miRNAture

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CONTENTS:

Computational detection of microRNA candidates

CHAPTER

ONE

INTRODUCTION

MicroRNAs (miRNAs) have been characterized as an important regulators in almost all animals and plants, as well as in unicellular eukaryotes [11]. Since their discovery in 1993 [10] and subsequently their recognition as a biological entity later from 2000 [14], microRNAs were identified as key regulators on the temporal control of heterochronic genes on the nematode *Caenorhabditis elegans* as well as in another metazoan species, too [12]. This recognition was complemented by a complete characterization of their typical features as: a stem-loop structure, well-conservation over multiple metazoan clades and typical expression patterns as isolated locus or co-expressed as polycistronic miRNA transcripts [7][8][9][14].

Currently, metazoan miRNAs have been recognized as a conserved group of short ncRNAs, typically ~ 22 nt, with important roles as post-transcriptional regulators of the gene expression affecting a sizeable number of mRNAs and expressed in all developmental process and diseases [2]. Their canonical biogenesis starts in the form of primary precursors (pri-miRNAs) transcribed from long non-coding RNAs or protein-coding transcripts, mostly from introns [17]. Later, derived from hairpin-like precursors excised in the nucleus (pre-miRNAs), their acting form is subsequently further processed as miRNA/miRNA* duplexes on the cytoplasm and incorporated into the RISC complex. Target specificity is achieved by complementarity between the miR and mRNA sequence. (see more details in [2]).

Current classification of annotated miRNAs into families are available in miRBase[1] and mirGeneDB[2] databases. As an example, the human genome reported 1984 miRNA precursors in the miRBase v.22.1 [6] and the corresponding mature products were estimated ~2300 [1]. Focusing on the *confidently canonical* miRNAs reported in [4], the number of miRNAs is 519. Those differences are explained on the basis of multiple *miRNA* detection methods as well as the intrinsic definitions to define a *canonical miRNA*.

Despite the small size of the precursors (80-100 nt), sequence comparison methods are able to detected them, due a high level of sequence conservation [13]. In one hand, it is important to point that the use of blast-based homology searches alone tend to produce false positives that require extensive curation, which relies on properties obtained from miRNAs [15]. On the other hand, the inclusion of the consensus structure complements the homology search methods, for example using covariance models (CMs) [3][5]. The accuracy and sensitivity of CMs depends critically on the sequence alignment and the annotated consensus used to build the model. Those observations call for an integrated workflow to perform homology search and to evaluate their results in a consistent manner.

In this computational approach, focused on the *canonical* miRNAs processed by Drosha and Dicer, we improve on ideas from MIRfix [16] and integrate it with homology search. `miRNAture` is used to identify and annotate homologs of metazoan microRNAs in a homology-based setting.

[1] https://www.mirbase.org/
[2] https://www.mirgenedb.org/

CHAPTER

TWO

INSTALLATION

The easiest way to install `miRNAture` is through *conda*. To do so, please first install Conda[1].

To speed up installation of dependencies and packages we suggest to use mamba[2], for this just run:

```
conda install mamba -c conda-forge
```

You can use *mamba* as drop-in replacement for *conda* by simply replacing the call to *conda* with a call to *mamba*.

Install via Conda

To install `miRNAture` from *conda* in a specific *mirnature* environment simply run:

```
mamba create -n mirnature mirnature
```

if *mamba* is available, else run:

```
conda create -n mirnature mirnature
```

Manual install, resolve dependencies via Conda

Create a *mirnature conda* environment with the file *miRNAture.yml*:

```
mamba env create -n mirnature -f miRNAture.yml
```

Activate the environment containing all dependencies:

```
conda activate mirnature
```

followed by the manual steps:

```
perl Build.PL
./Build
./Build test
./Build install
```

which will install `miRNAture` in the *mirnature conda* environment.

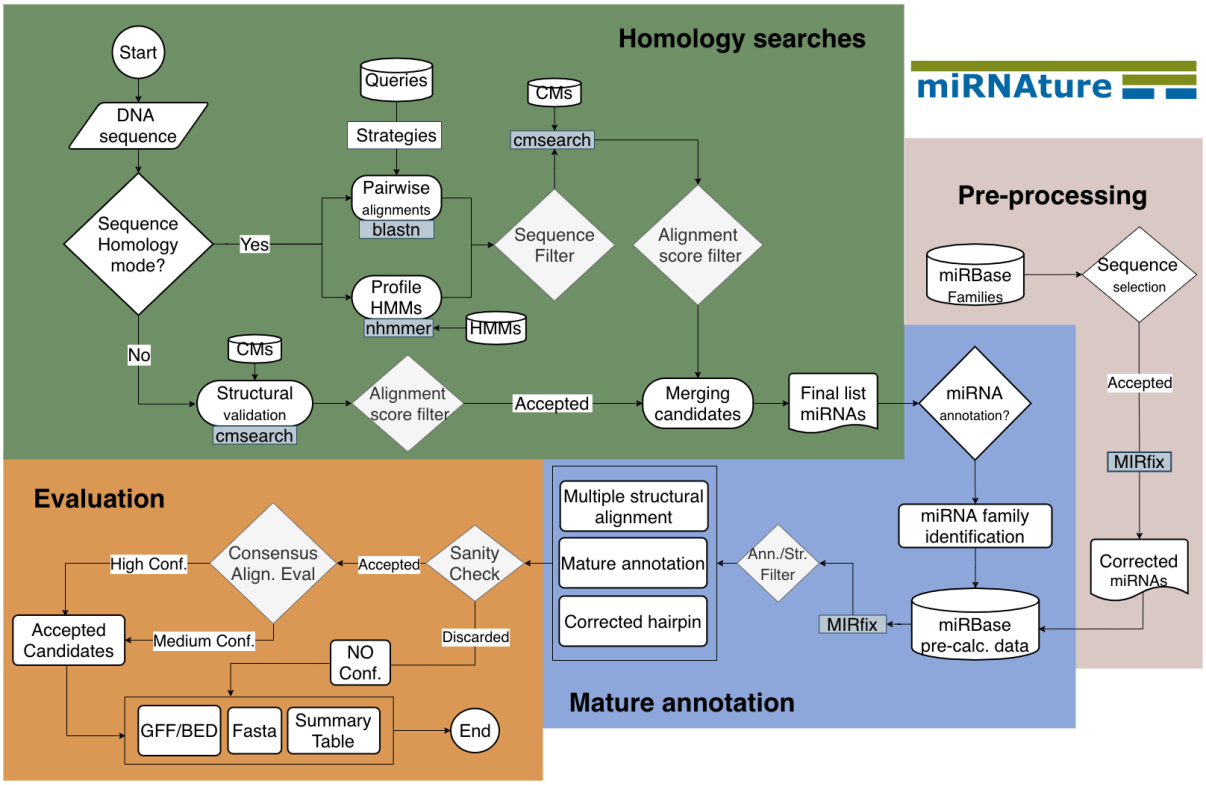
[1] https://docs.conda.io/projects/conda/en/latest/user-guide/install/
[2] https://github.com/mamba-org/mamba

CHAPTER

THREE

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 `miRNAture`. This current approach combines two different sequence-homology modes, using `blast` or `HMMer`, and a secondary structure validation step, performed by the `INFERNAL` package. Current workflow is depicted as follows:



CHAPTER

FOUR

TUTORIAL

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. All the required files to execute this tutorial are included in the `miRNAture` source files in the *miRNAture/Tutorial* folder.

4.1 Annotating let-7 on coelacanth

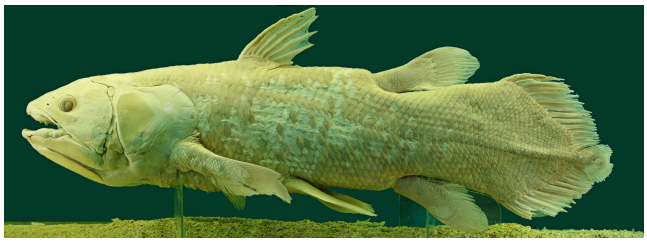


Fig. 1: *Latimeria chalumnae*. Source: Alberto Fernandez Fernandez / CC BY-SA

Based on the miRNA annotation retrieved from `Ensembl` release 100, the coelacanth genome featured 8 let-7 locus distributed along 6 contigs. For purposes of this tutorial, 3 contigs were selected with variable number of miRNA/let-7 annotations, as follows:

Contig	Length (Mb)	Numb. miRNAs	Let-7 loci
JH126571.1	5.981	5	1
JH129429.1	0.248	3	3
AFYH01291077.1	0.001	1	0

The main goal is the identification of the let-7 loci on the referred contigs. To do so, the *Tutorial* folder contained all the input files in *Data/*, required wrapper to *miRNAture* on *Code/* and a *Results/* folder where all prediction will be stored. As you can imagine, homology comparisons are prone to create both, a high number of input and output files. *miRNAture* avoids manual curation to the detected hits, life is too short to perform all of those steps by hand!

4.1.1 Folder structure

The folder tree on miRNAture looks like:

```
$ tree -L 1 miRNAture/
miRNAture/
├── Build.PL
├── Changes
├── ignore.txt
├── index.md
├── lib/
├── LICENSE
├── MANIFEST
├── META.json
├── META.yml
├── mirnature_logo.png
├── miRNAture-Manual/
├── miRNAture.yml
├── README
├── README.md
├── README.rst
├── script/
├── t/
├── Tutorial/
└── xt/
```

Our target folder is located in `Tutorial/`:

```
$ cd Tutorial/
$ tree -L2 .
Tutorial/
├── Code
│   ├── list_miRNAs_to_search.txt
│   ├── Precalculated-Data-tutorial
│   ├── tutorial_test_selected_models.sh
│   └── User_Test_Data
├── Data
│   ├── latimeria_chalumnae_genome.fa
│   └── 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 contigs from coelacanth in a multi-fasta file:`latimeria_chalumnae_genome.fa`. In the same folder, in `QueriesToTest/` let-7 annotations from 11 metazoans[1] were provided as queries.

Note: Together with the query files, the file *queries_description.txt* is required to control which dataset of sequences that will be used by the *blastn* comparisons. Three columns are needed to be recognized:

<Name_fasta_file.fa> miRNA <Origin_of_sequence>

The first one corresponds to the file name, the second one have to be miRNA, the third one is the name of the source specie in the format: *Genera specie*. If you do not known the source, a valid name would be: Unknown specie. If ommited, `miRNAture` will create automatically this file using all fasta files in this folder with an Unknown origin.

---

[1] *Anolis carolinensis*, *Branchiostoma belcheri*, *Branchiostoma floridae*, *Ciona robusta*, *Ciona savignyi*, *Danio rerio*, *Eptatretus burgeri*, *Petromyzon marinus*, *Strongylocentrotus purpuratus*, *Xenopus laevis* and *Xenopus tropicalis*.

The `Results/` folder will conserve all the output files generated by `miRNAture`.

4.1.2 Input files

To run `miRNAture` just go directly to `Code/` folder:

```
$ cd Code/
$ tree -L 1 .
.
├── list_miRNAs_to_search.txt
├── Precalculated-Data-tutorial
├── tutorial_test_selected_models.sh
└── User_Test_Data
```

In this path, the `tutorial_test_selected_models.sh` file is `bash` script that will organize all our code to run `miRNAture`. This way is preferred in terms of reproducibility means of your computational experiments. This code will give you a general idea to run `miRNAture`, let's explain this in detail:

```
#!/bin/bash

current=$( pwd )
specie_tag="Lach"
specie_genome="$current/../Data/latimeria_chalumnae_genome.fa"
specie_name="Latimeria_chalumnae"

workdir="$current/../Results"
mkdir -p $workdir
mode="Blast,HMM,Infernal,Other_CM,Final"
strategy="5,6,ALL"
blastQueriesFolder="$current/../Data/QueriesToTest"
user_models="$current/User_Test_Data"
data_precalculated_folder="$current/Precalculated-Data-tutorial"

### Step by step: homology->validation->evaluation->summarise
# Run only homology-searches
#miRNAture -stage homology -sublist $current/list_miRNAs_to_search.txt \
#    -dataF $data_precalculated_folder -speG $specie_genome -speN $specie_name \
#    -speT $specie_tag -w $workdir -m $mode -pe 0 -str $strategy \
#    -blastq $blastQueriesFolder -rep relax,150,100 -usrM $user_models
# Run detection matures
#miRNAture -stage validation -dataF $data_precalculated_folder -speG $specie_genome \
#    -speN $specie_name -speT $specie_tag -w $workdir -m $mode -pe 0 -usrM $user_
↪models
# Run the complete analysis
#miRNAture -stage evaluation -dataF $data_precalculated_folder -speG $specie_genome \
#    -speN $specie_name -speT $specie_tag -w $workdir -m $mode -pe 0
# Create summarise report
#miRNAture -stage summarise -dataF $data_precalculated_folder -speG $specie_genome \
#    -speN $specie_name -speT $specie_tag -w $workdir -m $mode -pe 0

# Run miRNAture complete
miRNAture -stage complete -sublist $current/list_miRNAs_to_search.txt \
    -dataF $data_precalculated_folder -speG $specie_genome -speN $specie_name \
    -speT $specie_tag -w $workdir -m $mode -pe 0 -str $strategy \
    -blastq $blastQueriesFolder -rep relax,150,100 -usrM $user_models
```

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.

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

Declare the name of input and output locations. This will help to assign miRNAture flags and easily reproduce the experiment. In this case, we used the following options (flags indicated in parenthesis):

- Processing stage (-stage): Running stage on miRNAture. In this case was selected complete to run all the stages. To run step by step, this flag accepts: homology, validation, evaluation and summarise. You should run all of them in this order to obtain the same final results as complete option.
- Subset of miRNA models to run (-sublist): Subset of miRNA families references to be searched on the target sequence. See list_miRNAs_to_search.txt file as an example. If not provided all miRNA RFAM models will be searched.
- Pre-calculated data location (-dataF): Location of pre-calculated data required by miRNAture. It included hidden markov, covariance models and curated input files to annotate mature sequences[2].
- Specie genome (-speG): Current target sequence.
- Specie name (-speN): Scientific name of the specie which belongs the subject sequence(s).
- Specie tag (-speT): 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).
- Working directory (-w): Output directory, final path of miRNAture results.
- Running mode (-m): Select at least one, or any combination of the miRNA search strategies between: Blast, HMM, Infernal and Other_CM. At the same time, to merge the complete results from those homology search modes, write at the end Final.
- Parallel jobs using SLURM (-pe): Activate (1) or not (0).
- Blast strategies (-str): Write the numbers of desired blastn strategies. Possible strategies are: 1,2,3,4,5,6. To merge all results put at the end ALL.
- Path of blastn queries (-blastq): Declare the path of annotated query sequences of miRNAs. In this case is enough to indicate the folder name.
- Homology repetition detection (-rep): Setup number of maximum loci number that will be evaluated by the mature annotation stage. By default, miRNAture will detect miRNA families that report high number of loci (> 200 loci). Then, it will select the top 100 candidates in terms of alignment scores, as candidates for the validation stage (default,200,100). Modify this values using relax,Number_Loci,Candidates_to_evaluate.
- User hidden markov/covariance models (-usrM): Directory with additional hidden Markov (HMMs) or covariance models (CMs) provided by the user to be searched on the target sequence.

Then, run miRNAture through this script:

```
$ ./tutorial_test_selected_models.sh
```

Note: The list of complete flags can be found typing: miRNAture -h or miRNAture -man.

[2] Pre-calculated data should be downloaded from https://zenodo.org/record/4531376#.YDqO4-bTVTZ

CHAPTER

FIVE

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CHAPTER

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