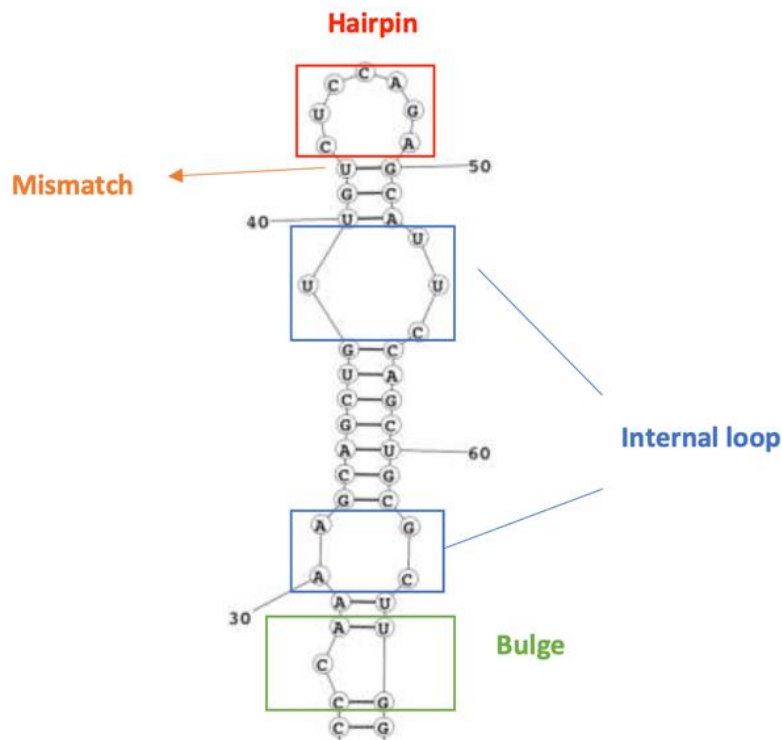


Instruction on miRmo Usage

1. Introduction

miRmo is a user-friendly secondary structure motif-based miRNA screening tool, which is implemented as a web server. **The primary purpose of miRmo is to screen user-defined structural motif (input) for identification of all possible miRNAs (output) for further studies, for instance, elucidating other potential functions of the motif/miRNAs or discover possible off-targets of the motif-targeting therapeutics.** The input includes several parameters about the motif, and the output consists of a list of miRNAs containing the input motif and related information (RNA secondary structures, motif location, etc.). The program is primarily coded in Python and PHP for the server side and the client side. Users can input the target motifs by following certain rules. Meanwhile, we provide options for users to search several databases including the Protein Data Bank (PDB), and motif types should be specified as part of the arguments.

2. RNA Motifs:



Users need to choose the type of motif structure they want to search. They need to know about their motif of interest and specify which motif type to search. Such knowledge will help to interpret the screening results (e.g., miRNA function annotations). Users may refer to the **Input Parameters** section for examples of each motif structure. Herein we provide 4 options: Hairpin, Bulge, Internal Loop, and Mismatch.

3. Input Parameters

The miRmo inputs include 6 parameters: **strand1, strand2, database, prediction software, motif type, and PDB search**. In the following session, we will explain in details on how to specify these inputs.

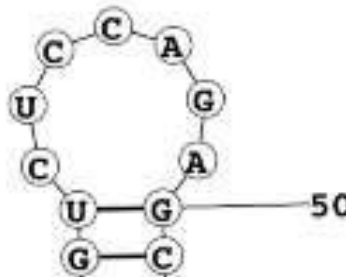
- 1) Strand1: The motif sequence on one strand (side) of RNAs
- 2) Strand2: The motif sequence one the other strand (side) of RNA

Special Notes:

- 1). It is not required to input the sequence direction (5'-3' or 3'-5'); miRmo screens both directions. This improves tolerance of input errors from users.
- 2). It is just for the sake of the program to define strand1 and Strand2, but they are interchangeable, and users can define either strand as the Strand1 or Strand2.
- 3). However, the two sides of the motif input should be "paired" correspondingly.
- 4). Use "-" as the non-paired part if users search for Hairpin or Bulge motif.

Illustration with Examples:

1) Hairpin:



a. Only include Hairpin:

Tips: As long as the sequence is in order, the input for the blank ("-") and the hairpin sequence can be reversed, either with the sequence or strand.

Strand1: cuccaga Strand 2: -

OR

Strand1: - Strand 2: agaccuc

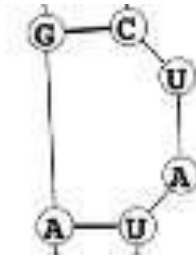
b. The neighbor sequences are included to increase the robustness and accuracy.

Tips: The easiest way to provide the input:

1. write the "Strand1" from left to right in order
2. write the "Strand2" from right to left in order; however, use a single "-" since there is no sequence for "Strand2".

Strand1: gucuccagagc Strand2: cg-ug

2) Bulge:



a) Only search Bulge:

Strand1: -

Strand2: ua

b) The neighbor sequences are included, and the rules are similar to Hairpin search, as illustrated below:

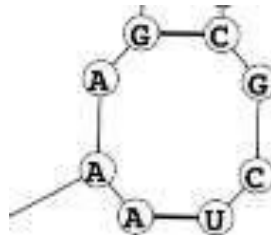
Strand1: g-a

Strand2: cuau

3) Internal Loop:

Special Note: the neighbor sequences are NOT allowed for Internal Loop search.

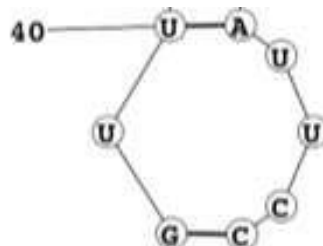
a. Have the same length for both sides of the motif:



Strand1: aa

Strand2: gc

b. Have different length for the two sides of the motif:



Strand1: u

Strand2: uuc

4) Mismatch:

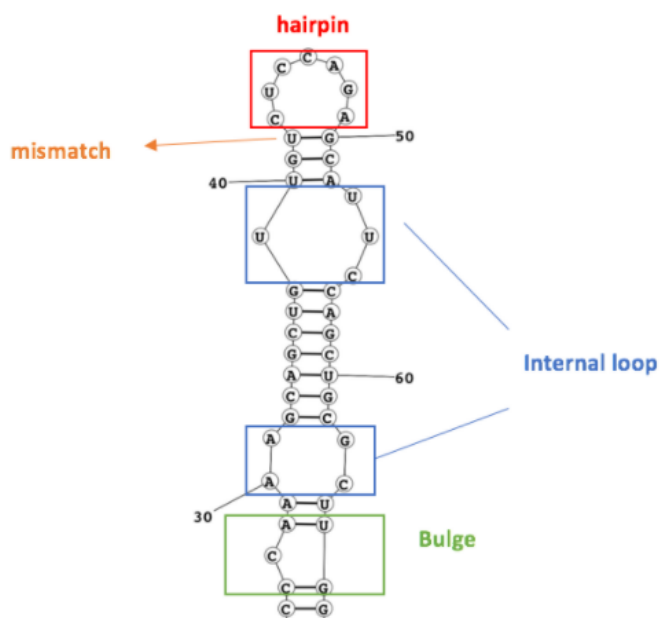
The two sides/strands of the motif need to have THE SAME LENGTH.

Mix of mismatch and normal pairing is also allowed.

Strand1: ug

Strand2: gc

Other specific parameters for the search:



miRmo Input

Strand1	<input type="text" value="ua"/>
Strand2	<input type="text" value="ug"/>
Database	<input type="text" value="Homo Sapiens"/>
Software	<input type="text" value="RNAstructure"/>
Motif Type	<input type="text" value="Internal Loop"/>
PDB Search	<input type="text" value="No"/>

Database:

Choose the miRNA database to screen. If you are interested in human miRNAs, you may choose *Homo sapiens* as your database. Otherwise, you may choose the *All Species* including miRNAs for >270 species. However, please note there would be relatively a long waiting time to screen *All Species* due to its significantly large size. We also included subsets of only miRNAs annotated with high confidence.

Option:

- Homo sapiens
- All Species
- Homo sapiens (high confidence)
- All Species (high confidence)

Software:

Choose the RNA secondary structure prediction tool. Due to the challenges in prediction, the predicted secondary structures for the same RNA may be slightly different from software to software. Thus, we included multiple algorithms for users to choose.

Option:

- RNAstructure
- RNAfold
- CentroidFold

PDB Search:

Option to find if the motif exists in RNAs with available 3D structures. Users have the option to search PDB to identify all RNAs with available 3D structures containing the motif. To achieve this, the 3D structures of RNAs from PDB database were converted to 2D structures and made available for searching. The purpose is to give users an idea of how the motif looks like in a known RNA 3D structures which will help elucidate other potential functions of the motif. However, this search will also increase the length of screening time.

Option:

- a. Yes
- b. No

4. Output Results

With all the necessary input parameters, users can hit the “**Submit**” button to start the motif search. Due to the high volume of jobs submitted and intensive computations, it may take some time (1-20 minutes) to search the desired motifs through the selected miRNA databases and show the result. For users’ convenience, a hyperlink will be provided so that users can check the result any time (within 30 days). If *PDB Search* is not selected, there will be only one table showing all miRNAs containing the queried motif. Otherwise, the result will include two tables: one is for the searching result of miRNAs, the other is for screening of all RNAs in PDB with known 3D structures.

Output of miRNA screening (curated data from miRbase)

- miRNAs: names and hyperlinks to miRBase entries.
- Species: which species a miRNA comes from.
- Location: Index of matched motif’s first nuclear acid.
- CT file: a file format containing the 2D structural information of RNA
- Image (2D): PNG image file generated based on the CT file provided.

Output of *PDB Search*

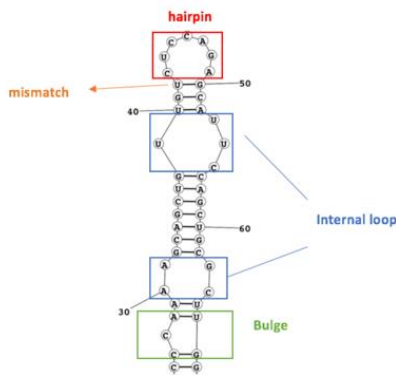
- PDB ID: 4 characters used as entry in PDB.
- PDB Link: hyperlink to the specific RNA entry in PDB.
- Motif Location: Index of matched motif’s first nuclear acid.

Please see the following example for illustration and explanation.

miRmo - A web server for microRNA motif screening

miRmo is a computational tool that enables fast motif screening in pre-microRNA (pre-miRNA) secondary structures. This may help conduct function annotation of miRNAs and elucidate the potential off-targeting properties of therapeutic agents that target those non-coding molecules.

A.



miRmo Input

Strand1:

Strand2:

Database:

Software:

Motif Type:

PDB Search:

miRmo Output

Results will be available at the following link. It may take 1-10 minutes to complete the search, depending on which database to screen and whether PDB Search is enabled. Please bookmark the link, which will be deactivated after 30 days.

https://www.imdlab.net/mirmo/results/results_ZHZEJC.php

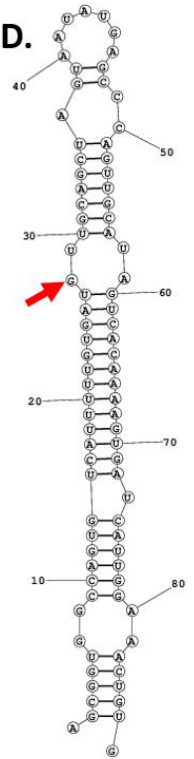
B. Identified miRNAs that contain the queried motif

miRNA	Species	Location	CT File	Structure Image
miR-499a	Homo sapiens	34	Download	Visualize
miR-548ay	Homo sapiens	49	Download	Visualize
miR-1296	Homo sapiens	40	Download	Visualize
miR-548g	Homo sapiens	39	Download	Visualize
miR-382	Homo sapiens	25	Download	Visualize
miR-153-2	Homo sapiens	28	Download	Visualize
miR-512-1	Homo sapiens	9	Download	Visualize
miR-508	Homo sapiens	39	Download	Visualize
miR-363	Homo sapiens	5	Download	Visualize
miR-512-2	Homo sapiens	15	Download	Visualize
miR-1271	Homo sapiens	8	Download	Visualize
miR-146b	Homo sapiens	30	Download	Visualize

C. Identified RNAs that contain the queried motif with available 3D structures in PDB

PDB ID	PDB Link	Motif Location
6ddg	Link	2048
3i55	Link	2553
1yjn	Link	2553
3g6e	Link	2553
5t7v	Link	2048
6qyz	Link	90
3ccl	Link	2553
3cc7	Link	2553
5an9	Link	1470
6s0z	Link	2048
5ah5	Link	8

D.



ENERGY = -42.5 hsa-mir-153-2

A. The input parameters include: 1. motif sequence for Strand1 (**ua**); 2. motif sequence for Strand2 (**ug**); 3. Option of species database (**Homo sapiens**); 4. Choice of secondary structure prediction software (**RNAstructure**); 5. Queried motif type (**Internal Loop**); and 6. Option to search PDB or not (**Yes**). Once search is submitted, miRmo provides a unique link for users to check back with the result. Users can submit as many searches as they want, no abuse of the system though. **B-D:** Part of the screening result with “ug” and “ua” as the query motif. **B.** A table of miRNAs containing the queried motif in their precursor pre-miRNAs. The first column includes the miRNA names linked to their annotations in miRbase; the second column indicates the miRNA species; the third column labels the starting position of the queried motif in each pre-miRNA; the fourth column is a link to download the secondary structures of pre-miRNAs in .ct format; the final column links to the secondary structure images of pre-miRNAs. If no miRNA is found, the table would be shown as blank. **C.** All RNA structures in PDB that contain the queried motif, with links to their PDB resources. **D.** An example of the secondary structure image of miR-153-2 which contains the queried internal loop motif starting at the 28th nucleoside, as indicated by the red arrow. The secondary structure energy is calculated by RNAstructure. MI0000464 is the miRBase ID of miR-153-2.