

A network diagram on a dark background showing interactions between miRNAs. Nodes are represented by small squares (light blue) and circles (orange). The nodes are interconnected by thin grey lines. Four specific nodes are labeled with their IDs: hsa-mir-369-5p (top right), hsa-mir-29b-3p (center), hsa-mir-23a-3p (bottom right), and hsa-mir-190a-5p (bottom left).

miRNet Tutorial Network Analysis

Goal for this tutorial

- Understand the main features of network viewer;
- Perform functional enrichment analysis according to the result network.

Understanding the Network Viewer

Background: Black View: Default Layout: Default Layout Scope: -- Specify -- Download: -- Specify --

Node Explorer

Node ID: Search

Information for nodes and they can be sorted by degree, betweenness and status (only for expression data).

<input type="checkbox"/>	BTG1	105	108970	1.9003
<input type="checkbox"/>	FHL2	85	82136	-1.5401
<input type="checkbox"/>	SLC29A1	83	67482	-1.3441
<input type="checkbox"/>	POLA2	73	84597	-1.6136
<input type="checkbox"/>	BAK1	73	62451	1.7522
<input type="checkbox"/>	EFNA1	66	68793	1.3762
<input type="checkbox"/>	CSE1L	65	52367	-1.1473
<input type="checkbox"/>	MTHFD1	64	57698	-1.5063
<input type="checkbox"/>	TFPI	64	71220	1.901
<input type="checkbox"/>	IGFBP4	60	45883	-1.4207
<input type="checkbox"/>	CDT1	55	67225	-1.0018
<input type="checkbox"/>	MCM7	50	41668	-2.1023
<input type="checkbox"/>	H2AFX	42	47479	-1.6104

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Current Selections

- Node: BTG2
- Link: [Entrez](#)
- Degree: 275
- Betweenness: 383380

Functional enrichment analysis

Function Explorer

Query: All genes
Algorithm: Hypergeometric test
Database: KEGG Submit

Name	Hits	Pval	Color
DNA replication	5	0.0000769	
Cell cycle	7	0.0000769	
Base excision repair	3	0.0311	
Hepatitis B	4	0.209	
Pyrimidine metabolism	3	0.464	
Viral carcinogenesis	4	0.464	
Bladder cancer	2	0.508	
HTLV-I infection	4	0.754	
p53 signaling pathway	2	0.808	
MicroRNAs in cancer	4	0.808	
Glioma	2	0.808	
Melanoma	2	0.808	
Chronic myeloid leukemia	2	0.808	
DNA replication	2	0.808	

View Options Path Finder Batch Hi

Node Effect

Set size for: All nodes
Increase ++ Submit
Note: you can increase size of any particular node by repeatedly clicking on it

Edge Effect

Edge shape: Line
Edge width: Default Submit

The detail for selected node

In the network viewer, the square nodes are miRNAs and the round nodes are the targets. The size of the node indicate its significance in the network.

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

3. Choose "Highlighted nodes", algorithm (hypergeometric test or empirical test) and pathway (KEGG, GO and Reactome). Click "submit".

1. Locate the node by clicking IDs, the size of nodes will be increased and zoom in. (they are sorted by the betweenness).

2. Double click nodes in the viewer and make sure they change color. You can drag to separate nodes.

Node Explorer

ID	Degree	Betweenness	Status
<input checked="" type="checkbox"/> BTG2	275	383380	1.2725
<input checked="" type="checkbox"/> CCND1	203	238610	-1.1596
<input checked="" type="checkbox"/> SLC7A5	153	181220	-1.5115
<input checked="" type="checkbox"/> CDKN1A	113	129780	1.416
<input checked="" type="checkbox"/> BTG1	105	108970	1.9003

Function Explorer

Query: Highlighted genes
Algorithm: Hypergeometric test
Database: KEGG

Name	Hits	Pval	Color
RNA degradation	2	0.0564	
p53 signaling pathway	2	0.0564	
Glioma	2	0.0564	
Melanoma	2	0.0564	
Bladder cancer	2	0.0564	
Chronic myeloid leukemia	2	0.0564	
Prostate cancer	2	0.0645	
Cell cycle	2	0.109	
FoxO signaling pathway	2	0.113	
Jak-STAT signaling pathway	2	0.117	
Oxytocin signaling pathway	2	0.117	
Hepatitis B	2	0.117	
Viral carcinogenesis	2	0.166	

Node Effect

Set size for: All nodes
Increase ++ Submit
Note: you can increase size of any particular node by repeatedly clicking on it

Edge Effect

Edge shape: Line
Edge width: Default Submit

Current Selections

- Node: hsa-let-7g-5p
- Link: mirBase
- Degree: 3
- Betweenness: 1288.5

Enrichment analysis by selecting pathway

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

ID	Degree	Betweenness	Status
<input type="checkbox"/> BTG2	275	383380	1.2725
<input type="checkbox"/> CCND1	203	238610	-1.1596
<input type="checkbox"/> SLC7A5	153	181220	-1.5115
<input type="checkbox"/> CDKN1A	113	129780	1.416
<input type="checkbox"/> BTG1	105	108970	1.9003
<input type="checkbox"/> FHL2	85	82136	-1.5401
<input type="checkbox"/> SLC29A1	83	67482	-1.3441
<input type="checkbox"/> POLA2	73	84597	-1.6136
<input type="checkbox"/> BAK1	73	62451	1.7522
<input type="checkbox"/> EFNA1	66	68793	1.3762
<input type="checkbox"/> CSE1L	65	52367	-1.1473
<input type="checkbox"/> MTHFD1	64	57698	-1.5063
<input type="checkbox"/> TFPI	64	71220	1.901
<input type="checkbox"/> IGFBP4	60	45883	-1.4207
<input type="checkbox"/> CDT1	55	67225	-1.0018
<input type="checkbox"/> MCM7	50	41668	-2.1023

Current Selections

- Bladder cancer
- CCND1
- CDKN1A

The genes in this pathway

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

Function Explorer

Query: All genes
Algorithm: Hypergeometric test
Database: KEGG Submit

Name	Hits	Pval	Color
DNA replication	5	0.0000769	
Cell cycle	7	0.0000769	
Base excision repair	3	0.0311	Blue
Hepatitis B	4	0.209	
Pyrimidine metabolism	3	0.464	Yellow
Viral carcinogenesis	4	0.464	
Bladder cancer	2	0.508	Green
HTLV/Infection	1	0.54	

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

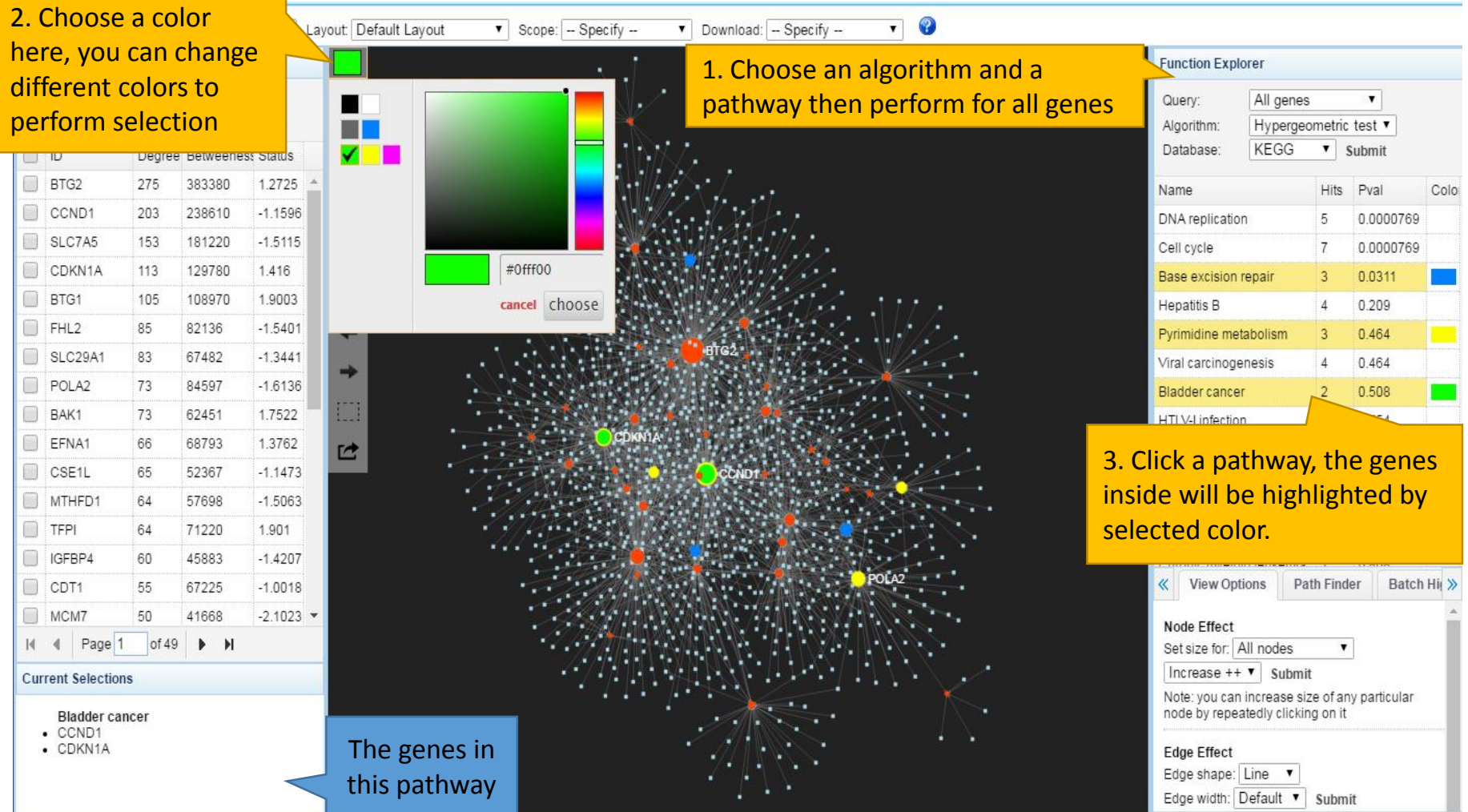
View Options Path Finder Batch Hi >>

Node Effect

Set size for: All nodes
Increase ++ Submit
Note: you can increase size of any particular node by repeatedly clicking on it

Edge Effect

Edge shape: Line
Edge width: Default Submit



Enrichment analysis by associated targets

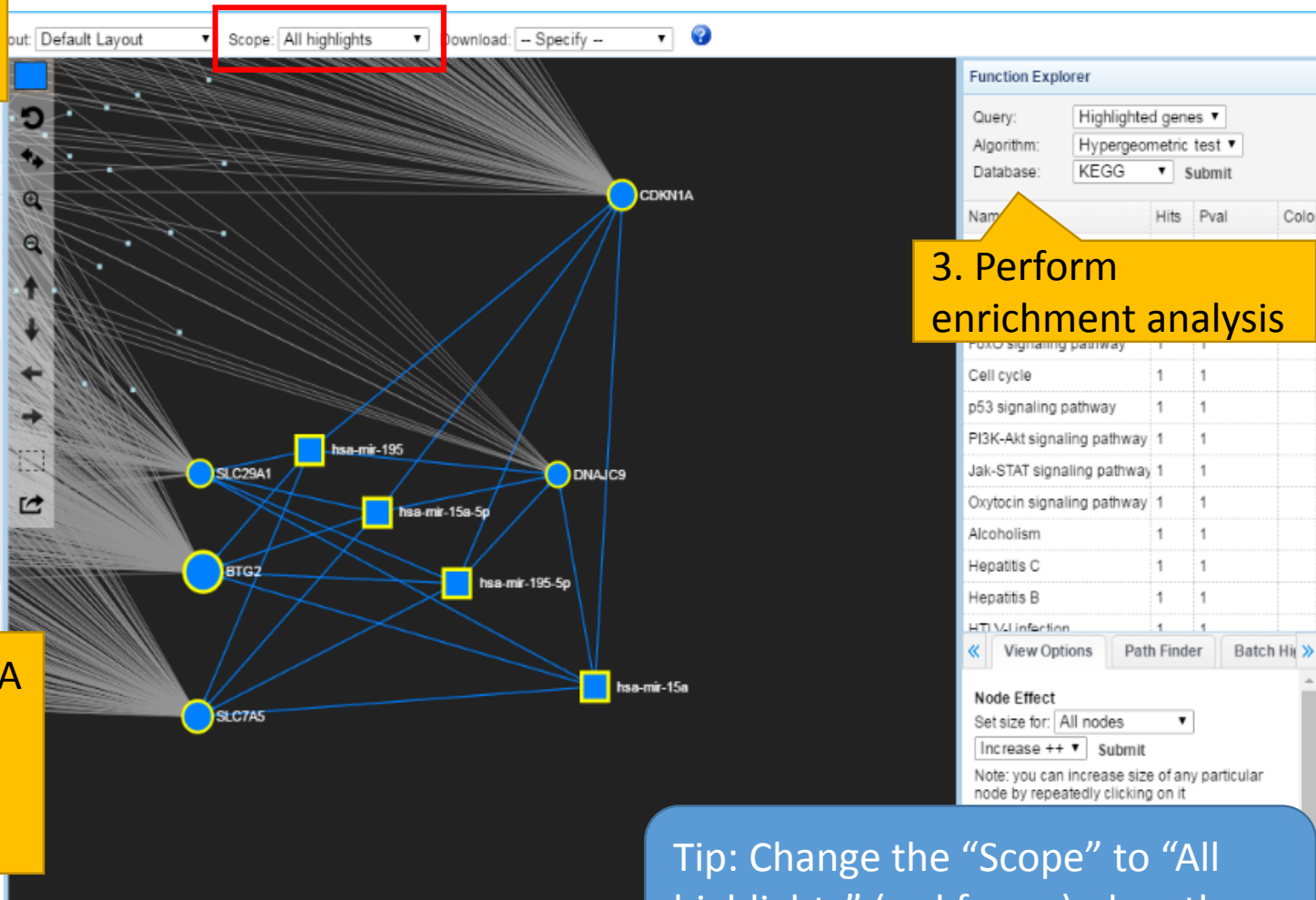
2. All – Selecting all associated targets
Shared – Selecting sharing targets

Node ID: Search

Highlight miRNA targets: **All** Submit
All
Shared

ID	Degr	Score	Status
<input type="checkbox"/> PMP22	11	12047	1.2851
<input type="checkbox"/> MYBL2	10	22.5	-2.9243
<input type="checkbox"/> ENC1	9	7908.7	1.1646
<input type="checkbox"/> BARD1	6	7285	-1.874
<input type="checkbox"/> hsa-mir-15b	6	13706	
<input type="checkbox"/> hsa-mir-15b-5p	6	13706	
<input checked="" type="checkbox"/> hsa-mir-195-5p	5	7266.1	
<input type="checkbox"/> hsa-mir-6077	5	22848	
<input checked="" type="checkbox"/> hsa-mir-15a-5p	5	7266.1	
<input type="checkbox"/> hsa-mir-574-5p	5	15739	
<input checked="" type="checkbox"/> hsa-mir-15a	5	7266.1	
<input checked="" type="checkbox"/> hsa-mir-195	5	7266.1	
<input type="checkbox"/> hsa-mir-10b-3p	4	1924.2	
<input type="checkbox"/> hsa-mir-10b-5p	4	4507.3	

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



3. Perform enrichment analysis

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

Dragging nodes-Current highlights

Background: Black View: Default Layout: Default Layout Scope: Current highlights Download: -- Specify --

Node Explorer

Node ID: Search

Highlight miRNA targets All Submit

ID	Degree	Betweenness	Status
<input type="checkbox"/> BTG2	275	383380	1.2725
<input type="checkbox"/> CCND1	203	238610	-1.1596
<input type="checkbox"/> SLC7A5	153	181220	-1.5115
<input type="checkbox"/> CDKN1A	113	129780	1.416
<input type="checkbox"/> BTG1	105	108970	1.9003
<input type="checkbox"/> FHL2	85	82136	-1.5401
<input type="checkbox"/> SLC29A1	83	67482	-1.3441
<input type="checkbox"/> POLA2	73	84597	-1.6136
<input type="checkbox"/> BAK1	73	62451	1.7522
<input type="checkbox"/> EFNA1	66	68793	1.3762
<input type="checkbox"/> CSE1L	65	52367	-1.1473
<input type="checkbox"/> MTHFD1	64	57698	-1.5063
<input type="checkbox"/> TFPI	64	71220	1.901
<input type="checkbox"/> IGFBP4	60	45883	-1.4207
<input type="checkbox"/> CDT1	55	67225	-1.0018
<input type="checkbox"/> MCM7	50	41668	-2.1023

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Current Selections

- Node: CCND1
- Link: [Entrez](#)
- Degree: 203
- Betweenness: 238610

Function Explorer

Query: All genes

Algorithm: Hypergeometric test

Database: KEGG Submit

Name	Hits	Pval	Color
DNA replication	5	0.0000769	
Cell cycle	7	0.0000769	
Base excision repair	3	0.0311	Blue
Hepatitis B	4	0.209	
Pyrimidine metabolism	3	0.464	Yellow
Viral carcinogenesis	4	0.464	
Bladder cancer	2	0.508	Green
HT infection pathway	4	0.754	
	2	0.808	

Node Effect

Set size for: All nodes

Increase ++ Submit

Note: you can increase size of any particular node by repeatedly clicking on it

Edge Effect

Edge shape: Line

Edge width: Default Submit

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

Background: Black View: Default Layout: Default Layout Scope: All highlights Download: -- Specify --

1. Choose "All highlights"

2. Drag all highlighted nodes aside and zoom in.

Node Explorer

Node ID: Search

Highlight miRNA targets: All Submit

ID	Degree	Betweenness	Status
<input type="checkbox"/> BTG2	275	383380	1.2725
<input type="checkbox"/> CCND1	203	238610	-1.1596
<input type="checkbox"/> SLC7A5	153	181220	-1.5115
<input type="checkbox"/> CDKN1A	113	129780	1.416
<input type="checkbox"/> BTG1	105	108970	1.9003
<input type="checkbox"/> FHL2	85	82136	-1.5401
<input type="checkbox"/> SLC29A1	83	67482	-1.3441
<input type="checkbox"/> POLA2	73	84597	-1.6136
<input type="checkbox"/> BAK1	73	62451	1.7522
<input type="checkbox"/> EFNA1	66	68793	1.3762
<input type="checkbox"/> CSE1L	65	52367	-1.1473
<input type="checkbox"/> MTHFD1	64	57698	-1.5063
<input type="checkbox"/> TFPI	64	71220	1.901
<input type="checkbox"/> IGFBP4	60	45883	-1.4207
<input type="checkbox"/> CDT1	55	67225	-1.0018
<input type="checkbox"/> MCM7	50	41668	-2.1023
<input type="checkbox"/> H2AFX	42	47479	-1.6104

Current Selections

- Bladder cancer
 - CCND1
 - CDKN1A

Function Explorer

Query: All genes

Algorithm: Hypergeometric test

Database: KEGG Submit

Name	Hits	Pval	Color
DNA replication	5	0.000769	
Cell cycle	7	0.000769	
Base excision repair	3	0.0311	Blue
Hepatitis B	4	0.209	
Pyrimidine metabolism	3	0.464	Yellow
Viral carcinogenesis	4	0.464	
Bladder cancer	2	0.508	Green
HTLV-I infection	4	0.754	
p53 signaling pathway	2	0.808	
MicroRNAs in cancer	4	0.808	
Glioma	2	0.808	
Melanoma	2	0.808	
Chronic myeloid leukemia	2	0.808	
RNA degradation	2	0.827	

Node Effect

Set size for: All nodes

Increase ++ Submit

Note: you can increase size of any particular node by repeatedly clicking on it

Edge Effect

Edge shape: Line

Edge width: Default Submit

Expression analysis in network

The screenshot displays a network analysis software interface. The central part is a network graph with nodes of various sizes and colors (red, green, grey) connected by edges. The interface includes several panels:

- Node Explorer:** A table with columns for gene IDs and expression values. It shows a list of genes with checkboxes and a 'Current Selections' section below it.
- Function Explorer:** A panel on the right with a query dropdown set to 'Highlighted genes', an algorithm dropdown set to 'Hypergeometric test', and a database dropdown set to 'KEGG'. Below this is a table of pathway results.
- View Options:** A panel at the bottom right with options for 'View Options', 'Path Finder', and 'Batch Hit'. It includes a 'Set size for' dropdown set to 'All nodes', an 'Increase ++' button, and a 'Submit' button. Below this are 'Edge Effect' options for 'Edge shape' (set to 'Line') and 'Edge width' (set to 'Default'), each with a 'Submit' button.

Four yellow callout boxes provide instructions:

1. Choose "Expression" here. In the network, red nodes are upregulated and green nodes are downregulated
2. Locate the nodes by clicking each ID or you can skip this step and directly highlight nodes in the network.
3. Double click node and make sure the color has been changed.
4. Choose "Highlighted genes" and pathway. Click "submit"

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.

Background: Black View: Default Layout: Default Layout Scope: Node-neighbours Download: -- Specify --

Node Explorer

No	ID	Degree	Betweenness	Status
<input type="checkbox"/>	BTG2	221	252550	1.2725
<input type="checkbox"/>	CCND1	157	152620	-1.1596
<input type="checkbox"/>	SLC7A5	121	117090	-1.5115
<input type="checkbox"/>	CDKN1A	87	82222	1.416
<input type="checkbox"/>	BTG1	81	72045	1.9003
<input type="checkbox"/>	POLA2	58	55530	-1.6136
<input type="checkbox"/>	FHL2	68	55278	-1.5401
<input type="checkbox"/>	TFPI	53	48783	1.901
<input type="checkbox"/>	SLC29A1	68	47401	-1.3441
<input type="checkbox"/>	EFNA1	54	45821	1.3762
<input type="checkbox"/>	CDT1	45	43079	-1.0018
<input type="checkbox"/>	BAK1	56	40929	1.7522
<input type="checkbox"/>	MTHFD1	55	40201	-1.5063
<input type="checkbox"/>	CSE1L	55	35628	-1.1473
<input type="checkbox"/>	FEN1	30	32802	-2.1068
<input type="checkbox"/>	H2AFX	34	31430	-1.6104
<input type="checkbox"/>	IGFBP4	51	30794	-1.4207
<input type="checkbox"/>	GREB1	32	26327	-2.1884

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Current Selections

Manual Selection

- hsa-let-7d-5p
- hsa-let-7f-5p
- hsa-mir-18a-5p
- hsa-mir-95-3p
- hsa-mir-96-5p
- hsa-mir-101-2p

Function Explorer

Query: Highlighted genes

Algorithm: Hypergeometric test

Database: Reactome Submit

Name	Hits	Color
Signaling by NOTCH	1	
Signal Transduction	1	

Cyclin D associated events 1 1

G1 Phase 1 1

S Phase 1 1

Cell Cycle Mitotic 1 1

View Options Path Finder Batch Hi

Node Effect

Set size for: All nodes

Increase ++ Submit

Note: you can increase size of any particular node by repeatedly clicking on it

Edge Effect

Edge shape: Line

Edge width: Default Submit

Configuring the general visualization feature

Background: White View: Default Layout: Large Graph Layout Scope: -- Specify -- Download: -- Specify --

Node Explorer

Change to white background

Change different layouts

Download the network in different formats.

Function Explorer

All genes
Hypergeometric test
KEGG Submit

Name	Hits	Pval	Color
Epstein-Barr virus infection	3	0.125	
Cell cycle	2	0.444	
Hepatitis B	2	0.444	
Measles	2	0.444	
Transcriptional misregulation	2	0.527	
Viral carcinogenesis	2	0.571	
Glycolysis / Gluconeogenesis	1	1	
Cysteine and methionine metabolism	1	1	
Pyruvate metabolism	1	1	
Propanoate metabolism	1	1	
Metabolic pathways	1	1	
RNA degradation	1	1	
Spliceosome	1	1	
ErbB signaling pathway	1	1	

View Options Path Finder Batch Hit

Node Effect
Set size for: All nodes
Increase ++ Submit
Note: you can increase size of any particular node by repeatedly clicking on it

Edge Effect
Edge shape: Line
Edge width: Default Submit

Current Selections

- Node: DDX5
- Link: Entrez
- Degree: 2
- Betweenness: 5.7342

View Options

View Options Path Finder Highlight

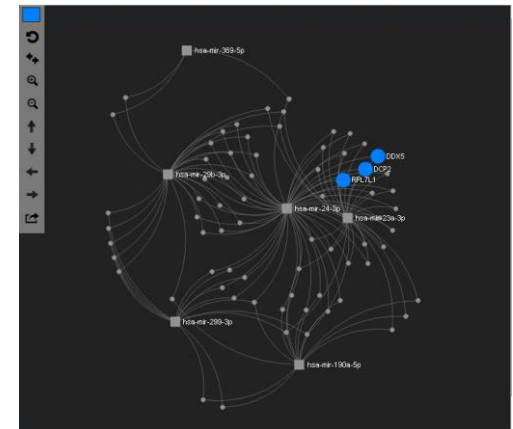
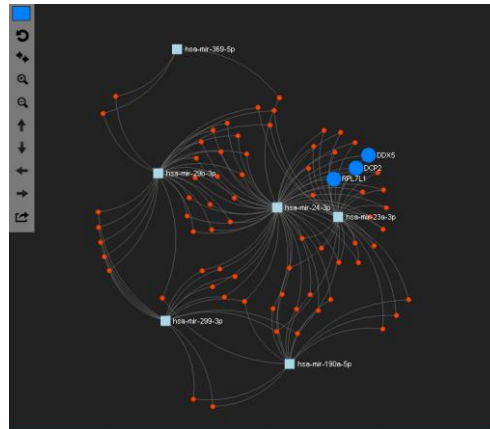
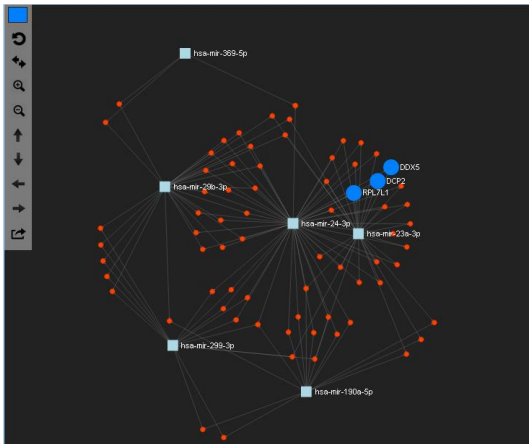
Node Effect
Set size for: Selected Nodes ▾
Increase ++ ▾ Submit
Note: you can increase size of any particular node by repeatedly clicking on it

View Options Path Finder Highlight

Edge Effect
Edge shape: Curve ▾
Edge width: Thick ▾ Submit

View Options Path Finder Highlight

Highlight Effect
Other Nodes: Dim down ▾ Submit
Tip: double clicking to manually highlight



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==