
miRNAture

Release Alpha

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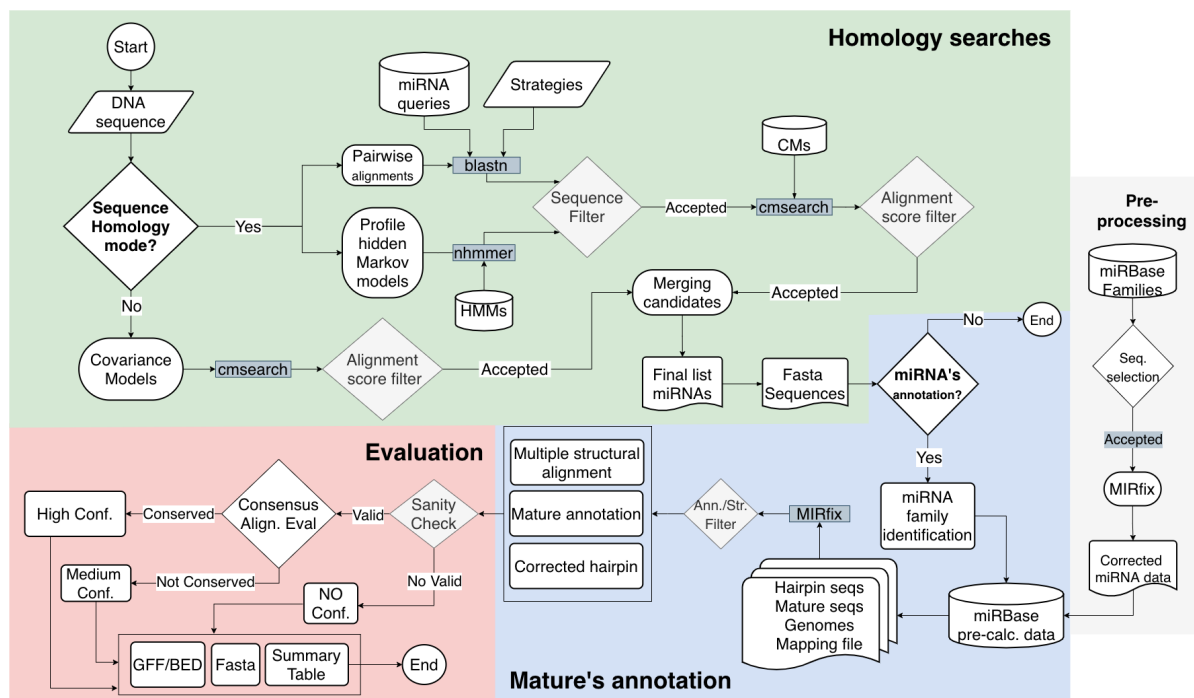
Computational detection of microRNA candidates.

INTRODUCTION

miRNAture is used to annotate homologs of metazoan microRNAs, discovered by means of sequence, structural validation and correct position of their *mature* sequences, detected on a harboring-hairpin-loop structure. This computational strategy enables an automated annotation workflow to search *bona fide* miRNA candidates, based on pairwise comparisons and hidden Markov profiles, at sequence level. The secondary structure is evaluated using covariance models and evaluating the correct folding of the hairpin sequences, based on the position of the *mature* sequences.

STRATEGY

Current approaches to computational miRNA detection relies on homology relationships or detection of hairpin-loop candidates with lower folding energy. A complete set of tools to automatize this task have been assembled on *miRNA*ture. This current approach combines two different sequence-homology modes, using *blast* or *HMMer*, and a secondary structure validation step, performed by the *INFERNAL* package. Combination of different strategies and the modification of default covariance models, let *miRNA*ture report not only the homology relationships, but also define positions of *mature* sequences and the correct miRNA annotation, supported by multiple family specific alignments. Current workflow is depicted as follows:



INSTALLATION

Download the source code or clone the miRNature project located on [Github](#). On the `Code/` folder execute the file:

```
./activate_environment_conda.sh
```

Which will create the miRNature conda repository (based on the `miRNature.yml` file) and will install all the required programs to run miRNature.

3.1 Tutorial

3.1.1 Annotating (some) coelacanth miRNAs

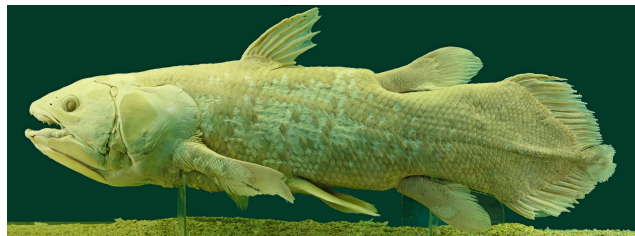


Fig. 1: *Latimeria chalumnae*. Source: [Alberto Fernandez Fernandez / CC BY-SA](#)

Through this step-by-step tutorial you could make use of key options from miRNature to annotate the *bona fide* miRNA complement on selected contigs from the coelacanth (*Latimeria chalumnae*) genome, based on the current miRNA annotation, retrieved from Ensembl release 100. The following table shows the features from selected contigs that composed the fasta file with subject sequences:

Contig	Length (Mb)	Numb. miRNAs
JH126571.1	5.98145	5
JH126620.1	3.03251	8
AFYH01291077.1	0.00106	1

Your task will be the identification of homologous miRNAs on the described contigs. To perform this task, miRNature makes use of pairwise alignments with `blastn` and the use of hidden Markov models using `nhmmer`. To the validation steps rounds of structural alignments, using `cmsearch`, would be applied. The final validation step, will be performed by `MIRfix` in order to annotate the correct positions of candidate mature regions along the detected hairpin sequence.

As you can imagine, there would be created both, a high number of input and output files and classification rules to parse and select the candidate miRNA regions. But, do not worry too much about this! life is too short to perform all of this task by hand! and miRNature will help to perform all the heavy and painstaking work.

Folder structure

The folder tree on miRNature looks like:

```
$ tree -L 1 miRNature/
miRNature/
├── Code
├── LICENSE
├── Manuscript
└── README.md
```

Out target folder is located on Code/Tutorial:

```
$ cd Code/Tutorial
$ tree -L2 .
Tutorial
├── Code
│   └── run_miRNature.sh
├── Data
│   ├── annotated_miRNAs_latch.gff3
│   ├── latimeria_chalumnae_genome.fa
│   ├── Latimeria_chalumnae.LatChal.100.gff3
│   └── QueriesToTest
└── Results
```

The Tutorial folder is composed by the subfolders: Code/, where all the necessary scripts to run miRNature are located. Data/ keeps the described genome from coelacanth in a multi-fasta format in latimeria_chalumnae_genome.fa. Another key folder is QueriesToTest/, where the miRNAs from 11 chordates were provided to serve as query sequences. Detailed list of query species and their correspondent files are described on QueriesToTest/queries_description.txt. The set of files inside Data/ corresponds to the current and filtered miRNA annotation of coelacanth retrieved from Ensembl release 100: Latimeria_chalumnae.LatChal.100.gff3 and annotated_miRNAs_latch.gff3 in GFF3 format, respectively. The last Results folder will conserve all the output files generated by miRNature.

Input files

As described earlier, to run miRNature just go directly to Code/, located on the Tutorial/ folder:

```
$ cd Code/
$ ls -ls
4 -rwxr-xr-x 1 cristian students 598 Jul  8 18:47 run_miRNature.sh
```

As you noted, exists a bash file inside this folder which will organize all our code to run miRNature. This way is preferred, if you think about increase the reproducibility of your computational experiments. Looking in detail this code will give you a general idea to run miRNature:

```
#!/bin/bash

# Declare input folder
current=$( pwd )
```

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```

# Step 1: Activate conda environment
conda activate miRNAture

# Step 2: Input files/folders
specie_tag="Lach"
specie_genome="$current/../../Data/latimeria_chalumnae_genome.fa"
specie_name="Latimeria_chalumnae"
workdir="$current/../../Results"
mirfix_path="/homes/biertank/cristian/Projects/MIRfix/scripts/MIRfix.py"
mode="Blast,HMM,Infernal,Final"
strategy="1,2,3,4,9,10,ALL"
blastQueriesFolder="$current/../../Data/QueriesToTest"

# Step 3: Running miRNAture
cd $current/../../Code/

# Step 3.1: Run homology-searches:
./miRNAture -stage homology -speG $specie_genome -speN $specie_name \
-speT $specie_tag -w $workdir -mfx $mirfix_path -m $mode -pe 0 \
-str $strategy -blastq $blastQueriesFolder

#Step 3.2: Validate miRNAs annotating their mature sequences:
./miRNAture -stage validation -speG $specie_genome -speN $specie_name \
-speT $specie_tag -w $workdir -mfx $mirfix_path -m $mode -pe 0 \
-str $strategy -blastq $blastQueriesFolder

```

The last script shows three steps that are required to run miRNAture:

1. Activate the conda environment called miRNAture. The installation and activation of this environment is required previously to run miRNAture. All the dependences are described on the file miRNAture.yml, located on the miRNAture/Code/ folder.
2. Declare the name of input and output locations. miRNAture detects different flags with their correspondent values. The basic configuration is composed by:
 - Specie genome: Current target sequence
 - Specie name: Scientific name of the specie which belongs the subject sequence(s).
 - Specie tag: Tag of the specie name, suggested one takes the first two letters from the Genera joined with the first two from the specie (i.e Homo sapiens = hosa, Didemnum vexillum = dive, Latimeria chalumnae = lach).
 - Working directory: Output directory, final path of miRNAture results.
 - MIRfix path: path of MIRfix on your system.
 - Running mode: Select at least one, or any combination of the miRNA search strategies between: Blast, HMM or/and Infernal. At the same time, to merge the complete results from those homology search modes, write at the end ``Final.
 - Blast strategies: Write the numbers of desired blastn strategies. Possible strategies are: 1, 2, 3, 4, 5, 6. At the same time, to merge all results put at the end ALL.
 - Path of blastn queries: Declare the path of annotated query sequences of miRNAs. In this case is enough to indicate the folder name.
3. Run miRNAture. Setup all the command line options based on the described input files on step 2. The list of complete flags can be found at:

```
$ ./miRNAture --help
Usage:
./miRNAture [-options]

Options:
-help          print this documentation

-man           Prints the manual page and exits.

-stage         Selects the running mode of miRNAture. The options are:
               'homology', 'validation' or 'complete'.

-speG          path of target genome or genomic sequence to be analyzed

-speN          Specie or sequence source's scientific name. The format must
               be: Genera_specie, separated by '_'.

-speT          Tag of the specie, sequence or project. Just for future
               reference.

-w            Path of working directory

-mfx           Path of the MIRfix
               <https://github.com/Bierinformatik/MIRfix> program:
               "MIRfix.py"

-m            Homology search modes: Blast,HMM,Infernal and Final. It is
               possible to perform individual analysis, but it is always
               desirable include the Final option.

-str           This flag is blast specific. It corresponds to the selected
               blast strategies used to search miRNAs. It might be
               indicated along with -m Blast or in case you refer it in
               your selected mode.

-blstq        Path of blast queries sequences in fasta format to be
               searched on the subject sequence.
```

Searching miRNAs

The most important step will be performed! Based on the last configurations on the script `run_miRNAture.sh`, `miRNAture` will be executed on the designed coelacanth sequences. The idea is to perform independently each of the stages, *homology-searches* and *detection of mature*, for demonstrative purposes. In case that you require to run the complete pipeline, just adjust the parameter **-stage** to the *complete* option.

Homology search

As mentioned, we are going to execute the *homology-search* stage. To activate this stage in miRNAture please verify the flag value to be **-stage homology**. In brief, our *target* coelacanth sequences would be annotated using a set of miRNA *queries* that belong from the following chordate species, (V: vertebrata, T: tunicata and C:cephalochordata) and one echinoderm (E):

- *Anolis carolinensis* (V)
- *Branchiostoma belcheri* (C)
- *Branchiostoma floridae* (C)
- *Ciona robusta* (T)
- *Ciona savignyi* (T)
- *Danio rerio* (V)
- *Eptatretus burgeri* (V)
- *Petromyzon marinus* (V)
- *Strongylocentrotus purpuratus* (E)
- *Xenopus laevis* (V)
- *Xenopus tropicalis* (V)

We are going to test all the capability of miRNAture, using at the same time all the available modes: Blast, HMM, Infernal and the final concatenation with Final. Specifically for the pairwise-comparisons with Blast mode, we are going to use only 3 strategies: 1,9,10 and the final concatenation and comparison with ALL, but feel free to choose more or less strategies.

Then, just let it run typing:

```
./run_miRNAture.sh homology
```

A long descriptive output will be printed on the screen. Keep an eye on the Results/ folder where the action is taking place.

Note: Keep in mind that `run_miRNAture.sh` was created as an example to run miRNAture but it does not mean that is the only way to do that. Change it according your requirements.

Homology search results

To refer directly to the results, type:

```
$ cd ../Results/
$ tree -L 1
Results/
├── Blast
├── Final_Candidates
├── HMMs
├── Infernal
├── miRNAture_log_190609072022.log
├── miRNAture_log_22505108072023.log
└── miRNAture_log_23590008072025.log
```

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```
└─ miRNature_log_4209072026.log
└─ mirnature_runLatch.sh
```

If everything goes well, you could see 4 log files `miRNA_log_*.log`, a script generated automatically to run the search strategy on miRNature (`mirnature_runLATCH.sh`) and the folders with homology comparisons: `Blast/`, `HMMs` and `direct structure comparison: Infernal` and the `Final_Candidates` with the final set of homology predicted miRNAs. Next, go directly to the `Final_Candidates` folder:

```
$ cd Final_Candidates/
$ tree -L 1
├── all_RFAM_Latch_Final.ncRNAs_homology.txt
├── all_RFAM_Latch_Final.truetable
├── all_RFAM_Latch_Final.truetable.discarded.table
├── all_RFAM_Latch_Final.truetable.joined.table
├── all_RFAM_Latch_Final.truetable.joined.table.db
├── all_RFAM_Latch_Final.truetable.temp
└── Fasta
```

Where the most important file is `all_RFAM_Latch_Final.ncRNAs_homology.txt`, which reported all the merged candidates to miRNAs on the subject contigs from coelacanth. The results are summarised on the following table:

Contig	Length (Mb)	Numb. miRNAs	miRNAture Pred.
JH126571.1	5.98145	5	122
JH126620.1	3.03251	8	106
AFYH01291077.1	0.00106	1	0

The final results could be discriminated by the annotation method (Blast, HMM or Infernal):

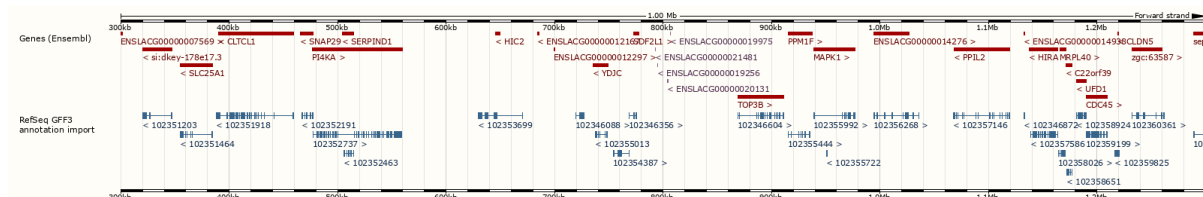
Contig	Blast	HMM	Infernal	miRNature Pred.
JH126571.1	22	7	93	122
JH126620.1	35	6	65	106

and even, this set of computational annotations could be visualized on a broad genome context, generating for example a **BED** file and uploading it at the Coelacanth Ensembl Genome Browser, using some Linux commands as follows:

```
$awk '{print $1"\t"$6"\t"$7"\t"$8"\t"$2"\t"$3}'
all_RFAM_Latch_Final.ncRNAs_homology.txt > predicted_miRNature.bed
```

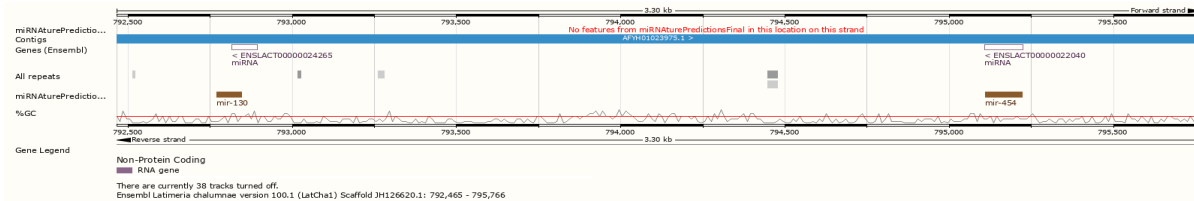
Next, just upload the track on the corresponding Genome Ensembl hub (as explained in more detail [here](#)) as a Custom Track.

Certainly, after uploading this miRNAs coordinates you would visualize this results:

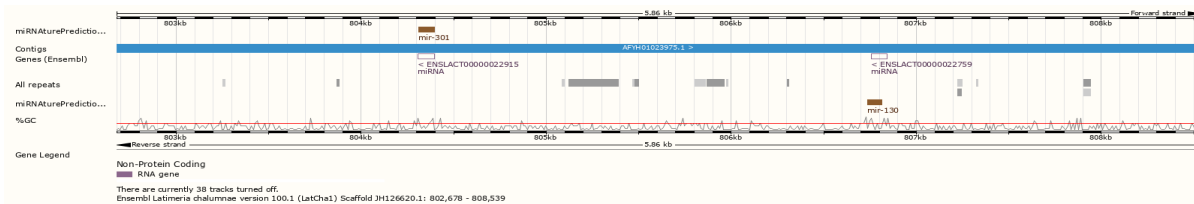


This image corresponds to the region JH126620.1:788915–822338, which according to the Ensembl annotation, exists 4 miRNA genes of the families: mir-130 (ENSLACG00000021481), mir-454 (ENSLACG00000019256), and two of mir-130 (ENSLACG00000020131, ENSLACG00000019975)

Here, miRNAture detected the same families, with overlapping regions on the previously reported miRNAs on *L. chalumnae*.



And this is the second cluster, with two families, the overlapping is the same but in one miRNA the family prediction and the strand differ:



For that reason, those candidates required a complementary evaluation of their current detection and correct positioning of the *mature* miRNA sequences. As a final result, you could check that all the reported miRNAs on the contigs JH126571.1 and JH126620.1 were identified. The reported miRNA on AFYH01291077.1, was predicted as a miRNA using RFAM, but currently there is no information about the family or mature products. miRNAture detected this candidate on the direct Infernal searches, but it did not show an acceptable homology (for mir-105 family) and folding values (Bitscore: 13.4 and E-value 7.8), see file Results/miRNA_predictionInfernal/Latch/RF00670_Latch.tab.

On the other side, miRNAture detected new candidates that currently are not reported on the genome annotation.

Validation of miRNA candidates

An additional output was generated on the Final_results/ folder and contained all the resulted fasta sequences from the last 228 hairpin candidates, organized by their Rfam family. Based on those regions, validated by sequence and structure homology, the idea is to evaluate the annotation of candidate *mature* regions that are contained in this hairpin-loop and validate their annotation with an additional layer, supported by the structural alignments of sequences selected from other organisms.

To do that, please execute again the script:

```
$. /run_miRNAture.sh validate
```

which essentially have the same input parameters, except for the `-stage validation` flag that was changed to tell miRNAture that the second stage have to be activated.

In this step, each detected miRNA candidate were grouped by their Rfam miRNA family. Based on this reference, previously calculated data from the family is retrieved. This input data, required to perform the correction of the *mature* sequences using the MIRfix program, was inferred as a product of this study¹ and comprises this set of files:

- The set of Rfam hairpin sequences.
- The mature sequences annotated for each Rfam hairpin sequence.
- The genomes/contigs/sequences that contained the Rfam sequences.

¹ From the *seed* sequences from Rfam v.12.2 and additionally the sequences from Rfam and miRBase that reported *mature* sequences.

- A mapping file, which explicitly declares the relation between hairpin and their mature sequences.

For more details refer to the [MIRfix repository](#). Automatically, miRNA^ture structure all your data and generate the required input files to perform the *mature* annotation.

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