

miRCuit: A Regulatory Circuit Analysis Tool

User Manual

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GitHub Repository: <https://github.com/miRcuit/miRcuit>

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***miRCuit*: A Regulatory Circuit Analysis Tool**

Abstract

miRCuit is a circuit analysis program developed for researchers from various disciplines working with mRNA, miRNA, lncRNA, and TF expression data. The program provides a comprehensive and integrative analysis platform for users in the fields of bioinformatics, molecular biology, genetics, and systems biology.

miRCuit utilizes data from 11 distinct databases (miRcode, DIANA TarBase, LncCeRBase, LncTarD, miRDB, miRTarBase, TFLink, TRRUST, RNA Interactome Database, TargetScan, and TransmiR) to offer researchers a detailed and thorough analysis of regulatory circuits. The program defines and categorizes different circuit types ranging from Type 1 to Type 8, while also presenting specialized circuit structures, such as miRNA-dependent lncRNA regulatory circuits and TF-dependent lncRNA regulatory circuits, thus providing a holistic framework for regulatory network analysis.

Additionally, with its Gene Set Enrichment Analysis (GSEA) option, *miRCuit* enables pathway analysis, revealing the pathways enriched by the circuit molecules, thereby offering users deeper insights into their regulatory networks.

Requirements

miRCuit is developed as a .py file and can be executed in environments such as Visual Studio Code, Jupyter Notebook, etc. To run this program, you will need the following software and libraries:

```
- Python 3.x  
- pandas (version 2.2.2)  
- PyQt5 (version 5.15.11)  
- gseapy (version 1.1.3)  
- scipy (version 1.14.0)  
- matplotlib (version 3.9.1)  
- networkx (version 3.3)
```

Installation Steps

1. Download and Install Python
 - Download the latest version of Python from the <https://www.python.org/downloads/>
 - Run the downloaded file to install Python. Make sure to check the "Add Python to PATH" option during installation.
2. Chose an IDE or Text Editor
 - Select and install an IDE or text editor, such as Visual Studio Code, PyCharm, or Jupyter Notebook.
 - Example: Download Visual Studio Code from <https://code.visualstudio.com>
3. Install Required Libraries
 - Open the terminal or command prompt.
 - Navigate to your project directory (e.g., `cd your_project`).
 - Use the following command to install the required libraries (The requirement.txt file is available):

```
pip install -r requirements.txt
```

- If you are unable to perform this operation, please download each of the libraries mentioned above using the following command through the VS Code terminal:

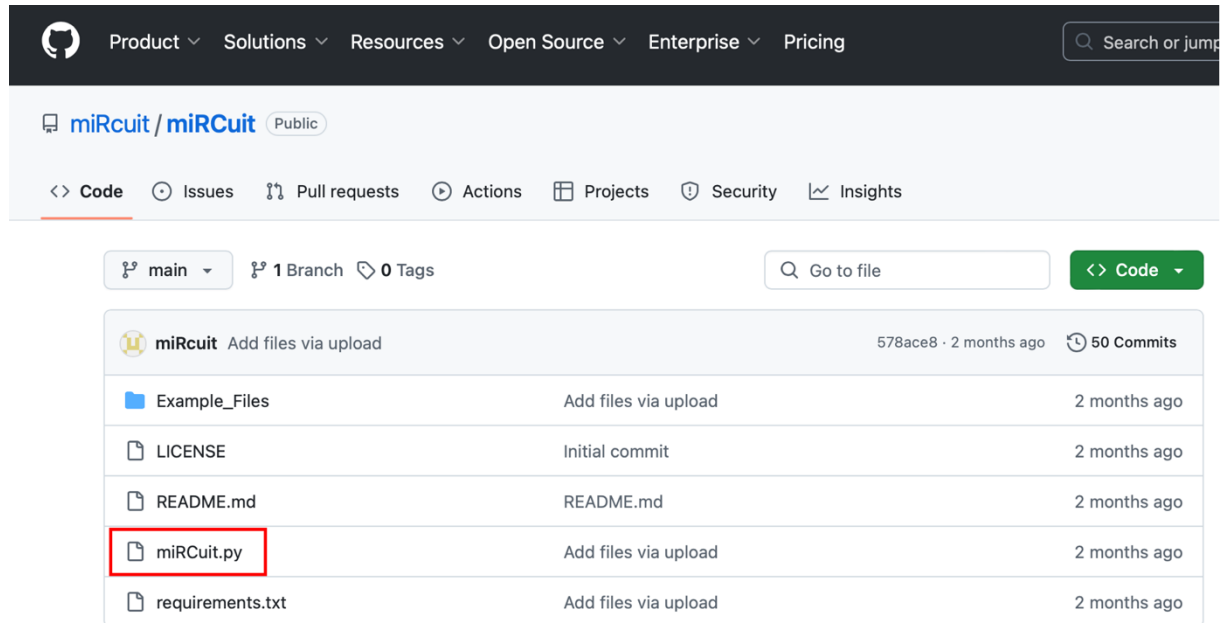
```
pip install <library_name>
```

4. Download the Program File
 - Download the .py file of the program or clone the source code.
5. Run the Program
 - Run the code using Visual Studio Code or another IDE.
6. Load Data Files
 - Upload the necessary data files containing mRNA, miRNA, lncRNA, and TF molecules for the program to function.

Check the 'Program Usage Steps' section to learn about the required formats of these files and how to upload them.

Program Usage Steps

1. Visit the *miRCuit* GitHub repository (<https://github.com/miRcuit/miRcuit>) and download the **miRCuit.py** file to your local machine.



2. Run the script as described in the previous steps. Upon successful execution, the program's graphical user interface (GUI) will open.

miRcuit: A REGULATORY CIRCUIT ANALYSIS PROGRAM

miRcuit: A Regulatory Circuit Analysis Program

miRcuit is a program designed to analyze the regulatory effects of miRNAs, lncRNAs, and transcription factors (TFs) on mRNAs. By comparing its analysis results with databases such as *miRcode*, *DIANA TarBase*, *LncCeRBase*, *LncTarD*, *miRDB*, *miRTarBase*, *TFLink*, *TRRUST*, *RNAInter*, *TargetScan*, and *TransmiR* miRcuit reveals the potential structure of molecular interactions, contributing to a deeper understanding of biological processes.

Before running the program, you must select the necessary files for the analysis. Once the files are selected, the analysis will begin using the threshold values and other parameters you have specified.

File Selection

Please upload your research results for mRNA, miRNA, lncRNA, and TF expression files to the system in CSV format, ensuring that they include the *Gene Symbol* and *log2FoldChange* values.

Please select the mRNA file. [Click here](#) for the sample file format

Select File

Please select the miRNA file. [Click here](#) for the sample file format

Select File

Please select the lncRNA file. [Click here](#) for the sample file format

Select File

Please select the TF file. [Click here](#) for the sample file format

Select File

Threshold Setting

Select the desired fold change threshold for all files to apply across your research groups.

mRNA Threshold:

▼

miRNA Threshold:

▼

lncRNA Threshold:

▼

TF Threshold:

▼

GSEA Enrichment Analysis

Please check the box to perform the GSEA enrichment analysis on your dataset using the MSigDB database.

☐ Perform GSEA Enrichment Analysis
 ☒ Do not perform GSEA Enrichment Analysis

START ANALYSIS

3. In the opened interface, navigate to the **File Selection** section. Select the required input files for analysis, including: mRNA expression file, miRNA expression file, lncRNA expression file and TF expression file (Please refer to the section titled “*Important Notes About Uploaded File Formats*”). Use the file browser buttons provided in the interface to locate and upload these files.

Important Notes About Uploaded File Formats:

- The files you upload must be in CSV format and consist of two columns. The first column should contain the names of your molecules under the heading "Annotation," and the second column should include the fold changes under the heading "log2FoldChange."
- Before uploading your files, please review the "Example_Files" under the miRcuit repository or check the "Examine the example file format" section in the interface (The p-value column is not required). If you wish, you can also run the program using the example files.
- Please ensure that the capitalization and spacing match the example exactly.

Note: Example formats of the files to be uploaded can be viewed by clicking on the "Click here" sections.

miRcuiT: A Regulatory Circuit Analysis Program

miRcuiT is a program designed to analyze the regulatory effects of miRNAs, lncRNAs, and transcription factors (TFs) on mRNAs. By comparing its analysis results with databases such as *miRcode*, *DIANA TarBase*, *LncCeRBase*, *LncTarD*, *miRDB*, *miRTarBase*, *TFLink*, *TRRUST*, *RNAInter*, *TargetScan*, and *TransmiR* miRcuiT reveals the potential structure of molecular interactions, contributing to a deeper understanding of biological processes.

Before running the program, you must select the necessary files for the analysis. Once the files are selected, the analysis will be performed using the threshold values and other parameters you have specified.

File Selection

Please upload your research results for mRNA, miRNA, lncRNA, and TF expression files to the system in CSV format, ensuring to include the Gene Symbol and log2FoldChange values.

Please select the mRNA file. [Click here](#) for the sample file format.

Select File

Please select the miRNA file. [Click here](#) for the sample file format.

Select File

Please select the lncRNA file. [Click here](#) for the sample file format.

Select File

Please select the TF file. [Click here](#) for the sample file format.

Select File

	A	B	C
1	Annotation	log2FoldChange	pvalue
2	CLCNKB	-1.4077448069438518	0.0010625756622217
3	RNU1-1	1.4071192162437791	0.0113952470218958
4	AL137798.1	-0.8833834330740418	0.0012541398330506
5	KLHDC7A	1.0292896971372207	0.0406505131200199
6	AKR7L	0.6603172606482554	0.049180794833407
7	RNF186	-2.008194475896393	0.0024431611607462
8	PLA2G5	-1.0135609111019508	0.0055290693255778
9	EIF4G3	0.2505363187122558	0.0311154594641312
10	CNR2	1.2392358766354132	0.0268369977846605
11	AL020996.1	-0.3544192079566973	0.0407646332334904
12	STMN1	0.7004940715472928	0.0121977604498432
13	SLC30A2	1.0418800188811352	0.0079804971617835
14	IFI6	1.144594941479737	0.0449748514080602
15	TMEM200B	-0.4680147663312355	0.0297045881385783
16	MATN1	-0.8661747121754974	0.0031303805087723
17	MATN1-AS1	-0.3902203265568862	0.0217883852787947
18	NKAIN1	1.7984319363449424	0.001864866695461
19	TSSK3	-0.4544768889491466	0.0467680118095755
20	MANEAL	0.9840727834054398	0.016802811283065
21	HPICAL4	1.1505696149859816	0.0145792102852657
22	RIMKLA	0.7127110189789336	0.0248825731117127
23	DNAP1	-0.2481482417936176	0.0048048811842669
24	ARMH1	0.4936944707155167	0.0025371991527991
25	CYP4A11	-1.323495046778632	0.002923848784019
26	SHISAL2A	0.7975812280302494	0.0381798679772103
27	ECHDC2	-0.4877864762705843	0.0310007232594619
28	SSBP3	-0.4051764862812506	0.0034961303683614
29	SLC44A5	1.1712404699781862	0.0170270619419911

4. Subsequently, specify the threshold value for fold change for each expression dataset to ensure accurate filtering and analysis (This value represents the minimum fold change you wish to observe between the two groups you are comparing in your research (tumor/normal, treated/untreated etc.)).

Threshold Setting

Select the desired fold change threshold for all files to apply across your research groups.

mRNA Threshold:

miRNA Threshold:

lncRNA Threshold:

TF Threshold:

GSEA Enrichment Analysis

Please check the box to perform the GSEA enrichment analysis on your dataset using the MSigDB database.

mRNA Threshold: ☒ 1.0

miRNA Threshold: ☐ 1.5

lncRNA Threshold: ☐ 2.0

TF Threshold: ☐ 2.5

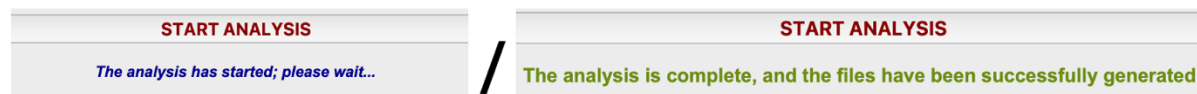
5. Finally, select whether to perform the Gene Set Enrichment Analysis (GSEA). By default, the "Do not perform GSEA Enrichment Analysis" option is selected.

GSEA Enrichment Analysis

Please check the box to perform the GSEA enrichment analysis on your dataset using the MSigDB database.

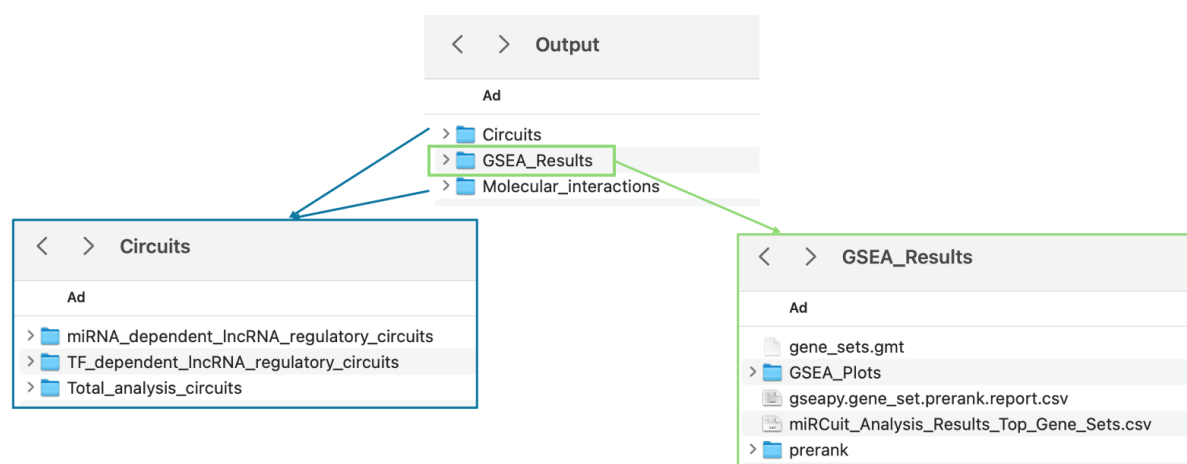
- ☐ Perform GSEA Enrichment Analysis
- ☒ Do not perform GSEA Enrichment Analysis

6. The analysis is initiated. During the analysis, the current status is displayed to the user as notifications both when the analysis starts and when it is completed.



7. The outputs are automatically saved in an **Output** folder, organized into three separate subfolders:

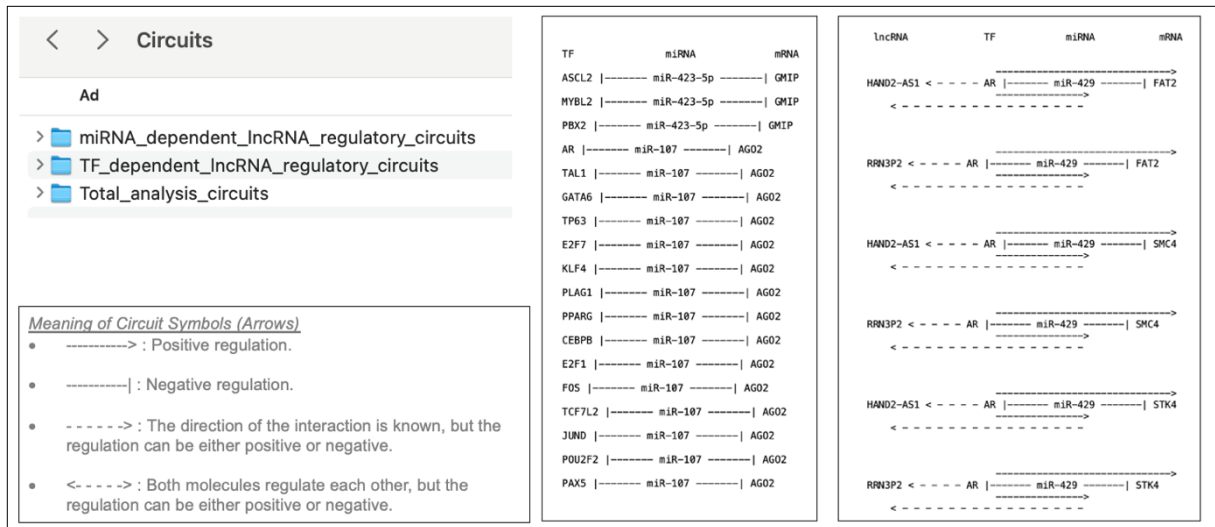
1. Linear-form outputs of the constructed circuits (**Circuits**).
2. Table-formatted CSV files generated for advanced analyses of these circuits (**Molecular interactions**).
3. Results of the GSEA analysis (**GSEA Results**).



The contents of the files are described as follows:

1) Linear-form Outputs of Circuits

This section contains the linear representations of molecular relationships in **txt format** for a total of 10 distinct circuit types: 8 standard circuit types and 2 special circuit types (*miRNA-dependent lncRNA regulatory circuits* and *TF-dependent lncRNA regulatory circuits*). These files provide a comprehensive overview of the molecular interactions in a linear format, ensuring clarity and ease of interpretation.



2) Table-formatted CSV Files for Advanced Analyses

Provides the tabular versions of the above files in CSV format.

<

>

Molecular_interactions

Ad

>

miRNA_dependent_lncRNA_regulatory_interaction

>

TF_dependent_lncRNA_regulatory_interaction

>

Total_analysis_interaction

	A	B	C
1	TF	miRNA	mRNA
2	ASCL2	miR-423-5p	GMIP
3	MYBL2	miR-423-5p	GMIP
4	PBX2	miR-423-5p	GMIP
5	ASCL2	miR-423-5p	CCDC117
6	MYBL2	miR-423-5p	CCDC117
7	PBX2	miR-423-5p	CCDC117
8	ASCL2	miR-423-5p	CCNB1
9	MYBL2	miR-423-5p	CCNB1
10	PBX2	miR-423-5p	CCNB1
11	ASCL2	miR-423-5p	ATAD2
12	MYBL2	miR-423-5p	ATAD2
13	PBX2	miR-423-5p	ATAD2
14	ASCL2	miR-423-5p	PLK1
15	MYBL2	miR-423-5p	PLK1
16	PBX2	miR-423-5p	PLK1
17	ASCL2	miR-423-5p	HASPIN
18	MYBL2	miR-423-5p	HASPIN
19	PBX2	miR-423-5p	HASPIN
20	ASCL2	miR-423-5p	MCM2
21	MYBL2	miR-423-5p	MCM2

	A	B	C	D
1	TF	miRNA	mRNA	lncRNA
2	AR	miR-429	MAP1B	HAND2-AS1
3	AR	miR-429	RUNX1T1	HAND2-AS1
4	AR	miR-429	DDX24	HAND2-AS1
5	AR	miR-429	IGF2	HAND2-AS1
6	AR	miR-429	ZFP36	HAND2-AS1
7	AR	miR-429	SEMA6D	HAND2-AS1
8	AR	miR-429	SCN3B	HAND2-AS1
9	AR	miR-429	EFNA1	HAND2-AS1
10	AR	miR-429	MMP16	HAND2-AS1
11	AR	miR-429	PI3	HAND2-AS1
12	AR	miR-429	PKP1	HAND2-AS1
13	AR	miR-429	EGFR	HAND2-AS1
14	AR	miR-429	ALDH1A3	HAND2-AS1
15	AR	miR-429	EPHA2	HAND2-AS1
16	AR	miR-429	CHST3	HAND2-AS1
17	AR	miR-429	CAV1	HAND2-AS1
18	AR	miR-429	PLCD3	HAND2-AS1
19	AR	miR-429	PRKN	HAND2-AS1
20	AR	miR-429	NECTIN1	HAND2-AS1
21	AR	miR-429	UBR7	HAND2-AS1
22	AR	miR-429	CYBRD1	HAND2-AS1
23	AR	miR-429	TRHDE	HAND2-AS1

3) GSEA Analysis Results

Provides the table, graphs, and networks of the pathways enriched by the genes obtained from the circuits.

