise, in the miRTarBase visualization, the selected miRNAs will be considered.

The selected miRNA families will appear in a table on the right along with the number of their interactions.

Functional Enrichments for microRNA target genes

Select microRNA from TargetScan miRNA-target Enrichment  
HSA-LET-7A-5P/HSA-LET-7B-5P/HSA-LET-7C-5P/HSA-LET-7D-5P/H...

Select background  
Whole Genome

Functional enrichment analysis will be performed with target genes of selected miRNAs using the following databases:  

- KEGG
- REACTOME
- WikiPathways
- Disease Ontology (only Human)

ENRICH!

TargetScan miRTarBase

microRNA selected

Showing 1 to 3 of 3 entries

| miRNA family  | Number of interactions |
|---|------------------------|
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | 20                     |
| hsa-miR-101-3p.1  | 10                     |
| hsa-miR-103a-3p/hsa-miR-107   | 11                     |

Show 10 entries Previous 1 Next

Secondly, the user can select the background set. Currently, the choice is between: “Whole Genome” (i.e., all annotated genes in the annotation database will be used as reference set), or “Your input list of genes” (i.e., the user’s input list of genes will be used as reference set).

Functional Enrichments for microRNA target genes

Select microRNA from TargetScan miRNA-target Enrichment  
HSA-LET-7A-5P/HSA-LET-7B-5P/HSA-LET-7C-5P/HSA-LET-7D-5P/H...

Select background  
Whole Genome

Functional enrichment analysis will be performed with target genes of selected miRNAs using the following databases:  

- KEGG
- REACTOME
- WikiPathways
- Disease Ontology (only Human)

ENRICH!

TargetScan miRTarBase

microRNA selected

Showing 1 to 3 of 3 entries

| miRNA family  | Number of interactions |
|---|------------------------|
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | 20                     |
| hsa-miR-101-3p.1  | 10                     |
| hsa-miR-103a-3p/hsa-miR-107   | 11                     |

Show 10 entries Previous 1 Next

To start the analysis, click the ENRICH button.

Functional Enrichments for microRNA target genes

Select microRNA from TargetScan miRNA-target Enrichment  
HSA-LET-7A-5P/HSA-LET-7B-5P/HSA-LET-7C-5P/HSA-LET-7D-5P/H...

Select background  
Whole Genome

Functional enrichment analysis will be performed with target genes of selected miRNAs using the following databases:  

- KEGG
- REACTOME
- WikiPathways
- Disease Ontology (only Human)

ENRICH!

TargetScan miRTarBase

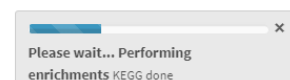
microRNA selected

Showing 1 to 3 of 3 entries

| miRNA family  | Number of interactions |
|---|------------------------|
| hsa-let-7a-5p/hsa-let-7b-5p/hsa-let-7c-5p/hsa-let-7d-5p/hsa-let-7e-5p/hsa-let-7f-5p/hsa-let-7g-5p/hsa-let-7i-5p/hsa-miR-4458/hsa-miR-4500/hsa-miR-98-5p | 20                     |
| hsa-miR-101-3p.1  | 10                     |
| hsa-miR-103a-3p/hsa-miR-107   | 11                     |

Show 10 entries Previous 1 Next

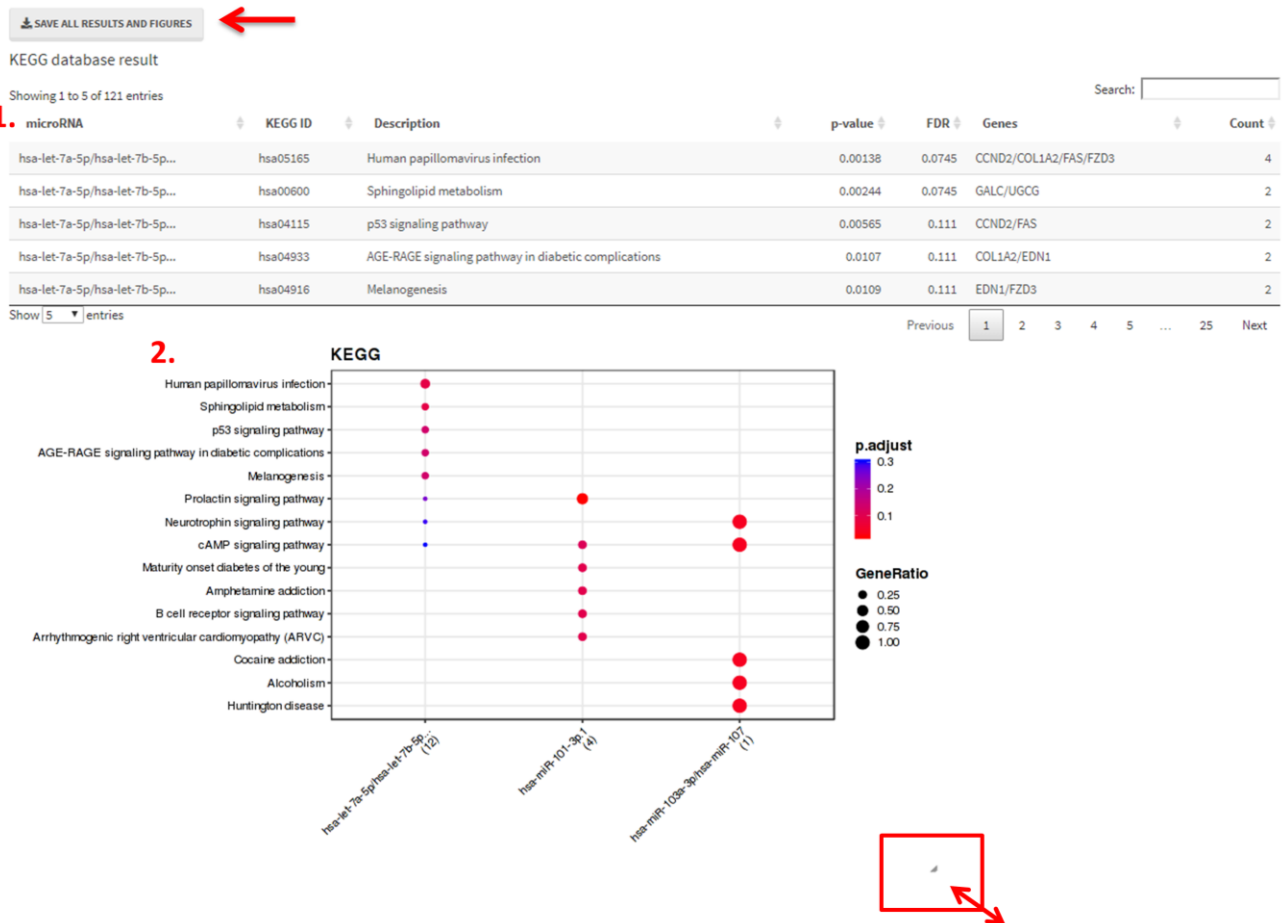
**! Caveat:** The function enrichment analysis will regard the targets of each selected miRNA, separately. The user could see a progress bar in action at the bottom-right of the page.



For each annotation database, the results of the functional enrichment analysis are presented as<sup>14</sup>:

3. an interactive table reporting: the miRNA name, the category name of the annotation database (e.g., KEGG ID), the category description, the p-value and the adjusted p-value (FDR), the list of target genes, the number of targets genes.
4. a dot plot, where the Y-axis reports the annotation categories (e.g., KEGG pathways) and the X-axis reports the miRNA family with the number of recognized targets in round brackets. The colors of the dots represent the adjusted p-values (FDR), whereas the size of the dots represents gene ratio (i.e., the number of miRNA targets found annotated in each category over the number of total genes annotated in that category). All the plots are resizable by grabbing and dragging an active corner at the bottom right of the page.

All the results can be saved by clicking on SAVE ALL RESULTS AND FIGURES button.



<sup>14</sup> The results presented here refer to the KEGG enrichment analysis.

## References

- [1] B. P. Lewis, I. Shih, M. W. Jones-Rhoades, D. P. Bartel, and C. B. Burge, “Prediction of mammalian microRNA targets,” *Cell*, vol. 115, no. 7, pp. 787–798, 2003.
- [2] C.-H. Chou *et al.*, “miRTarBase 2016: updates to the experimentally validated miRNA-target interactions database,” *Nucleic Acids Res.*, vol. 44, no. D1, pp. D239–D247, 2016.
- [3] B. P. Lewis, C. B. Burge, and D. P. Bartel, “Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets,” *Cell*, vol. 120, no. 1, pp. 15–20, Jan. 2005.
- [4] V. Agarwal, G. W. Bell, J.-W. Nam, and D. P. Bartel, “Predicting effective microRNA target sites in mammalian mRNAs.,” *Elife*, vol. 4, 2015.
- [5] R. C. Friedman, K. K.-H. Farh, C. B. Burge, and D. P. Bartel, “Most mammalian mRNAs are conserved targets of microRNAs,” *Genome Res.*, vol. 19, no. 1, pp. 92–105, Jan. 2009.
- [6] G. Fiscon, F. Conte, V. Licursi, S. Nasi, and P. Paci, “Computational identification of specific genes for glioblastoma stem-like cells identity,” *Sci. Rep.*, vol. 8, no. 1, p. 7769, May 2018.