miRNet Tutorial
Starting with expression data

Computer and Browser Requirements

• A modern web browser with Java Script enabled Chrome, Safari, Firefox, and Internet Explorer 9+

• For best performance and visualization, use:
– Latest Google Chrome
– At least 4GB of physical RAM
– A 15-inch screen or bigger

Goal for this tutorial

➢Perform differential expression analysis of data generated in miRNA functional analysis for miRNet

- Microarray
- RNA-seq
- RT-qPCR

Starting from the expression data

Data Formatting

- Manipulate data headings in a spreadsheet program like MS Excel
- Save as a **tab delimited** .txt file
- The headings #NAME and #CLASS: (**all capital letters**) must be used
  - #NAME is for sample names (first row in your data)
  - #CLASS is for the clinical metadata.

The screenshot below shows the labels for the experimental condition.

#NAME	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9
#CLASS	Y	N	N	Y	N	Y	Y	N	N
100_g_at	-3.06	-2.25	-1.15	-6.64	0.4	1.08	1.22	1.02	1.15
1000_at	-1.36	-0.67	-0.17	-0.97	-2.32	-5.06	0.28	1.32	0.73
1002_f_at	1.61	-0.27	0.71	-0.62	0.14		0.11	0.98	0.54
1008_f_at	0.93	1.29	-0.23	-0.74	-2	-1.25	1.07	1.27	1.02

Data Upload

Data Annotation

- Specifying the organism type and ID type allows miRNet to annotate your data;
- Eight organisms are supported;
- Entrez ID, Ensembl ID, gene symbol, miRNA ID and miRNA accession are supported for RNA-seq and RT-qPCR analysis;
- Affymetrix, Illumina and Agilent Microarray probe ID are supported for Microarray analysis;
- Supporting 53 tissues for human miRNA annotation.

Data Normalization

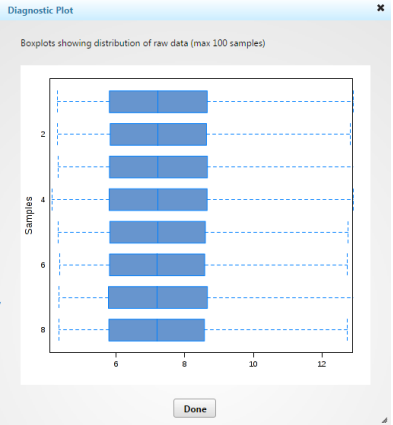- This example dataset has been normalized and needed no normalization
- If raw data is uploaded, normalization can be applied
  - ➢ Different normalization methods are provided for different data types

Data Analysis

Pruning or Expanding Network Data

Perform Data Filtering

Step 1 : Choose a target column which you want to perform the filter.

Step 2 : Choose the filter option, "Matching" is filtering by the exact words, "Containing" is filtering by keywords, "At least" is filtering by predicted score (only for *S.mansoni*)

Step 3 : Input the keywords and perform the filtering to keep or remove

The table after performing data filter

Only keep the method contains "CLIP"

Click "Proceed" to the network builder

Pruning or Expanding Network Data

Network tools

- ➢ The degree of a node is the number of connections it has to other nodes. Nodes with higher node degree act as hubs in a network.
- **Degree cutoff**: default 1.0, the minimal degree you want to choose.
- **All network nodes** : default option, choose all nodes in the network.
- **miRNA nodes only**: the degree filter will only perform in miRNA nodes.
- **All but miRNA nodes** : the degree filter will perform to other nodes except miRNA.
- **None**: Do not perform the filter.

Network tools
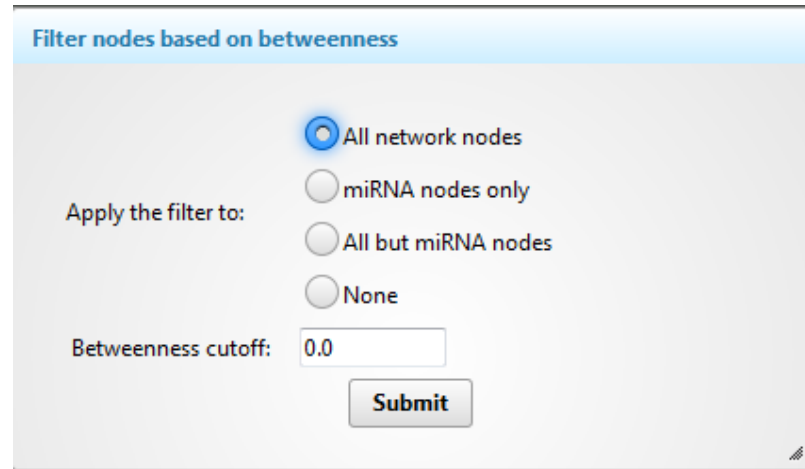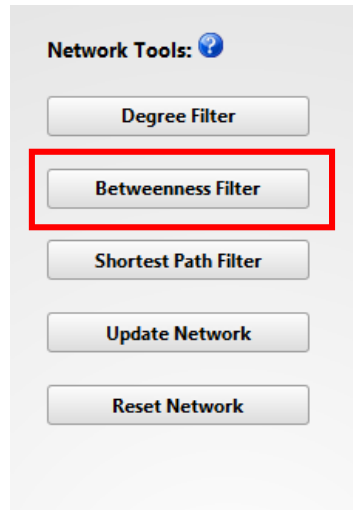
- The **betweenness centrality** measures the number of shortest paths going through the node. It takes into consideration the global network structure. For example, nodes that occur between two dense clusters will have a high betweenness centrality even if their degree centrality values are not high.
- **Degree cutoff**: default 0.0 (all nodes), the minimal betweenness you want to choose.
- **All network nodes** : default option, choose all nodes in the network.
- **miRNA nodes only**: the betweenness filter will only perform in miRNA nodes.
- **All but miRNA nodes** : the betweenness filter will perform to other nodes except miRNA.
- **None**: Do not perform the filter.

Network tools

Network Tools:

- Degree Filter
- Betweenness Filter
- Shortest Path Filter
- Update Network — Refresh network as new changes.
- Reset Network — Reset the network to default.

Reduce connections between densely connected nodes

Apply the filter to:

- ● All network nodes
- ○ miRNA nodes only
- ○ All but miRNA nodes
- ○ None

Submit

- **Shortest Path Filter** : If there are multiple paths that can link two nodes together, only one shortest path will be chose to reduce dense networks.
- **All network nodes** : default option, choose all nodes in the network.
- **miRNA nodes only**: the filter will only perform in miRNA nodes.
- **All but miRNA nodes** : the filter will perform to other nodes except miRNA.
- **None**: Do not perform the filter.

Understanding the Network Viewer

Choose different algorithms

- **Hypergeometric tests :**

Using the hypergeometric distribution to measure the statistical significance of those genes are identified from the miRNA target analysis.

- **Unbiased Empirical tests (only for miRNA):**

Being used to estimate the null distribution of the target genes as selected based on the input miRNAs. The procedures can be divided into three steps:

1) A list of miRNAs of the same size are randomly selected from all the miRNAs with known targets in the database;

2) The functional annotations (i.e. GO or KEGG) are then performed for the list;

3) The process is repeated 1000 times (default);

4) Compare the hits in each GO or KEGG pathways and the empirical p (Emp. p) values are calculated as the proportion of overlaps (with pathways or GO) from 1000 random process that equal or larger than the original.

5) User can perform the functional analysis again under the same parameters, the results will be combined. i.e. clicking five times will generate empirical p values based on 5000 random samplings.

Enrichment analysis by highlighting nodes

Enrichment analysis by selecting pathway

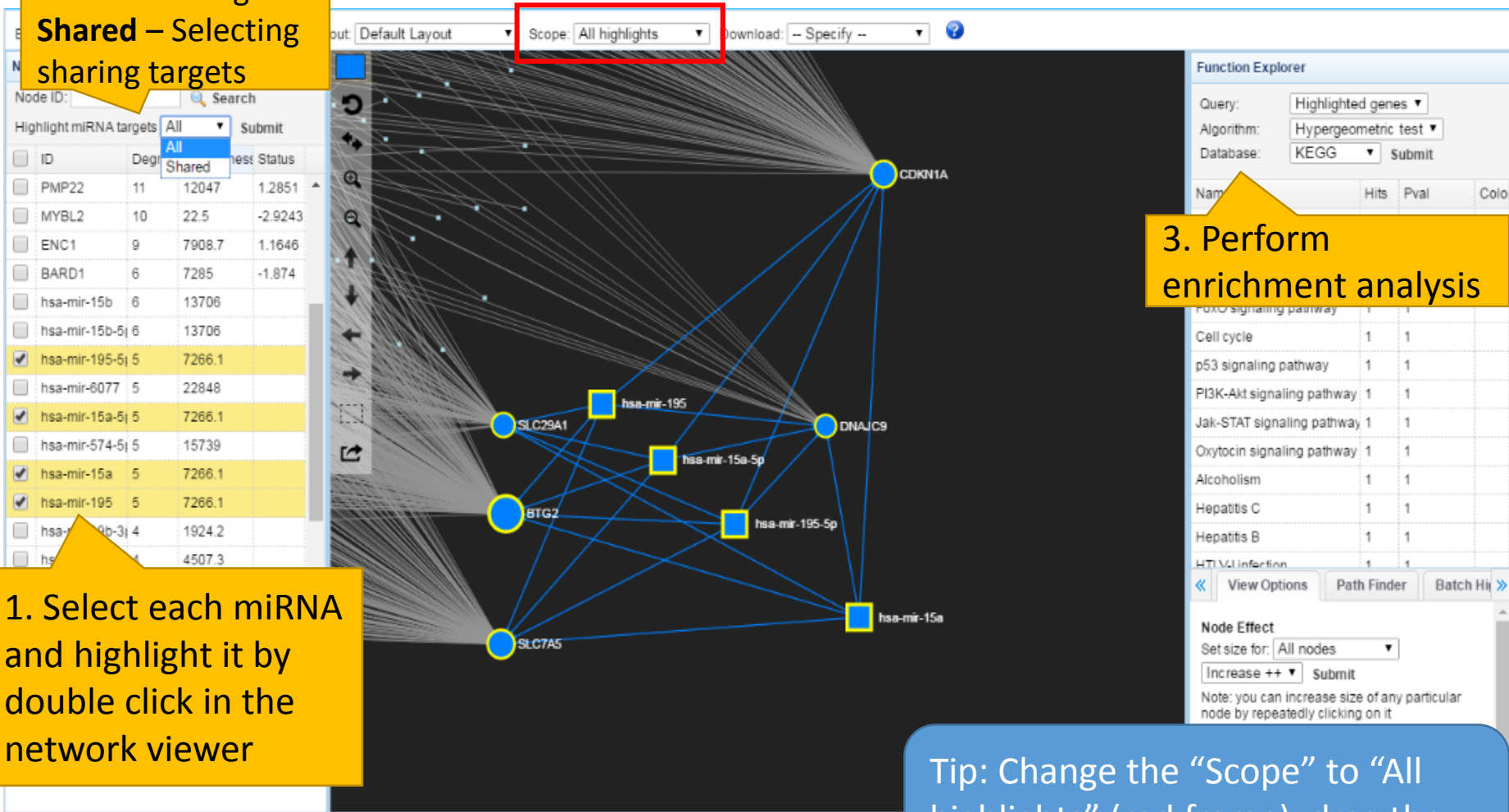2. Choose a color here, you can change different colors to perform selection

1. Choose an algorithm and a pathway then perform for all genes

3. Click a pathway, the genes inside will be highlighted by selected color.

The genes in this pathway

Enrichment analysis by associated targets

2. **All** – Selecting all associated targets
Shared – Selecting sharing targets

3. Perform enrichment analysis

1. Select each miRNA and highlight it by double click in the network viewer

Tip: Change the "Scope" to "All highlights" (red frame), drag the whole group aside and zoom in.

Dragging nodes-Current highlights

1. Choose "Current highlights"

2. Click a pathway, the current selected nodes are circled by golden line.

3. Drag current highlighted nodes aside and zoom in.

Dragging nodes-All highlights

Expression analysis in network

Selecting a group in the network

1. Choose a color

2A. Choose "Node-neighbours"

2B. Click here

3A. Double click the center node.

3B. Circle a group of nodes and they will be highlighted.

4. Choose "Highlighted nodes", algorithm and pathway, click "submit" to perform the enrichment analysis.

Configuring the general visualization feature

View Options

Node Effect: adjust the node size. You can increase or decrease the nodes.

Edge Effect : change the edge shape as curve or line. And the edge width as thin, medium and thick

Highlight Effect : for other nodes you can choose dim down or hide.

==END==