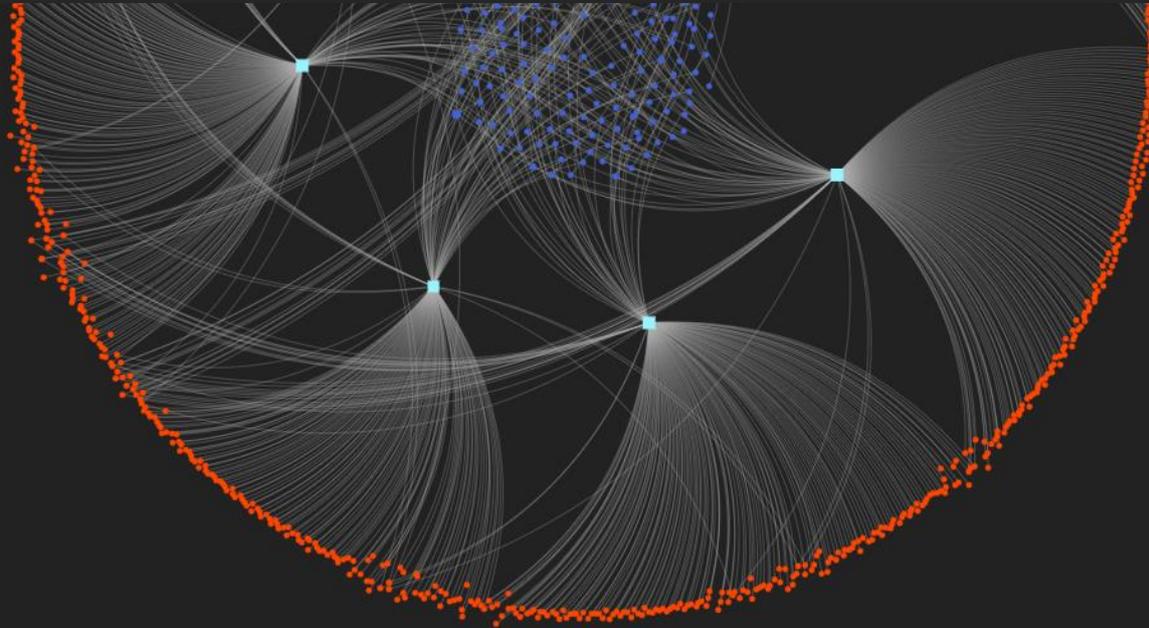


miRNet 2.0 Tutorial 2

Transcription factor-microRNA feed-forward loop analysis



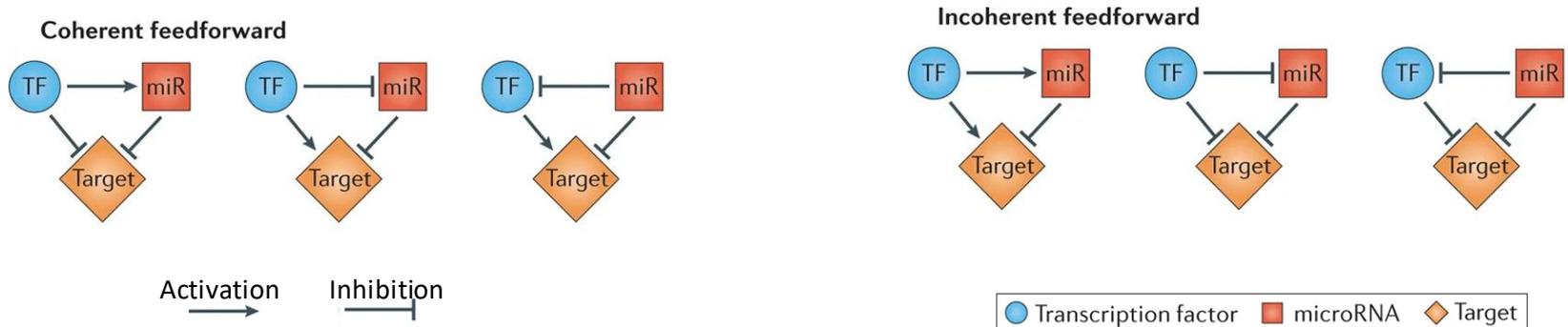
Computer and Browser Requirements

- A modern web browser with JavaScript enabled
 - Supported browsers include Chrome, Safari, Firefox, and Internet Explorer 9+
- For best performance and visualization, use:
 - Latest version of Google Chrome
 - A computer with at least 4GB of physical RAM
 - A 15-inch screen or bigger (larger is better)

Background

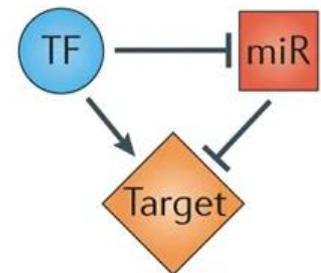
What is TF-miRNA feed-forward loop (FFL)?

- In TF-miRNA feed-forward loops, TF and miRNA **co-regulate** the target genes
 - **Coherent feed-forward loops:** the TF and miRNA have the *same* effects on their common targets
 - **Incoherent feed-forward loops:** the TF and miRNA carry out *opposing (buffering)* effects, which precisely fine-tune gene expressions to minimize noise and maintain stability (*Bracken et al., Nat Rev Genet. 2016*)



Goal for This Tutorial

- How to perform transcription factor (TF)-miRNA feed-forward loop analysis



Choose a Module



2.0 | miRNet -- an integrated platform linking miRNAs, targets & functions

Home Gallery FAQs Tutorials Resources APIs Updates About Support

Expression table miRNAs miR-SNPs Genes ncRNAs Transcription factors Xeno-miRs Xeno-miR explore Epigenetic modifiers Diseases Small compounds RT-qPCR data

Multiple query types

Input types:

- Upload a query list
- Select from database
- Upload a data table
- Mixed input types

News & Updates

- Added two layout algorithms (Concentric and Backbone) for multipartite networks (02/24/2020); **NEW**
- Code cleaning and refactoring (02/14/2020); **NEW**

Go to the miRNet home page (www.mirnet.ca) and click "**Multiple query types**" to enter the module

Input 1: Choose Molecular Types

The screenshot displays the miRNet 2.0 web application interface. At the top, the text reads "miRNet -- an integrated platform linking miRNAs, targets & functions". A navigation bar includes links for Home, FAQs, Tutorials, Resources, Gallery, APIs, Updates, About, and Support. The main content area features a central circular menu with various data types: Expression table, miRNAs, miR-SNPs, RT-qPCR data, Small compounds, Diseases, Xeno-miR explore, and Xeno-miRs. A "Choose Items" dialog box is open in the center, prompting the user to select multiple items. The dialog includes dropdown menus for "Organism" (set to "H. sapiens (human)") and "Tissue (human)" (set to "----Not specified----"). A list of molecular types is shown with checkboxes: miRNAs (checked), lncRNAs, Pseudogene, Transcription factors (checked), Small compounds, Genes, circRNAs, sncRNAs, Diseases, and Epigenetic modifiers. "OK" and "Cancel" buttons are at the bottom of the dialog. On the right side, an "Input types:" section lists options: Upload a query list, Select from database, Upload a data table, and Mixed input types. At the bottom of the page, there is a "News & Updates" section with two entries: "Updated FAQs and the miRNet Overview tutorial (03/13/2020); NEW" and "Added the 'Module Explorer' panel for better network visual analytics (03/08/2020); NEW". The footer text reads "Xia Lab @ McGill (last updated 2020-03-13)".

Select "miRNAs" and "Transcription factors", then click "OK" to proceed

Input 2: Upload miRNA Data

2.0

Click tab to TF upload view after submitting miRNA data

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OK
A total of 4 unique items were uploaded.

Upload about Support

Upload

Please use the tabs below to prepare your input for: **H. sapiens (human)**. Make sure to click **Submit** button before switching to the next tab.

miRNAs TFs

ID type: miRBase ID

Target type: Genes

miRNA list (one entry per line):
hsa-mir-302a-3p
hsa-mir-302d-3p
hsa-mir-367-3p
hsa-mir-373-3p

Submit

Try example

Data uploaded: mirna tf

Include PPI: Database selection

Proceed

Xia Lab @ McGill (last updated 2020-03-13)

Example data: [Qin,et al.,\(2020\)](#)

Input 3: Upload TF Data

miRNet -- an integrated platform linking miRNAs, targets & functions

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Upload

Please use the tabs below to prepare your input for: **H. sapiens (human)**. Make sure to click **Submit** button before switching to the next tab.

miRNAs **TFs**

ID type: Official gene symbol

Target type: miRNA Gene ENCODE

TF list (one entry per line): JUN, ERG, SOX9, NR2F2

Submit Try example

Data uploaded: mirna tf Include PPI: Database selection

Proceed

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After data uploaded for both miRNAs and TFs, click "**Proceed**"

Network Creation

2.0 | miRNet -- an integrated platform linking miRNAs, targets & functions

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Upload Network Builder

Interaction Tables

The pair-wise interaction tables together with the supporting information are listed below. You can click the corresponding link to download (**Download**) or browse (**View**) the interaction tables, or click the **Proceed** button at the bottom to directly explore the results in a network context.

mir2gene [miRNA:4, gene: 866]	± Download	+ View
tf2mir [TF:3, miRNA: 28]	± Download	+ View
tf2gene [TF:4, gene: 131]	± Download	+ View

You can also "Download" or "View" pair-wise interaction tables

Networks Created

In some cases, multiple isolated networks will be generated, with a big 'continent' containing most of queries, and several small 'islands' containing one or a few queries. These networks will be available for visual analysis in the next step.

Networks	Nodes	Edges	Queries	
mirnet1	1017	1991	TF: 3; Gene: 1; miRNA: 4;	± Download

Network Tools: ⓘ

- Degree Filter
- Betweenness Filter
- Shortest Path Filter
- Manual Batch Filter
- Minimum Network
- Steiner Forest Network

Update Network

Reset Network

Previous Downloads Proceed

Xia Lab @ McGill (last updated 2020-03-13)

Click "Proceed" to the Network Viewer

Network Viewer

Change layout to Circular Bipartite/Tripartite

The screenshot displays the Network Viewer interface with a circular bipartite network layout. The network consists of three layers of nodes: a central zone of blue nodes (miRNAs), a middle layer of red nodes (target genes), and an outer layer of green nodes (TFs). The interface includes a top navigation bar, a control panel on the left for node coloring and exploration, a central network visualization, and a right-hand panel for functional enrichment analysis.

Functional enrichment analysis results:

Pathway	Hits	Pval	Color
Pathways in cancer	39	0.000009	
Chronic myeloid leukemia	17	0.000009	
p53 signaling pathway	13	0.00133	
Influenza A	17	0.00133	
Transcriptional misregulation in cancer	7	0.00133	
Measles	16	0.00181	
HTLV-1 infection	24	0.00197	
ErbB signaling pathway	14	0.0027	
Prostate cancer	14	0.0027	
Cell cycle	17	0.00358	
Epstein-Barr virus infection	14	0.00363	
Non-small cell lung cancer	10	0.00443	
mTOR signaling pathway	9	0.00606	
Glioma	11	0.00606	
Jak-STAT signaling pathway	14	0.00655	
Acute myeloid leukemia	10	0.00723	
Melanoma	11	0.00745	
Pancreatic cancer	11	0.008	
Colorectal cancer	9	0.00816	

A TF-miRNA-gene network. The blue nodes (central zone) represent miRNAs, red nodes (middle layer) represent target genes and green nodes (outer layer) represent TFs.

Subnetwork Extraction

1) Choose "Node-neighbours"

The screenshot displays the Network Builder interface with a network graph. The graph shows a dense network of nodes and edges. Three callout boxes provide instructions: 1) '1) Choose "Node-neighbours"' points to the 'Scope' dropdown menu. 2) '2) Double click on node of interest' points to a yellow node labeled 'NR2F2'. 3) '3) Click "Extract" icon' points to a magnifying glass icon in the left sidebar.

Node Explorer

ID	Degree	Betweenness	Status
<input type="checkbox"/> hsa-mir-373-3p	531	180324.5	*
<input type="checkbox"/> hsa-mir-302a-3p	465	116436.3	*
<input type="checkbox"/> hsa-mir-302d-3p	460	110328.9	*
<input type="checkbox"/> hsa-mir-367-3p	331	266505.3	*
<input type="checkbox"/> JUN	97	71560.33	*
<input type="checkbox"/> NR2F2	65	55919.59	*
<input type="checkbox"/> SOX9	43	26606.43	*
<input type="checkbox"/> REST	5	7272.357	
<input type="checkbox"/> SUZ12	5	10682.83	
<input type="checkbox"/> FOXJ2	5	7272.357	
<input type="checkbox"/> CREB1	4	7272.357	
<input type="checkbox"/> GATA6	4	9547.157	
<input type="checkbox"/> MKNK2	4	9547.157	
<input type="checkbox"/> IPP	4	9547.157	
<input type="checkbox"/> IRF2	4	4.5956	
<input type="checkbox"/> MAP1B	4	9547.157	
<input type="checkbox"/> GCNT3	4	9547.157	
<input type="checkbox"/> ZNF264	4	9547.157	
<input type="checkbox"/> ZBTB33	4	4.5956	
<input type="checkbox"/> KLHDC10	4	9547.157	

Function Explorer

Query: All genes
Algorithm: Hypergeometric test
Database: KEGG

Name	Hits	Pval	Color
Pathways in cancer	39	0.000009	
Chronic myeloid leukemia	17	0.000009	
p53 signaling pathway	13	0.00133	
Influenza A	17	0.00133	
Transcriptional misregulation i	7	0.00133	
Measles	16	0.00181	
HTLV-1 infection	24	0.00197	
ErbB signaling pathway	14	0.0027	
Prostate cancer	14	0.0027	
Cell cycle	17	0.00358	
Epstein-Barr virus infection	14	0.00363	
Non-small cell lung cancer	10	0.00443	
mTOR signaling pathway	9	0.00606	
Glioma	11	0.00606	
Jak-STAT signaling pathway	14	0.00655	
Acute myeloid leukemia	10	0.00723	
Melanoma	11	0.00745	
Pancreatic cancer	11	0.008	
Colorectal cancer	9	0.00816	

Module Explorer

Algorithm: InfoMap

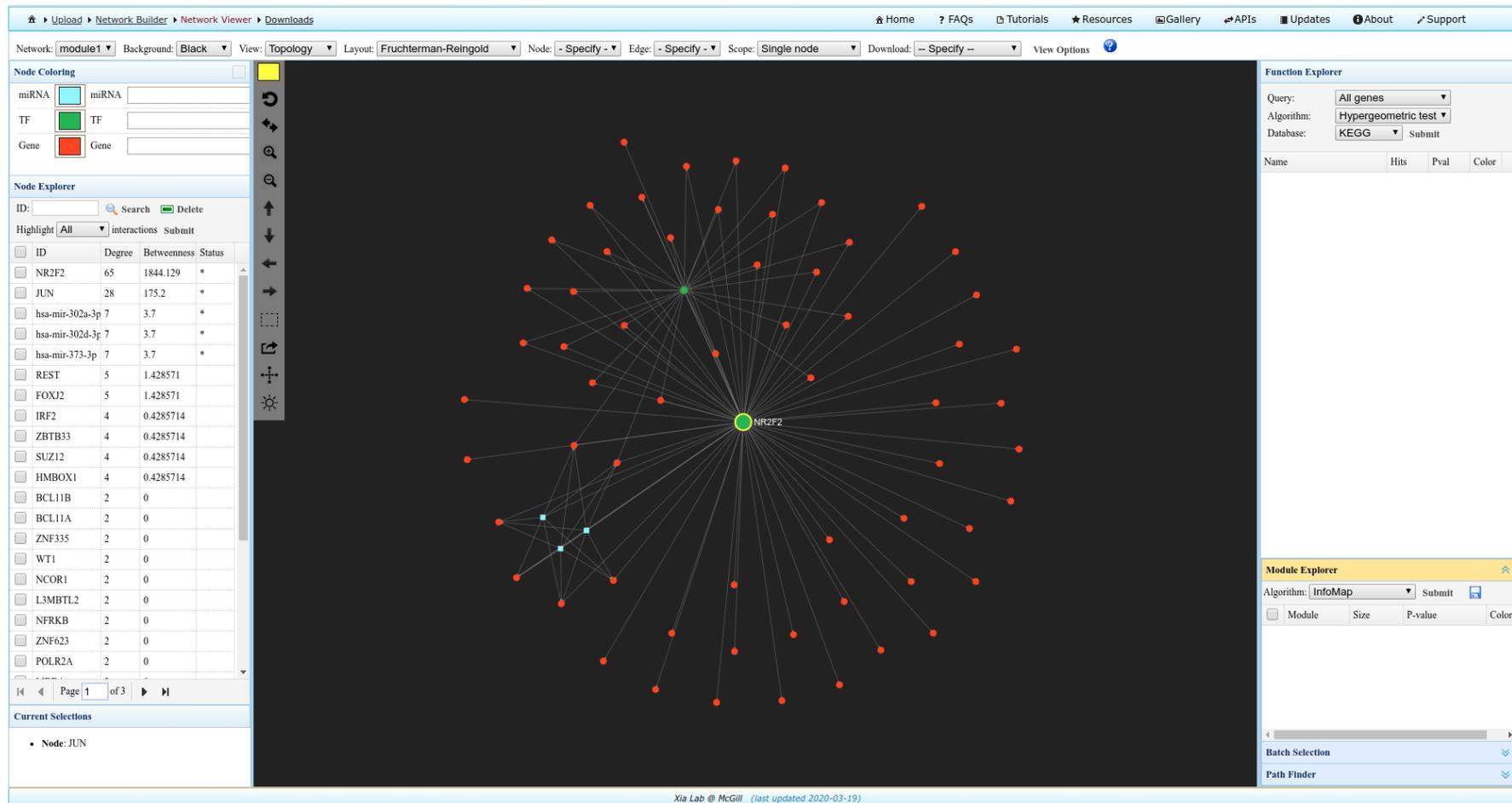
Module	Size	P-value	Color
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Current Selections: Node: NR2F2

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A few TFs and miRNAs regulate a large number of target genes. You can extract a subnetwork to further investigate.

TF-miRNA Feed-forward Loops



An example subnetwork containing TF-miRNA feed-forward loops (FFL). *NR2F2* is a TF-coding gene, which activates gene expression; whereas the miRNAs (*hsa-miR-302a-3p*, *hsa-miR-302d-3p*, *hsa-miR-373-3p*) repress gene expression, suggesting that the FFL might have a significant role in pathogenesis of testicular germ cell tumors (example data: Qin,et al.,2020).

==END==