

RWRMTN: a tool for predicting disease-associated microRNAs based on a microRNA-target gene network

Duc-Hau Le^{1,2,*}, Trang T.H. Tran^{1,2}

¹Computational Biomedicine Dept., Vingroup Big Data Institute, No 7, Bang Lang 1 Street, Viet Hung Ward, Long Bien District, Hanoi, Vietnam.

²School of Computer Science and Engineering, Thuyloi University, 175 Tay Son, Dong Da, Hanoi, Vietnam.

*To whom correspondence should be addressed (hauldhut@gmail.com).

Additional file 1

User Manual & Case studies

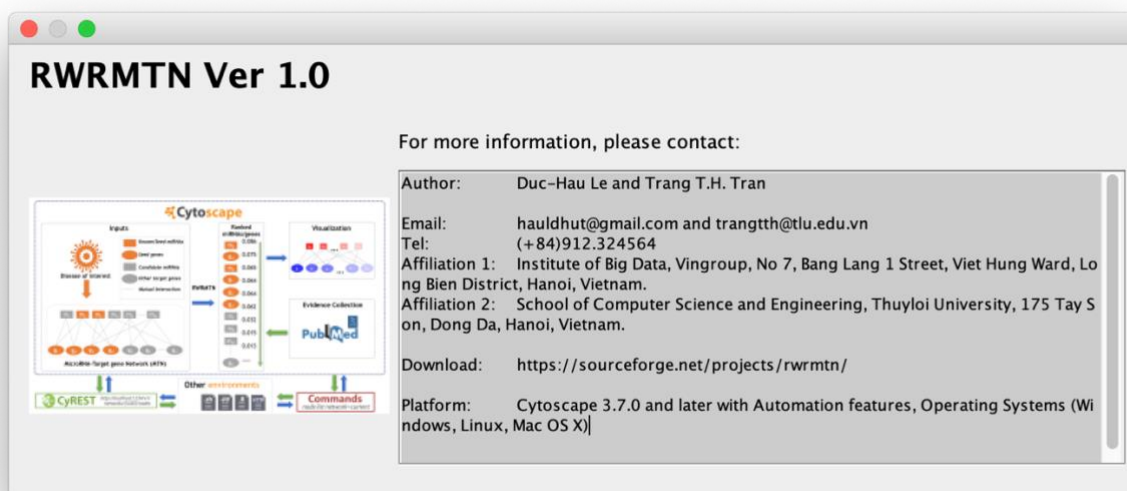


Table of Contents

I.	Setup	3
1.	Install Cytoscape.....	3
2.	Install RWRMTN app.....	3
II.	Overview of RWRMTN	4
III.	Case study: Prediction of breast cancer-associated miRNAs	5
1.	Run RWRMTN using Cytoscape menu and CyREST Command API	6
	Step 1: Load datasets	6
	Step 2: Rank candidate miRNAs	8
	Step 3: Search Evidences.....	12
	Step 4: Visualize	14
2.	Run RWRMTN by calling CyREST API.....	16
	Overview of CyREST APIs.....	16
	Using RWRMTN in a workflow in R environment	19
IV.	Reference	22

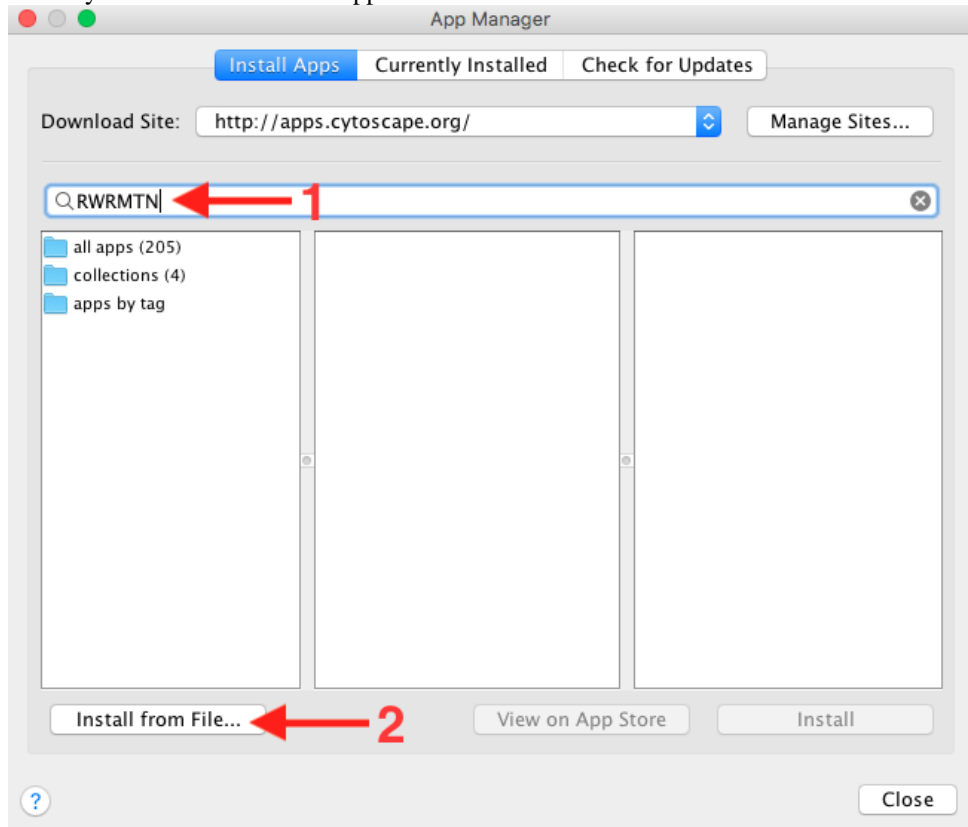
I. Setup

1. Install Cytoscape

- RWRMTN1.0 can only run on **Cytoscape 3.6 (or later)** platform, **which has Automation features**, therefore user should download this version at <http://cytoscape.org/>
- Cytoscape need JRE to run, therefore download JRE version 8.x or later from <http://www.oracle.com/technetwork/java/index.html> and install it.
- Install Cytoscape to the root folder (e.g., /Applications/Cytoscape_v3.6.0).

2. Install RWRMTN app

There are two ways to install RWRMTN app.

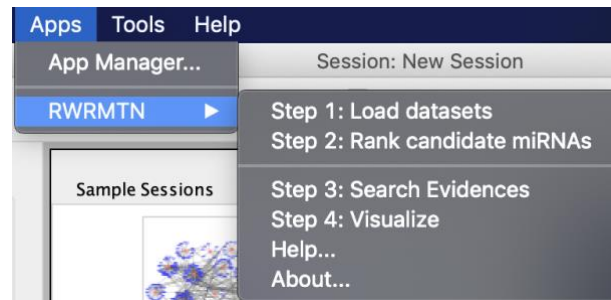


- *Method 1: Automatically install from Cytoscape Appstore:* Select menu **App → AppManager** in Cytoscape. Then type RWRMTN in search box to install directly from Appstore of Cytoscape.
- *Method 2: Manual install:*
 - Download RWRMTN_v1.0.jar file from <https://github.com/hauldhut/RWRMTN>
 - Then, install it by going to **Apps → App Manager**.... After that, choose **Install from file...**, then browse the downloaded RWRMTN_v1.0.jar file.

Note that: RWRMTN_v1.0 can work on Windows, Ubuntu and Mac OS. The following manual was prepared when running RWRMTN on Mac OS.

II. Overview of RWRMTN

After installing, RWRMTN will be automatically loaded in the App menu of Cytoscape



The main tasks (Rank candidate miRNAs, Evidence Search and Visualization) of RWRMTN are completed after four steps:

- **Step 1:** Load data sets (miRNA-target gene interactions and known disease-miRNA associations)
- **Step 2:** Rank candidate miRNAs (including 4 sub-steps)
 - o 1. Select a disease of interest
 - o 2. Input candidate miRNAs to rank
 - o 3. Parameters setting (for advanced users)
 - o 4. Rank
- **Step 3:** Search Evidences
- **Step 4:** Visualize

These steps can be performed, and results of each step can be exposed

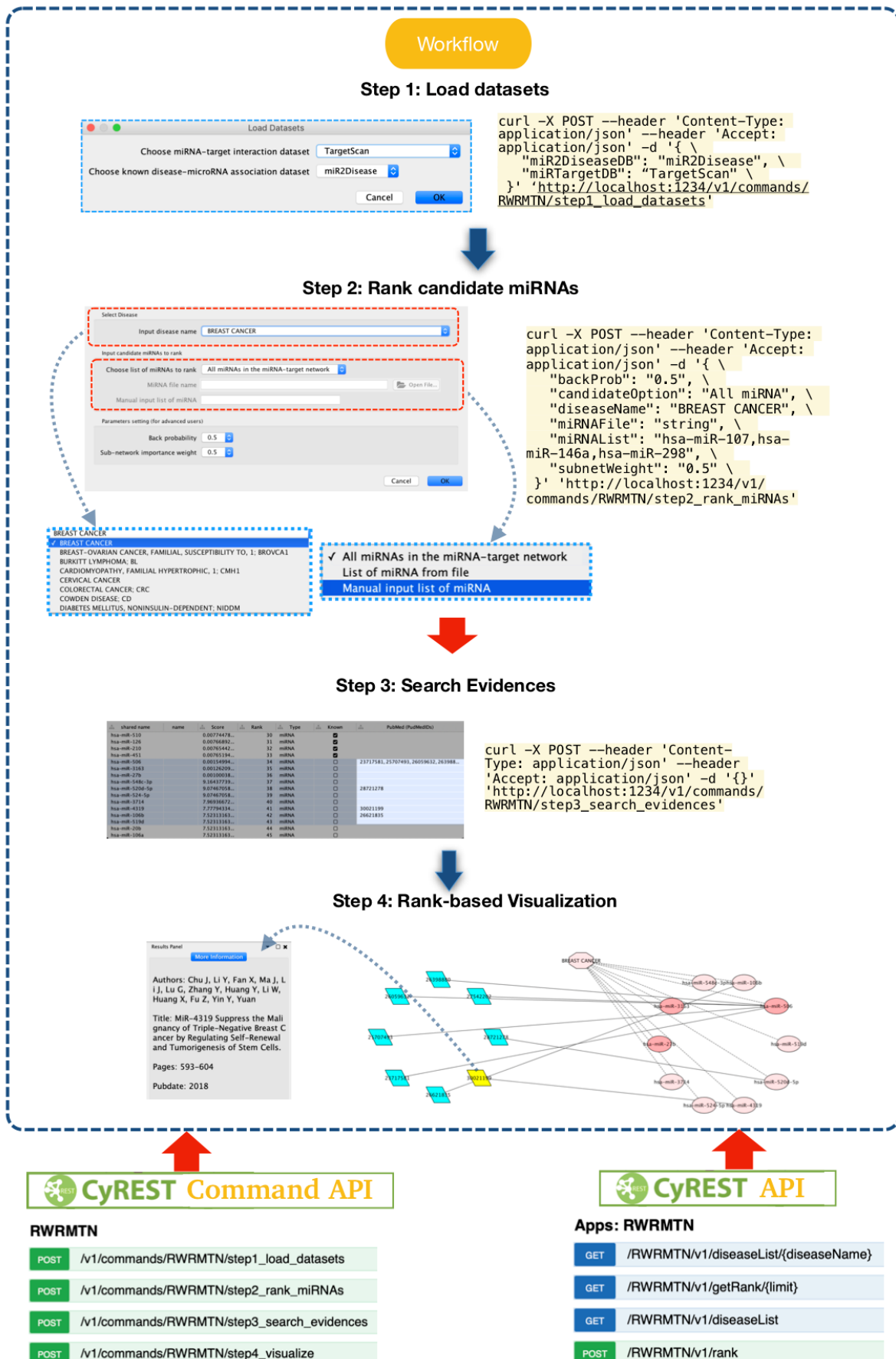
- Using Cytoscape menu

Beside the Cytoscape GUI, new upgraded automation feature of Cytoscape allows functions of Cytoscape and apps called via REST API. Therefore, we can call the functions of Cytoscape and apps in workflows in other environments such as R, Python, etc... Thus, RWRMTN functions can be used by

- CyREST Command API
- CyREST API

III. Case study: Prediction of breast cancer-associated miRNAs

In this section, we demonstrate the use of RWRMTN in predicting novel breast cancer-associated miRNAs by following workflow. The workflow can be done using Cytoscape menu or CyREST Command API. In addition, intermediate results can be exposed from other environments (e.g., R statistics) using CyREST API.



1. Run RWRMTN using Cytoscape menu and CyREST Command API

Step 1: Load datasets

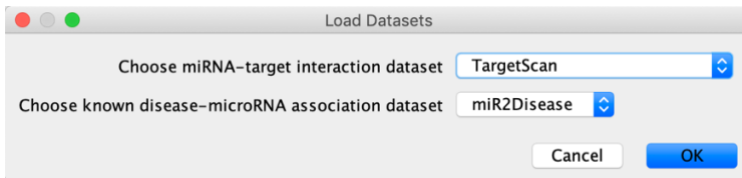
Load datasets for miRNA-target interaction network and known disease-miRNA associations:

- miRNA-target gene interaction dataset (*miRTargetDB*): choose built-in dataset **TargetScan** or **miRWalk** or your own dataset to build miRNA-target interaction network.
- Known disease-miRNA association dataset (*miR2DiseaseDB*): choose built-in dataset **miR2Disease** or **HMDD**.

Step 1 can be performed by two ways:

- **Cytoscape menu: Apps → RWRMTN → Step 1: Load datasets**

Here, a miRNA-target interaction dataset TargetScan (Lewis, et al., 2003) and a known disease-miRNA association dataset miR2Disease (Jiang, et al., 2009) were used.

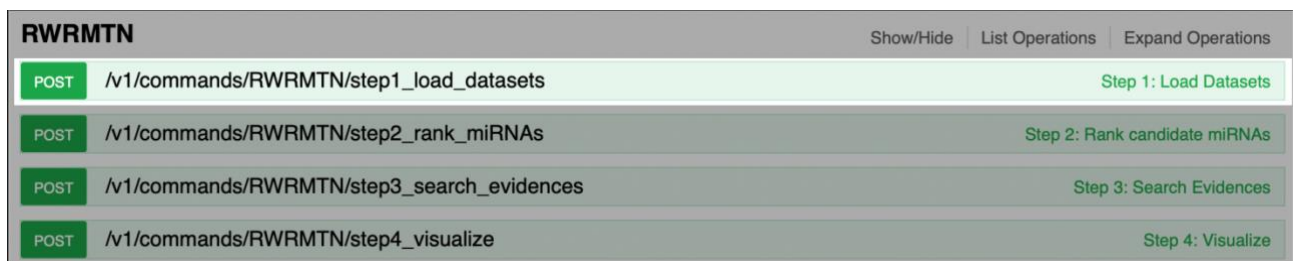


To build miRNA-target interaction network and load known disease-miRNA associations:

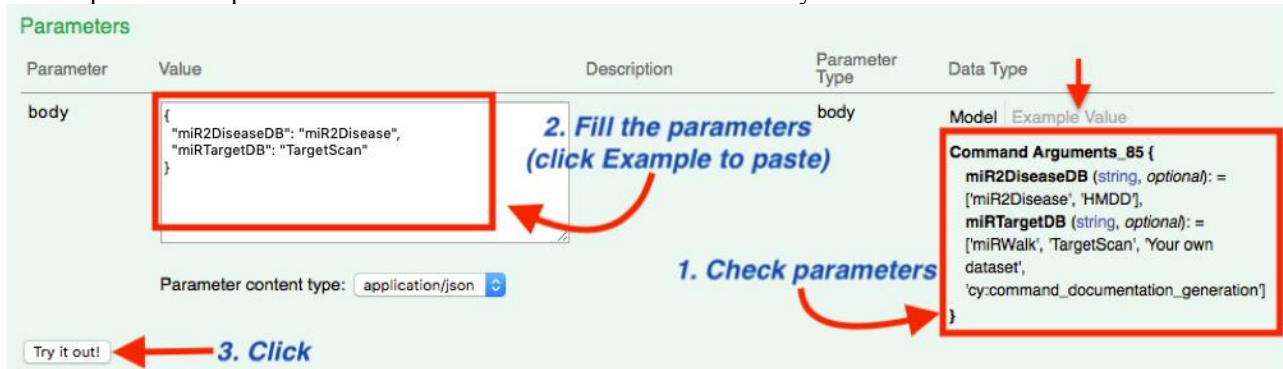
1. Choose miRNA-target interaction dataset.
2. Choose known disease-microRNA association dataset
3. Click **OK** to load datasets.

- **CyREST command API: Help → Automation → CyREST Command API.**

Choose RWRMTN in the SwaggerUI.

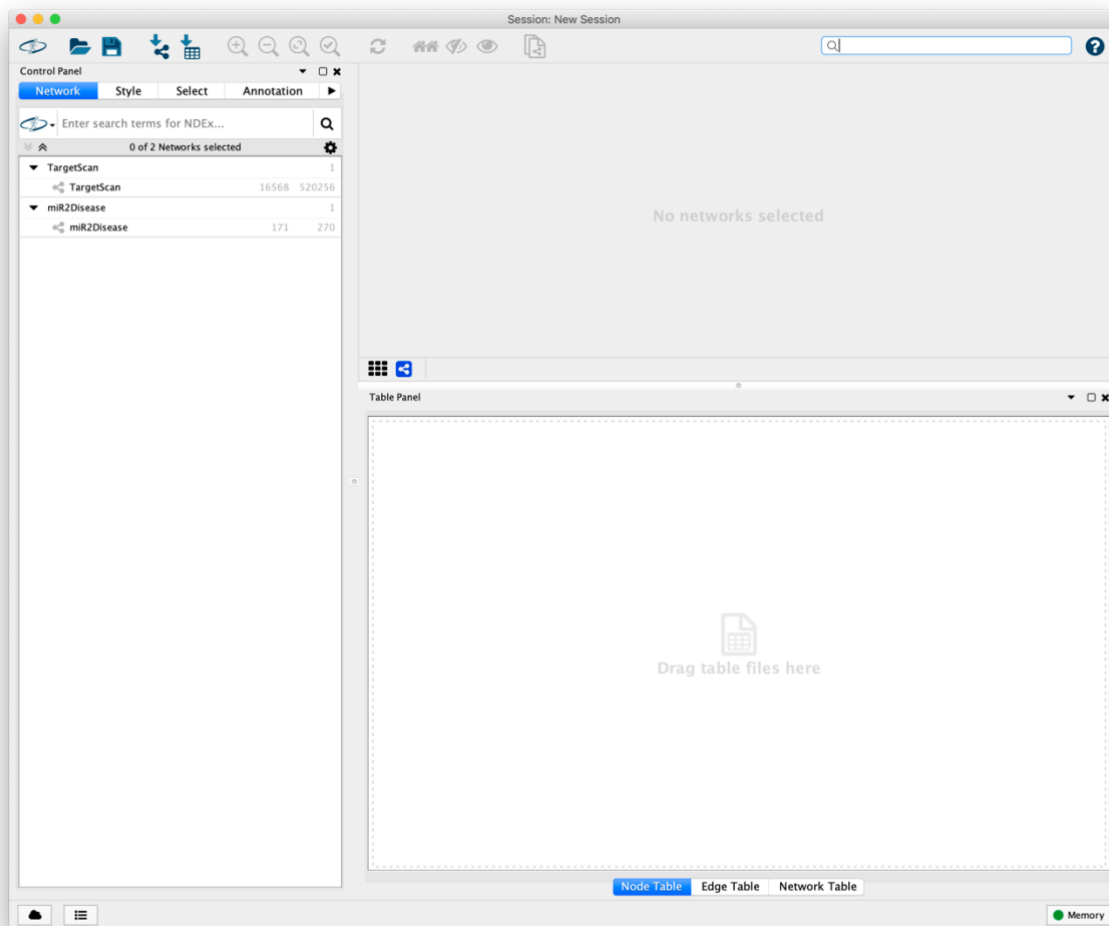


Fill the parameter requirement in the Parameter box and hit the button “Try it out”



(For more details on using automation features (CyREST API and CyREST Command API) of Cytoscape, visit the site: <https://github.com/cytoscape/cytoscape-automation/wiki/App-Developers:-Cytoscape-Command-Best-Practices>)

- This will load corresponding datasets into Network tab of Cytoscape (see following figure).



Note: a miRNA-target interaction dataset *TargetScan* (Lewis, et al., 2003) and a known disease-miRNA association dataset *miR2Disease* (Jiang, et al., 2009) were used.

For CyREST command API, return successful message:

Response Body

```
{
  "data": {
    "message": "Load Heterogeneous Network successfully"
  },
  "errors": []
}
```

Note that:

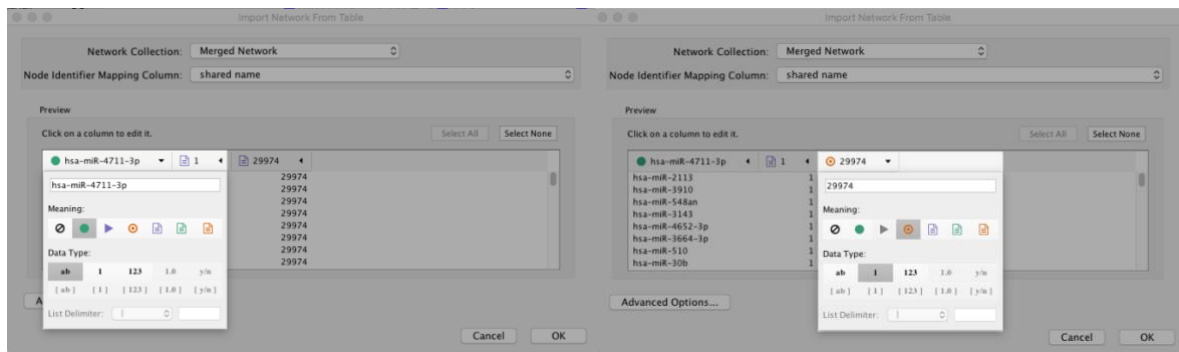
- For miRNA-target datasets: We pre-installed 2databases **miRWalk** (database of experimentally validated miRNA-target interactions) and **TargetScan** (a dataset containing predicted miRNA-target interactions). If you want to use your own dataset, it must follow the following format, and be imported into Cytoscape beforehand.

hsa-let-7a	1	52
hsa-let-7a	1	639
hsa-let-7a	1	836
hsa-let-7a	1	1603
hsa-let-7a	1	3265
hsa-let-7a	1	3690
hsa-let-7a	1	3845
hsa-let-7a	1	4771
hsa-let-7a	1	4893
hsa-let-7a	1	4988

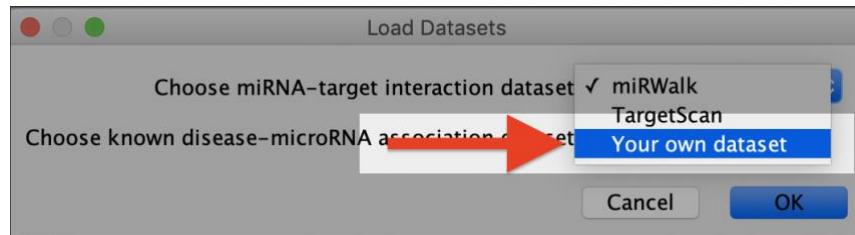
Column 1: miRNA
Column 2: Weight
Column 3: Gene ID

separate by TAB

Your own dataset can be imported into Cytoscape by clicking menu **File** → **Import** → **Network** → **File**. Set column 1 as source node and column 3 as target node.



Then, it will appear in miRNA network option:



- For known disease-miRNAs dataset: We pre-installed 2 datasets **miR2Disease** and **HMDD** (an up-to-date human disease-miRNA association database).

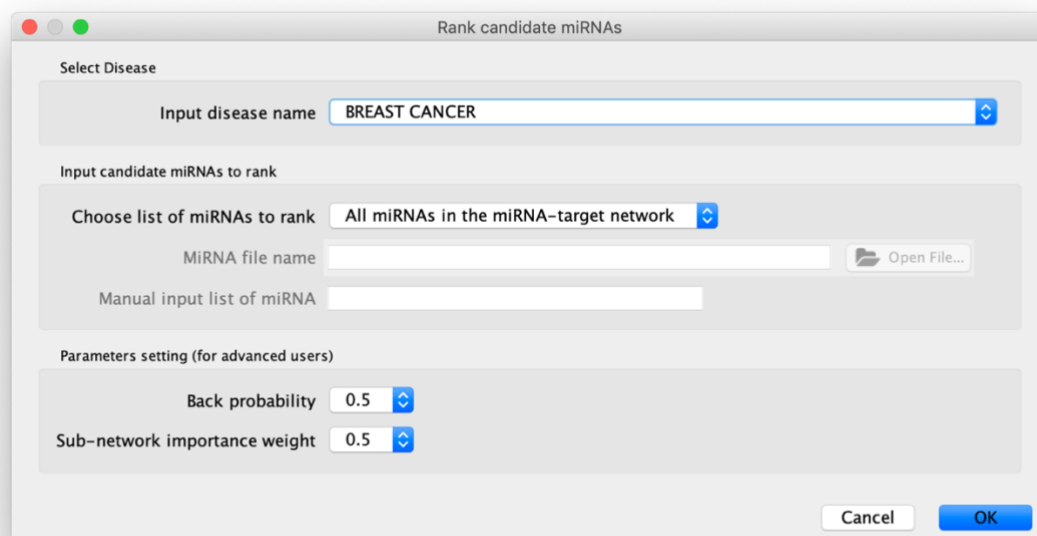
Step 2: Rank candidate miRNAs

This step includes 4 sub-steps:

- Select a disease: For example, Breast cancer (OMIM ID: 114480) is selected.
- Input candidate miRNAs to rank: there are 3 options to choose
 - o All miRNAs in the miRNA-target network
 - o List of miRNAs from file: input the file.
 - o Manual input list of miRNAs.
- Parameters setting (for advanced users)
 - o Back probability (default setting is 0.5)
 - o Sub-network importance weight (default setting is 0.5)
- Rank

Step 2 can be performed by two ways:

Cytoscape menu: Apps → RWRMTN → Step 2: Rank candidate miRNAs



- **Step 2.1:** Choose Disease name from the dropdown list:

Rank candidate miRNAs

Select Disease

Input disease name: BREAST CANCER

Input candidate miRNAs to rank

Choose list of miRNAs to rank

MiRNA file name

Manual input list of miRNA

Parameters setting (for advanced users)

Back probability: 0.5

Sub-network importance weight: 0.5

Cancel OK

- **Step 2.2:** Input candidate miRNAs to rank:
Default setting is “All miRNAs in the network”. If you want to choose specific list of miRNAs, you can select other options.

Select a file containing candidate miRNAs to be ranked.

Rank candidate miRNAs

Select Disease

Input disease name: BREAST CANCER

Input candidate miRNAs to rank

Choose list of miRNAs to rank: List of miRNA from file

MiRNA file name:

Open File...

Parameters setting (for advanced users)

Back probability: 0.5

Sub-network importance weight: 0.5

Cancel OK



Directly input list of candidate miRNAs


Rank candidate miRNAs


Select Disease

Input disease name


Input candidate miRNAs to rank


Choose list of miRNAs to rank   1

MiRNA file name  Open File...

Manual input list of miRNA  2

Parameters setting (for advanced users)

Back probability 

Sub-network importance weight 

- **Step 2.3:** Parameters setting (for advanced users)

Choose to set Back probability and Sub-network importance weight in case of using different values other than default values.

- **Step 2.4:** Rank (Click OK)

- **CyREST command API: Help → Automation → CyREST Command API**

RWRMTN		Show/Hide	List Operations	Expand Operations
POST	/v1/commands/RWRMTN/step1_load_datasets			Step 1: Load Datasets
POST	/v1/commands/RWRMTN/step2_rank_miRNAs			Step 2: Rank candidate miRNAs
POST	/v1/commands/RWRMTN/step3_search_evidences			Step 3: Search Evidences
POST	/v1/commands/RWRMTN/step4_visualize			Step 4: Visualize

Fill all the parameter requirement in the Parameter box and hit the button “Try it out”

Model Example Value

```
{
  "data": [
    {
      "name": "hsa-miR-124",
      "score": 0.01861165,
      "rank": 1,
      "type": "miRNA",
      "known": true
    },
    {
      "name": "hsa-miR-125a-5p",
      "score": 0.00533224,
      "rank": 2,
      "type": "miRNA",
      "known": true
    }
  ]
}
```

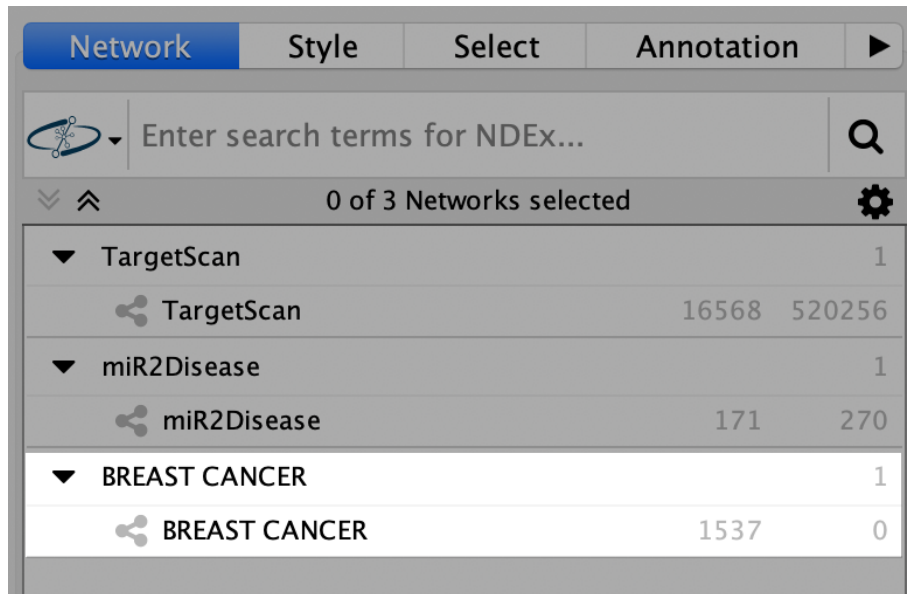
Response Content Type

Parameters

Parameter	Value	Description	Parameter Type	Data Type
body	<pre>{ "backProb": "0.5", "candidateOption": "All miRNA", "diseaseName": "BREAST CANCER", "miRNAFile": "string", "miRNAList": "hsa-miR-107,hsa-miR-146a,hsa-miR-298", "subnetWeight": "0.5" }</pre>		body	<p>Model Example Value</p> <pre>{ "backProb": "0.5", "candidateOption": "All miRNA", "diseaseName": "BREAST CANCER", "miRNAFile": "string", "miRNAList": "hsa-miR-107,hsa-miR-146a,hsa-miR-298", "subnetWeight": "0.5" }</pre>

Parameter content type:

Step 2 will generate list of ranked miRNAs displayed in a network named by the selected disease (i.e. BREAST CANCER)



The following table lists known miRNAs associated with the selected diseases and the inputted candidate miRNAs

shared name	name	Score	Rank	Type	Known
hsa-miR-124		0.00912531...	1	miRNA	<input checked="" type="checkbox"/>
hsa-miR-27a		0.00857614...	2	miRNA	<input checked="" type="checkbox"/>
hsa-miR-128		0.00840957...	3	miRNA	<input checked="" type="checkbox"/>
hsa-miR-200c		0.00838510...	4	miRNA	<input checked="" type="checkbox"/>
hsa-miR-200b		0.00838510...	5	miRNA	<input checked="" type="checkbox"/>
hsa-miR-429		0.00838123...	6	miRNA	<input checked="" type="checkbox"/>
hsa-miR-125b		0.00835355...	7	miRNA	<input checked="" type="checkbox"/>
hsa-miR-125a-5p		0.00835355...	8	miRNA	<input checked="" type="checkbox"/>
hsa-miR-17		0.00832807...	9	miRNA	<input checked="" type="checkbox"/>
hsa-miR-20a		0.00832807...	10	miRNA	<input checked="" type="checkbox"/>
hsa-let-7a		0.00826798...	11	miRNA	<input checked="" type="checkbox"/>
hsa-miR-206		0.00818675...	12	miRNA	<input checked="" type="checkbox"/>
hsa-miR-200a		0.00817063...	13	miRNA	<input checked="" type="checkbox"/>
hsa-miR-141		0.00817063...	14	miRNA	<input checked="" type="checkbox"/>
hsa-miR-204		0.00814230...	15	miRNA	<input checked="" type="checkbox"/>
hsa-miR-373		0.00805362...	16	miRNA	<input checked="" type="checkbox"/>
hsa-miR-516a-3p		0.00803928...	17	miRNA	<input checked="" type="checkbox"/>
hsa-miR-155		0.00802905...	18	miRNA	<input checked="" type="checkbox"/>
hsa-miR-7		0.00794792...	19	miRNA	<input checked="" type="checkbox"/>
hsa-miR-205		0.00794064...	20	miRNA	<input checked="" type="checkbox"/>
hsa-miR-221		0.00790413...	21	miRNA	<input checked="" type="checkbox"/>
hsa-miR-222		0.00790413...	22	miRNA	<input checked="" type="checkbox"/>
hsa-miR-127-5p		0.00790080...	23	miRNA	<input checked="" type="checkbox"/>
hsa-miR-335		0.00786437...	24	miRNA	<input checked="" type="checkbox"/>
hsa-miR-146a		0.00784430...	25	miRNA	<input checked="" type="checkbox"/>
hsa-miR-146b-5p		0.00784430...	26	miRNA	<input checked="" type="checkbox"/>
hsa-miR-21		0.00781843...	27	miRNA	<input checked="" type="checkbox"/>
hsa-miR-520c-5p		0.00778787...	28	miRNA	<input checked="" type="checkbox"/>
hsa-miR-10b		0.00778371...	29	miRNA	<input checked="" type="checkbox"/>
hsa-miR-510		0.00774478...	30	miRNA	<input checked="" type="checkbox"/>
hsa-miR-126		0.00766892...	31	miRNA	<input checked="" type="checkbox"/>
hsa-miR-210		0.00765442...	32	miRNA	<input checked="" type="checkbox"/>
hsa-miR-451		0.00765194...	33	miRNA	<input checked="" type="checkbox"/>
hsa-miR-506		0.00154994...	34	miRNA	<input type="checkbox"/>
hsa-miR-3163		0.00126209...	35	miRNA	<input type="checkbox"/>
hsa-miR-27b		0.00100038...	36	miRNA	<input type="checkbox"/>
hsa-miR-548c-3p		9.16437739...	37	miRNA	<input type="checkbox"/>
hsa-miR-520d-5p		9.07467058...	38	miRNA	<input type="checkbox"/>
hsa-miR-524-5p		9.07467058...	39	miRNA	<input type="checkbox"/>
hsa-miR-3714		7.96936672...	40	miRNA	<input type="checkbox"/>
hsa-miR-4319		7.77794334...	41	miRNA	<input type="checkbox"/>
hsa-miR-106b		7.52313163...	42	miRNA	<input type="checkbox"/>
hsa-miR-519d		7.52313163...	43	miRNA	<input type="checkbox"/>

And JSON result is exposed in CyREST Command API

Request URL

http://localhost:1234/v1/commands/RWRMTN/step2_rank_miRNAs

Response Body

```
{
  "data": [
    {
      "name": "hsa-miR-124",
      "score": 0.009125315577015921,
      "rank": 1,
      "type": "miRNA",
      "known": true
    },
    {
      "name": "hsa-miR-27a",
      "score": 0.008576144025852219,
      "rank": 2,
      "type": "miRNA",
      "known": true
    },
    {
      "name": "hsa-miR-128",
      "score": 0.008409577922964604,
      "rank": 3,

```

Response Code

200

Step 3: Search Evidences

By selecting option “All miRNAs in the miRNA-target network” in Step 2, all miRNAs in the selected miRNA-target gene network are ranked and displayed in Node Table of the network (in Network Tab of Cytoscape) which has the same name as the disease of interest (i.e., BREAST CANCER).

To find the evidences in literature (PubMed), you need to select highly ranked miRNAs by highlighting rows in the network (i.e., BREAST CANCER) and choose between these two ways:

- **Cytoscape menu:** Select menu **Apps** → **RWRMTN** → **Step 3: Search Evidences**
- **CyREST command API:** **Help** → **Automation** → **CyREST Command API**.

RWRMTN		Show/Hide	List Operations	Expand Operations
POST	/v1/commands/RWRMTN/step1_load_datasets			Step 1: Load Datasets
POST	/v1/commands/RWRMTN/step2_rank_miRNAs			Step 2: Rank candidate miRNAs
POST	/v1/commands/RWRMTN/step3_search_evidences			Step 3: Search Evidences
POST	/v1/commands/RWRMTN/step4_visualize			Step 4: Visualize

Hit the button “Try it out” without any parameters (remember to highlight rows first).

Note: If you receive the alert that none row is selected. You need to show the column “selected” in Cytoscape table and set value to **true** once to trigger the function “selected” column to work.

The result of Step 3 is a list of PubMed IDs of the publications containing evidences about associations between selected miRNAs and the disease of interest displayed in . For more information (e.g., paper title, author list, publication date, etc..), refer to Step 4 or use command API of step 3.

shared name	name	Score	Rank	Type	Known	PubMed (PudMedIDs)
hsa-miR-510		0.00774478...	30	miRNA	<input checked="" type="checkbox"/>	
hsa-miR-126		0.00766892...	31	miRNA	<input checked="" type="checkbox"/>	
hsa-miR-210		0.00765442...	32	miRNA	<input checked="" type="checkbox"/>	
hsa-miR-451		0.00765194...	33	miRNA	<input checked="" type="checkbox"/>	
hsa-miR-506		0.00154994...	34	miRNA	<input type="checkbox"/>	23717581, 25707493, 26059632, 263988...
hsa-miR-3163		0.00126209...	35	miRNA	<input type="checkbox"/>	
hsa-miR-27b		0.00100038...	36	miRNA	<input type="checkbox"/>	
hsa-miR-548c-3p		9.16437739...	37	miRNA	<input type="checkbox"/>	
hsa-miR-520d-5p		9.07467058...	38	miRNA	<input type="checkbox"/>	28721278
hsa-miR-524-5p		9.07467058...	39	miRNA	<input type="checkbox"/>	
hsa-miR-3714		7.96936672...	40	miRNA	<input type="checkbox"/>	
hsa-miR-4319		7.77794334...	41	miRNA	<input type="checkbox"/>	30021199
hsa-miR-106b		7.52313163...	42	miRNA	<input type="checkbox"/>	26621835
hsa-miR-519d		7.52313163...	43	miRNA	<input type="checkbox"/>	
hsa-miR-20b		7.52313163...	44	miRNA	<input type="checkbox"/>	
hsa-miR-106a		7.52313163...	45	miRNA	<input type="checkbox"/>	

This result is exposed by using CyREST Command API

Request URL

http://localhost:1234/v1/commands/RWRMTN/step3_search_evidences

Response Body

```
{
  "data": [
    {
      "miRnaName": "hsa-miR-520d-5p",
      "PubMedIds": [
        "28721278"
      ],
      "info": {
        "28721278": {
          "pubdate": "2016",
          "authors": [
            "Ishihara Y",
            "Tsuno S",
            "Ping B",
            "Ashizaki T",
            "Nakashima M",
            "Miura K",
            "Miura Y",
            "Yamashita T",
            "Hasegawa J"
          ]
        }
      }
    }
  ]
}
```

Response Code

200

As can be seen from the above screenshot, the result is returned as an array of JSON objects. Each object includes 3 pairs of key/value:

- “miRnaName” is the selected miRNA.
- “PubMedIds” lists the ids found in the PubMed which provided evidences of associations between selected miRNA and the disease of interest.
- “info” shows the detail information of each PubMed ID including publication date, authors, title and pages, which then can be seen by visualization functions of RWRMTN.

In this case study, four of ten highly ranked miRNAs are provided with evidences.

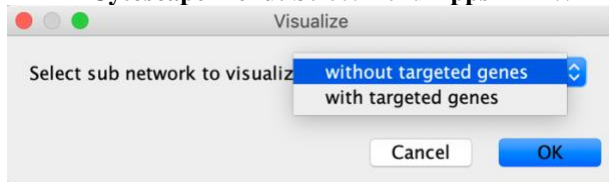
- “hsa-miR-506” supported by five studies (PubMed IDs: 23717581, 25707493, 26059632, 26398880 and 27542202). The study 23717581 showed that has-miR-506 regulates epithelial mesenchymal transition in breast cancer cell lines. Meanwhile, the study 26059632 proved notable inhibition of hsa-miR-506 over-expression to proliferation and metastasis of breast cancer cells. In addition, study 26398880 indicated that mechanism underlying miRNA-506 is a contributing factor in breast carcinogenesis (has-miR-506 was proven to be a tumor suppressor).
- “hsa-miR-520d-5p” supported by a study with PubMed ID 28721278. More specifically, it was reported that this miRNA upregulates the activation of BRCA1 (breast cancer 1, early onset) in the DNA repair process – 35 days after transfection.
- “hsa-miR-4319” was showed in study PubMed ID 30021199 as a suppressor of the malignancy of triple-negative breast cancer by regulating self-renewal and tumorigenesis of stem cells.
- “has-miR-106b” was proven by the experiment carried on patient samples and cell lines in the study (PubMed ID 26621835)

Step 4: Visualize

Selected miRNAs can be visualized in a network based on the rankings. In addition, target genes, the disease of interest and detail information of PubMed IDs collected from Step 3 such as *paper title, author list, journal name* can be displayed aside in this network.

Choose between two options for visualization (**Remember to highlight selected rows first**)

- **Cytoscape menu:** Select menu **Apps** → **RWRMTN** → **Step 4: Visualize**



Visualization options:

1. Visualize **without** targeted genes of selected miRNAs
2. Visualize **with** targeted genes of selected miRNAs
3. Click **OK** to visualize.

- **CyREST command API:** Help → Automation → CyREST Command API

RWRMTN			Show/Hide	List Operations	Expand Operations
POST	/v1/commands/RWRMTN/step1_load_datasets	Step 1: Load Datasets			
POST	/v1/commands/RWRMTN/step2_rank_miRNAs	Step 2: Rank candidate miRNAs			
POST	/v1/commands/RWRMTN/step3_search_evidences	Step 3: Search Evidences			
POST	/v1/commands/RWRMTN/step4_visualize	Step 4: Visualize			

Fill the parameter requirement in the Parameter box and hit the button “Try it out”

Parameters

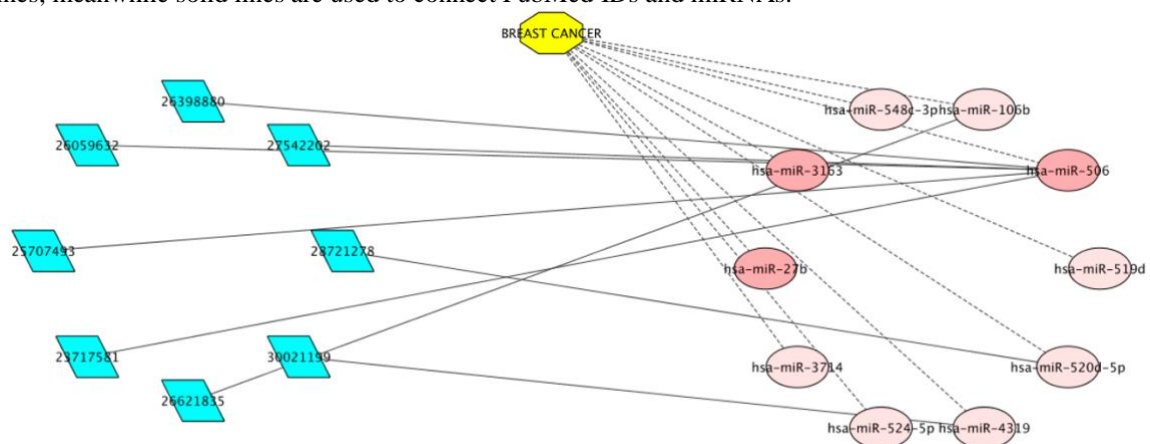
Parameter	Value	Description	Parameter Type	Data Type				
body	<pre>{ "visualizeOptions": "without targeted genes" }</pre> <p>Parameter content type: application/json</p>		body	<table> <thead> <tr> <th>Model</th><th>Example Value</th></tr> </thead> <tbody> <tr> <td></td><td> <pre>{ "visualizeOptions": "without targeted genes" }</pre> </td></tr> </tbody> </table>	Model	Example Value		<pre>{ "visualizeOptions": "without targeted genes" }</pre>
Model	Example Value							
	<pre>{ "visualizeOptions": "without targeted genes" }</pre>							

Try it out!

Step 4 will create network view in two cases:

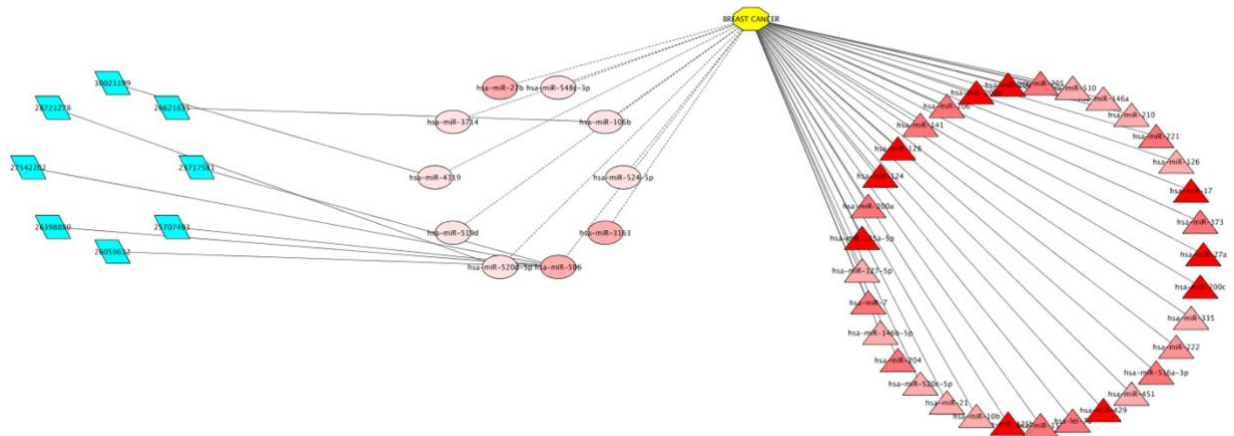
- o Without targeted genes

Here, we selected top 10 ranked candidate miRNAs and then visualized based on their rankings. In addition, evidences (PubMed IDs) for each selected miRNAs were also visualized. Disease and candidate miRNAs are connected by long-dash lines, meanwhile solid lines are used to connect PubMed IDs and miRNAs.



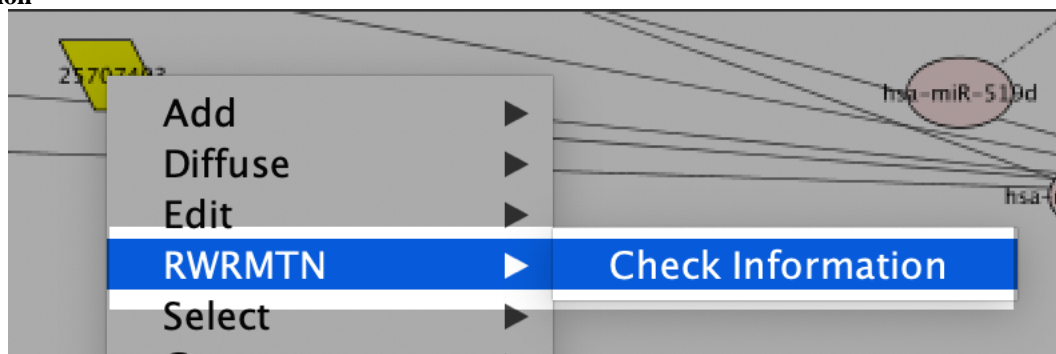
(Note: PubMed IDs, disease, and miRNAs are represented in parallelogram, octagon and ellipse shapes, respectively)

One may want to view the relationships with miRNAs known to be associated with the disease of interest (i.e., BREAST CANCER). Thus, we can additionally select known miRNAs to visualize

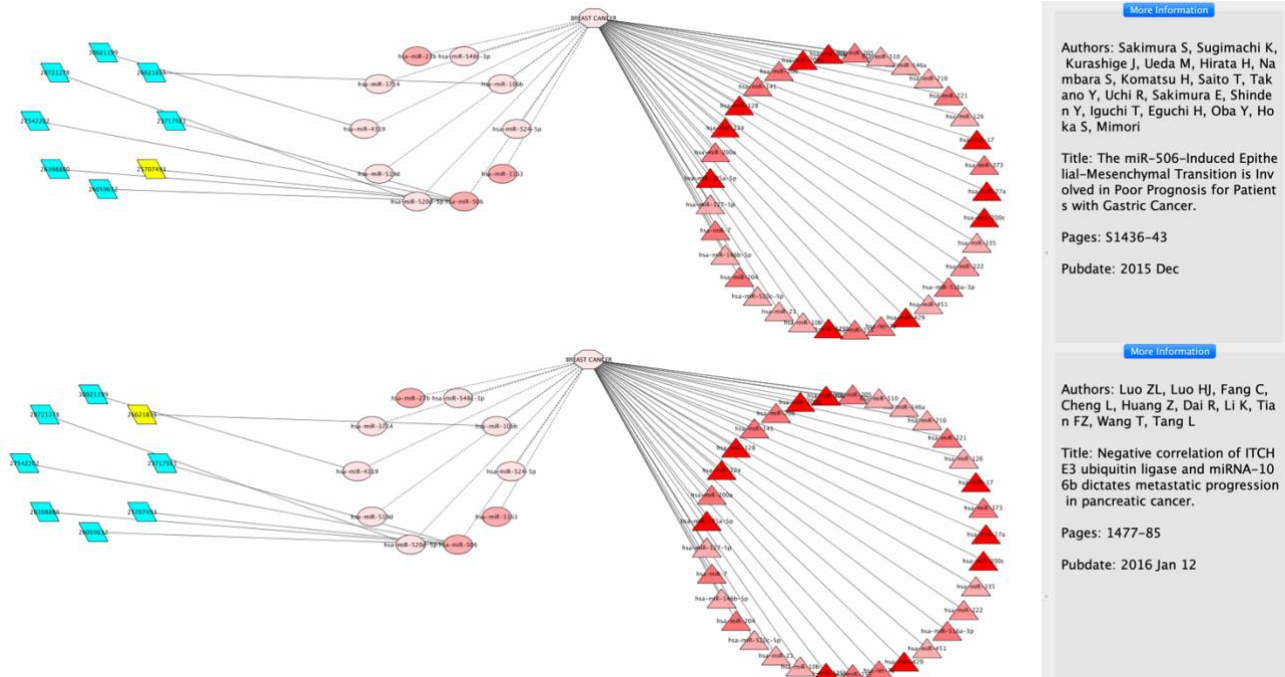


(Note: Known miRNAs are represented by triangles which are connected with the disease of interest by dot lines).

For detail information of each PubMed ID, right click on PubMed ID node and select menu **RWRMTN → Check Information**

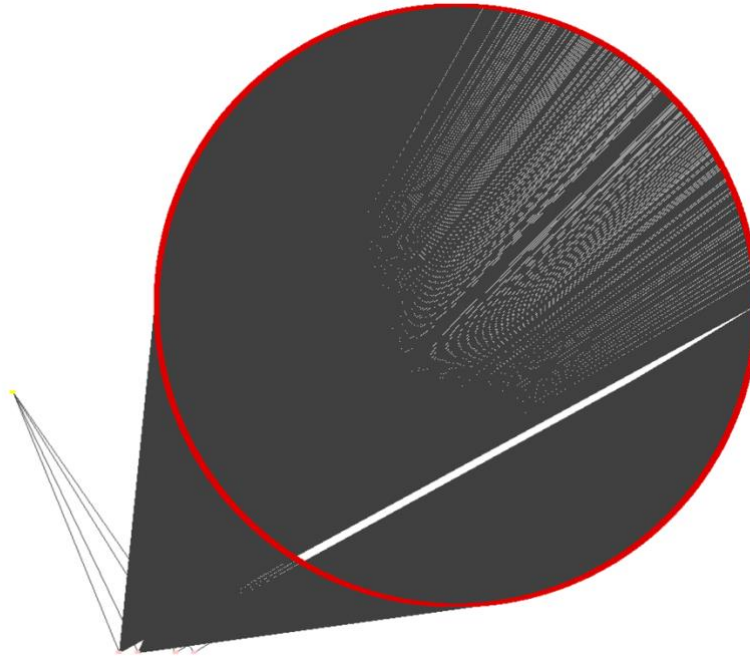


The East panel will appear to display detail information of the selected PubMed ID.



- *With targeted genes*

Select this option if user wants to see the relationships between the selected miRNAs and their target genes.



(Note: Each miRNA often targets to many genes)

2. Run RWRMTN by calling CyREST API

In this section, we first introduce some developed CyREST APIs which provides some helpful functions. Second, we demonstrate of their use in a workflow in R statistic environment.

Overview of CyREST APIs

To reveal all APIs, select menu **Help → Automation → CyREST API** to open Swagger UI of CyREST API. Here is the list of CyREST APIs of RWRMTN.

CyREST API

A RESTful service for accessing Cytoscape 3.

15/15 Automation Apps started.

Cytoscape
<http://cytoscape.org/>

Apps		Show/Hide	List Operations	Expand Operations
Apps: CyNDEx-2		Show/Hide	List Operations	Expand Operations
Apps: Diffusion		Show/Hide	List Operations	Expand Operations
Apps: RWRMTN		Show/Hide	List Operations	Expand Operations
POST	/RWRMTN/v1/rank	Rank candidate miRNAs		
GET	/RWRMTN/v1/diseaseList	List all diseases		
GET	/RWRMTN/v1/getRank/{limit}	Return top ranked miRNAs		
GET	/RWRMTN/v1/diseaseList/{diseaseName}	List the diseases match keyword search		

The detail of each API is available in Swagger Documentation. Here is short description.

GET	/RWRMTN/v1/diseaseList	List all diseases
-----	------------------------	-------------------

This API returns list of all diseases (OMIM ID and disease name) available in the selected known disease-miRNA association database (i.e., miR2Disease). Based on this list, users can select a disease of interest.

Request URL

http://localhost:1234/RWRMTN/v1/diseaseList

Response Body

```
[
  {
    "diseaseID": "MIM104300",
    "diseaseName": "ALZHEIMER DISEASE; AD"
  },
  {
    "diseaseID": "MIM109800",
    "diseaseName": "BLADDER CANCER"
  },
  {
    "diseaseID": "MIM113970",
    "diseaseName": "BURKITT LYMPHOMA; BL"
  },
  {
    "diseaseID": "MIM114480",
    "diseaseName": "BREAST CANCER"
  },
  {
    "diseaseID": "MIM114500",
    "diseaseName": "COLORECTAL CANCER; CRC"
  }
]
```

GET /RWRMTN/v1/diseaseList/{diseaseName}

List the diseases match keyword search

This API provides a list of diseases whose names match the query parameter (e.g., cancer). This API help user narrow down list of diseases to the disease of interest (e.g., disease ID MIM114480 for BREAST CANCER).

For example:

Request URL

http://localhost:1234/RWRMTN/v1/diseaseList/cancer

Response Body

```
[
  {
    "diseaseID": "MIM109800",
    "diseaseName": "BLADDER CANCER"
  },
  {
    "diseaseID": "MIM114480",
    "diseaseName": "BREAST CANCER"
  },
  {
    "diseaseID": "MIM114500",
    "diseaseName": "COLORECTAL CANCER; CRC"
  },
  {
    "diseaseID": "MIM133239",
    "diseaseName": "ESOPHAGEAL CANCER"
  },
  {
    "diseaseID": "MIM137215",
    "diseaseName": "GASTRIC CANCER, HEREDITARY DIFFUSE; HDGC"
  }
]
```

POST /RWRMTN/v1/rank

Rank candidate miRNAs

This API will use RWRMTN to rank candidate miRNAs and return the result in JSON format. The request is POST request with input parameters as follows:

required:
Disease OMIM ID: String
List of miRNA: String

optional:
miRTargetDB: String
miR2DiseaseDB: String
backProb: String
subnetWeight: String

Model	Example Value
RankParameters {	
diseaseOMIMID (string): Disease OMIM ID,	
listOfmiRNAs (string): List of miRNA to rank,	
miRTargetDB (string, optional): MicroRNA Dataset,	
miR2DiseaseDB (string, optional): Disease-miRNA Dataset,	
backProb (number, optional): Back-probability,	
subnetWeight (number, optional): Sub-network importance weight	
}	

Model	Example Value
{	
"diseaseOMIMID": "MIM114480",	
"listOfmiRNAs": "All miRNAs",	
"miRTargetDB": "TargetScan",	
"miR2DiseaseDB": "miR2Disease",	
"backProb": 0.5,	
"subnetWeight": 0.5	
}	

The result is:

Request URL

http://localhost:1234/RWRMTN/v1/rank

Response Body

```
[
  {
    "rnaName": "hsa-miR-124",
    "rnaScore": 0.009125315577015921,
    "rnaRank": 1,
    "type": "miRNA",
    "known": true
  },
  {
    "rnaName": "hsa-miR-27a",
    "rnaScore": 0.008576144025852219,
    "rnaRank": 2,
    "type": "miRNA",
    "known": true
  },
  {
    "rnaName": "hsa-miR-128",
    "rnaScore": 0.008409577922964604,
    "rnaRank": 3,
    "type": "miRNA",
    "known": true
  }
]
```

Response Code

200

GET /RWRMTN/v1/getRank/{limit}

Return top ranked miRNAs

This API returns top ranked miRNAs by setting {limit} parameter.

For example: The following query returns top 10 ranked miRNAs

Request URL

```
http://localhost:1234/RWRMTN/v1/getRank/10
```

Response Body

```
[
  {
    "rnaName": "hsa-miR-124",
    "rnaScore": 0.009125315577015921,
    "rnaRank": 1,
    "type": "miRNA",
    "known": true
  },
  {
    "rnaName": "hsa-miR-27a",
    "rnaScore": 0.008576144025852219,
    "rnaRank": 2,
    "type": "miRNA",
    "known": true
  },
  {
    "rnaName": "hsa-miR-128",
    "rnaScore": 0.008409577922964604,
    "rnaRank": 3,
    "type": "miRNA",
  }
]
```

Response Code

```
200
```

Using RWRMTN in a workflow in R environment

In this case study, we used a dataset GSE19783 from GEO (Enerly, et al., 2011), which was created using Agilent-019118 Human miRNA Microarray 2.0 G4470B platform (GPL8227) and Agilent-014850 Whole Human Genome Microarray 4x44K G4112F (GPL6480) (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE19783>). The study characterizes breast cancer subtypes from joint analysis of high throughput miRNA (using GPL8227) and mRNA (using GPL6480) Data.

In this case study, we explored the 799 miRNAs that were differentially expressed between the 64 wild-type samples (WT) and 36 *TP53* mutant samples via a workflow in R environment using CyREST API.

Briefly, here is the workflow:

1. Download the datasets
2. Perform differential expression analysis with *limma* package
3. Select a list of miRNAs, whose differential expression between cases and controls is statistically significant, as candidates
4. Rank the candidate miRNAs by RWRMTN via a CyREST API using a miRNA-target interaction dataset miRWalk (Dweep, et al., 2011) and a known disease-miRNA association dataset HMDD (Li, et al., 2014).

Before running the analysis, make sure RWRMTN and necessary packages are installed and they are functional:

- Please run `Check_CytoscapeConnection_LibraryInstallation_RWRMTN.R` (download at <https://github.com/hauldhut/RWRMTN>) for checking connection with Cytoscape and whether necessary packages are installed.

Then, run the following source code in R (the source code can be found in `CaseStudy_Final.R` (download at <https://github.com/hauldhut/RWRMTN>))

```
1. #####
2. library(Biobase)
3. library(GEOquery)
4. library(limma)
5. library(httr)
6. library(jsonlite)
7.
8. library(httr)
9. library(jsonlite)
10.
```

```

11. ### Load series and platform data from GEO
12. gset <- getGEO("GSE19783", GSEMatrix =TRUE, AnnotGPL=FALSE)
13. if (length(gset) > 1){
14.   idx <- grep("GPL8227", attr(gset, "names"))
15. }else{
16.   idx <- 1
17. }
18.
19. gset <- gset[[idx]]
20.
21. # make proper column names to match toptable
22. fvarLabels(gset) <- make.names(fvarLabels(gset))
23.
24. TP53Status<-gset$`tp53 mutation status:ch1`
25. # labeling for all samples
26. gsms<-""
27.
28. sml<-c()
29. for(i in 1:length(TP53Status)){
30.   if(TP53Status[i]=="Mut"){
31.     gsms<-paste0(gsms,"1")
32.     sml[i]<- "Group1"
33.   }else{
34.     gsms<-paste0(gsms,"0")
35.     sml[i]<- "Group0"
36.   }
37. }
38.
39. #Retrieve Expression Data From ESets
40. ex <- exprs(gset)
41. ex[which(ex <= 0)] <- NaN
42. # log2 transform
43. exprs(gset) <- log2(ex)
44.
45. ### Differential expression analysis with limma package
46. # set up the data and proceed with analysis
47. fl <- as.factor(sml)
48. gset$description <- fl
49. #creates a design (or model) matrix
50. design <- model.matrix(~ description + 0, gset)
51. colnames(design) <- levels(fl)
52. fit <- lmFit(gset, design)
53. cont.matrix <- makeContrasts(Group1-Group0, levels=design)
54. fit2 <- contrasts.fit(fit, cont.matrix)
55. fit2 <- eBayes(fit2, 0.01)
56. diffmiRNAlist <- topTable(fit2, adjust="fdr", number=nrow(fit2))
57.
58. #Only select miRNAs whose differential expression between the two group (Group1 & Group0)
59. #is statistically significant (adj.P.val <=0.05) for ranking with RWRMTN
60. sigmiRNAlist <- subset(diffmiRNAlist,adj.P.Val<=0.05) #This returns 85 miRNAs
61. sigmiRNAlist <- subset(sigmiRNAlist, select=c("ID","adj.P.Val","P.Value"))
62. colnames(sigmiRNAlist)<-c("rnaName", "adj.P.Val", "P.Value")
63.
64. #Save statistically significant miRNAs standard output
65. write.table(sigmiRNAlist, file=stdout(), row.names=F, sep="\t")
66.
67.
68. ###Rank statistically significant miRNAs (candidate miRNAs) with RWRMTN
69. #Get miRNA list
70. lr<-sigmiRNAlist$rnaName
71. lor<-""
72. n<-length(lr)
73. for(i in 1:n){
74.   lor<-paste(lor, lr[i],", ",sep='')
75. }
76.
77. #Select datasets (miRTargetDB, miR2DiseaseDB), the disease of interest (MIM114480: Breast cancer)
78. #and pass the candidata miRNAs list
79. login <- list(

```

```

80. diseaseOMIMID= "MIM114480",#OMIM ID of Breast cancer
81. listOfmiRNAs= lor,
82. miRTargetDB= "miRWalk",
83. miR2DiseaseDB= "HMDD"
84. )
85.
86. #Run Cytoscape CyREST API
87. request_body_json <- toJSON(login)
88. res <- POST("http://localhost:1234/RWRMTN/v1/rank", body = login, encode="json")
89. y<-httr::content(res,"text", encoding = 'UTF-8')
90. get_prices_json <- fromJSON(y, flatten = TRUE)
91.
92. Output <- fromJSON((y))
93. #Remove miRNA with rank=0 (which are not available on the miRNA-target network)
94. rankedmiRNAlist <- Output[which(Output$rnaRank!=0),]
95. rankedmiRNAlist
96. write.csv(rankedmiRNAlist, file="rankedmiRNAlist.csv", row.names=F)

```

Here is the result:

	rnaName	rnaScore	rnaRank	type	known
1	hsa-miR-375	1.719388e-02	1	miRNA	TRUE
2	hsa-miR-107	4.059087e-03	2	miRNA	TRUE
3	hsa-miR-15a	3.420814e-03	3	miRNA	TRUE
4	hsa-miR-326	3.377178e-03	4	miRNA	TRUE
5	hsa-miR-155	2.825815e-03	5	miRNA	TRUE
6	hsa-miR-145	2.716448e-03	6	miRNA	TRUE
7	hsa-miR-148b	2.686323e-03	7	miRNA	TRUE
8	hsa-miR-224	2.610611e-03	8	miRNA	TRUE
9	hsa-let-7e	2.553925e-03	9	miRNA	TRUE
10	hsa-miR-29c	2.545947e-03	10	miRNA	TRUE
11	hsa-miR-26b	2.467229e-03	11	miRNA	TRUE
12	hsa-miR-30a	2.455210e-03	12	miRNA	TRUE
13	hsa-let-7b	2.428472e-03	13	miRNA	TRUE
14	hsa-let-7c	2.411595e-03	14	miRNA	TRUE
15	hsa-miR-34b	2.396425e-03	15	miRNA	TRUE
16	hsa-miR-10b	2.389018e-03	16	miRNA	TRUE
17	hsa-miR-18a	2.352102e-03	17	miRNA	TRUE
18	hsa-miR-328	2.344313e-03	18	miRNA	TRUE
19	hsa-miR-143	2.331756e-03	19	miRNA	TRUE
20	hsa-miR-214	2.313317e-03	20	miRNA	TRUE
21	hsa-miR-152	2.283654e-03	21	miRNA	TRUE
22	hsa-miR-135b	2.273221e-03	22	miRNA	TRUE
23	hsa-miR-195	2.270405e-03	23	miRNA	TRUE
24	hsa-miR-125a-5p	7.286119e-04	24	miRNA	FALSE
25	hsa-miR-342-3p	5.469969e-04	25	miRNA	FALSE
26	hsa-let-7a	4.714080e-04	26	miRNA	FALSE
27	hsa-miR-769-5p	4.352923e-04	27	miRNA	FALSE
28	hsa-miR-361-5p	3.802039e-04	28	miRNA	FALSE
29	hsa-miR-142-3p	3.341059e-04	29	miRNA	FALSE
30	hsa-miR-34c-5p	2.341773e-04	30	miRNA	FALSE
31	hsa-miR-146b-5p	1.953439e-04	31	miRNA	FALSE
32	hsa-miR-449a	1.556556e-04	32	miRNA	FALSE
33	hsa-miR-199a-5p	1.228826e-04	33	miRNA	FALSE
34	hsa-miR-489	1.197518e-04	34	miRNA	FALSE
35	hsa-miR-9	9.189293e-05	35	miRNA	FALSE
36	hsa-miR-30a*	9.030027e-05	36	miRNA	FALSE
37	hsa-miR-135a	8.225978e-05	37	miRNA	FALSE
38	hsa-miR-181c	7.444931e-05	38	miRNA	FALSE
39	hsa-miR-378*	7.208481e-05	39	miRNA	FALSE
40	hsa-miR-30c	7.006724e-05	40	miRNA	FALSE
41	hsa-let-7f	6.601124e-05	41	miRNA	FALSE
42	hsa-miR-483-3p	5.512170e-05	42	miRNA	FALSE
43	hsa-miR-342-5p	5.253228e-05	43	miRNA	FALSE
44	hsa-miR-501-3p	4.575108e-05	44	miRNA	FALSE
45	hsa-miR-101	4.479886e-05	45	miRNA	FALSE
46	hsa-miR-574-3p	4.081944e-05	46	miRNA	FALSE
47	hsa-miR-26a	3.510984e-05	47	miRNA	FALSE
48	hsa-miR-103	3.103517e-05	48	miRNA	FALSE
49	hsa-miR-378	2.822501e-05	49	miRNA	FALSE
50	hsa-miR-99b	2.788255e-05	50	miRNA	FALSE
51	hsa-miR-590-5p	1.735067e-05	51	miRNA	FALSE
52	hsa-miR-362-5p	1.092474e-05	52	miRNA	FALSE
53	hsa-miR-9*	2.842895e-06	53	miRNA	FALSE
54	hsa-miR-142-5p	1.520914e-06	54	miRNA	FALSE
55	hsa-miR-101*	1.019211e-06	55	miRNA	FALSE
56	hsa-miR-30e*	7.906392e-07	56	miRNA	FALSE
57	hsa-miR-650	4.129138e-07	57	miRNA	FALSE

IV. Reference

- Dweep, H., *et al.* miRWalk - Database: Prediction of possible miRNA binding sites by "walking" the genes of three genomes. *Journal of Biomedical Informatics* 2011;44(5):839-847.
- Enerly, E., *et al.* miRNA-mRNA Integrated Analysis Reveals Roles for miRNAs in Primary Breast Tumors. *PLOS ONE* 2011;6(2):e16915.
- Jiang, Q., *et al.* miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic acids research* 2009;37(suppl 1):D98-D104.
- Lewis, B.P., *et al.* Prediction of Mammalian MicroRNA Targets. *Cell* 2003;115(7):787-798.
- Li, Y., *et al.* HMDD v2.0: a database for experimentally supported human microRNA and disease associations. *Nucleic Acids Research* 2014;42(D1):D1070-D1074.