# Contents

1 Installation ................................................. 3
   1.1 Seqcluster ........................................... 3
   1.2 Tools dependencies for a full small RNA pipeline .... 4
   1.3 Data .................................................. 4
   1.4 R package ............................................ 4

2 Citation ..................................................... 7

3 Getting started ............................................ 9
   3.1 Clustering of small RNA sequences .................... 9
   3.2 Interactive HTML Report ............................... 11
   3.3 Easy start with bcbio-nextgen.py .................. 12

4 Outputs .................................................... 13
   4.1 seqcluster .......................................... 13
   4.2 Report .............................................. 13

5 Examples of small RNA analysis .......................... 15
   5.1 miRQC data .......................................... 15

6 miRNA annotation .......................................... 17
   6.1 Processing of reads .................................. 17
   6.2 Prepare databases .................................... 18
   6.3 miRNA/isomiR annotation with JAVA ............... 18
   6.4 Convert to GFF3-srna ................................ 18
   6.5 Post-analysis with R ................................ 19
   6.6 Manual of miraligner(JAVA) .......................... 19

7 Collapse fastq(.gz) files .................................. 21

8 Handling multi-mapped reads .............................. 23

9 Tools for downstream analysis ............................ 25
   9.1 Web-servers .......................................... 25
   9.2 Command-lines ....................................... 25

10 Relevant papers about isomiRs and other novel small RNAs with functional relevance 27
   10.1 Validation ........................................... 27
Analysis of small RNA sequencing data. It detect unit of transcription over the genome, annotate them and create an HTML interactive report that helps to explore the data quickly.

Contents:
CHAPTER 1

Installation

1.1 Seqcluster

With bcbio installed

If you already have `bcbio`, seqcluster comes with it. If you want the last development version:

```
/bcbio_anaconda_bin_path/seqcluster_install.py --upgrade
```

Docker:

```
docker pull lpantano/smallsrna
```

Bioconda binary

install conda if you want an isolate env:

```
wget http://repo.continuum.io/miniconda/Miniconda-latest-Linux-x86_64.sh
bash Miniconda-latest-Linux-x86_64.sh -b -p ~/install/seqcluster/anaconda
```

You can install directly from binstar (only for linux):

```
~/install/seqcluster/anaconda/conda install seqcluster seqbuster bedtools samtools
   --> pip nose numpy scipy pandas pyvcf -c bioconda
```

With that you will have everything you need for the python package. The last step is to add seqcluster to your PATH if conda is not already there.

Go to Tools dependecies below to continue with the installation.

Note: After installation is highly recommended to get the last updated version doing:

```
seqcluster_install.py --upgrade
```

automated installation
Strongly recommended to use `bcbio` installation if you work with sequencing data. But if you want a minimal installation:

```
pip install fabric
seqcluster_install --upgrade
mkdir -p $PATH_TO_TOOLS/bin
seqcluster_install --tools $PATH_TO_TOOLS
```

After that you will need to add to your path: `export PATH=$PATH_TO_TOOLS/bin:$PATH`

### 1.2 Tools dependencies for a full small RNA pipeline

For seqcluster command:

- bedtools
- samtools
- rnafold (for HTML report)

For some steps of a typical small RNA-seq pipeline (recommended to use directly `bcbio`):

- STAR, bowtie
- fastqc
- cutadapt (install with bioconda using the same python env than seqcluster.

You will need to link the cutadapt binary to your PATH!

### 1.3 Data

Easy way to install your small RNA seq data with cloudbiolinux. Seqcluster has snipped code to do that for you. Recommended to use `bcbio` for the pipeline since will install everything you need in a single step `bcbio_nextgen.py upgrade -u development --tools --genomes hg19 --aligners bowtie`

But if you want to run seqcluster step by step an example of hg19 human version it will be (another well annotated supported genome is mm10):

```
seqcluster_install --data $PATH_TO_DATA --genomes hg19 --aligners bowtie2 --datatarget smallrna
```

If you want to install STAR indexes since gets kind of better results than bowtie2 (warning, 40GB memory RAM needed):

```
seqcluster_install --data $PATH_TO_DATA --genomes hg19 --aligners star
```

### 1.4 R package

Install isomiRs package for R using devtools:

```
devtools::install_github('lpantano/isomiRs')
```
To install all packages used by the Rmd report:

```
Rscript -e 'source(https://raw.githubusercontent.com/lpantano/seqcluster/master/
  scripts/install_libraries.R)'
```
Please if you use seqcluster make sure to cite the other tools are integrated here:


Best practices are implemented in a python framework.

### 3.1 clustering of small RNA sequences

seqcluster generates a list of clusters of small RNA sequences, their genome location, their annotation and the abundance in all the sample of the project

---

**REMOVE ADAPTER**

I am currently using `cutadapt`:

```
cutadapt --adapter=$ADAPTER --minimum-length=8 --untrimmed-output=sample1_notfound. --fastq -o sample1_clean.fastq -m 17 --overlap=8 sample1.fastq
```

**COLLAPSE READS**
To reduce computational time, I recommend to collapse sequences, also it would help to apply filters based on abundances. Like removing sequences that appear only once.

```
seqcluster collapse -f sample1_clean.fastq -o collapse
```

Here I am only using sequences that had the adapter, meaning that for sure are small fragments.

**PREPARE SAMPLES**

```
seqcluster prepare -c file_w_samples -o res --minl 17 --minc 2 --maxl 45
```

the file_w_samples should have the following format:

```
lane1_sequence.txt_1_1_phred.fastq cc1
lane1_sequence.txt_2_1_phred.fastq cc2
lane2_sequence.txt_1_1_phred.fastq cc3
lane2_sequence.txt_2_1_phred.fastq cc4
```

two columns file, where the first column is the name of the file with the small RNA sequences for each sample, and the second column in the name of the sample.

The fastq files should be like this:

```
@seq_1_x11
CCCCGTCCCTCCTCCTCC
+
QUALITY_LINE
@seq_2_x20
TGCGCAGTGGCAGTATCGT
+
QUALITY_LINE
</pre>
```

Where _x[09] indicate the abundance of that sequence, and the middle number is the index of the sequence.

This script will generate: seqs.fastq and seqs.ma. * seqs.fastq: have unique sequences and unique ids * seqs.ma: is the abundance matrix of all unique sequences in all samples

**ALIGNMENT**

You should use an aligner to map seqs.fa to your genome. A possibility is bowtie or STAR. From here, we need a file in BAM format for the next step. VERY IMPORTANT: the BAM file should be sorted

```
bowtie -a --best --strata -m 5000 INDEX seqs.fastq -S | samtools view -Sbh /dev/stdin
  | samtools sort -o /dev/stdout temp > seqs.sort.bam
```

or

```
STAR --genomeDir $star_index_folder --readFilesIn res/seqs.fastq --alignIntronMax 1 --outFilterMultimapNmax 1000 --outSAMattributes NH HI NM --outSAMtype BAM --SortedByCoordinate
```

**CLUSTERING**

```
seqcluster cluster -a res/Aligned.sortedByCoord.out.bam -m res/seqs.ma -g $GTF_FILE
  -o res/cluster -ref PATH_TO_GENOME_FASTA --db example
```

- `-a` is the SAM file generated after mapped with your tool, which input has been seqs.fa
- `-m` the previous seqs.fa
• `-b` annotation files in bed format (see below examples) [deprecated]
• `-g` annotation files in gtf format (see below examples) [recommended]
• `-i` genome fasta file used in the mapping step (only needed if `-s` active)
• `-o` output folder
• `-ref` genome fasta file. Needs fai file as well there. (i.e `hg19.fa, hg19.fa.fai`)
• `-d` create debug logging
• `-s` construction of putative precursor (NOT YET IMPLEMENTED)
• `-db` (optional) will create sqlite3 database with results that will be used to browse data with html web page (under development)

Example of a bed file for annotation (the fourth column should be the name of the feature):

```
chr1  157783  157886  snRNA  0  -
```

Strongly recommend gtf format. Bed annotation is deprecated. Go here to know how to download data from hg19 and mm10.

Example of a gtf file for annotation (the third column should be the name of the feature and the value after gene name attribute is the specific annotation):

```
chr1 source miRNA 1 11503 . + . gene name 'mir-102' ;
```

hint: scripts to generate human and mouse annotation are inside `seqcluster/scripts` folder.

**OUTPUTS**

• `counts.tsv`: count matrix that can be input of downstream analyses
• `size_counts.tsv`: size distribution of the small RNA by annotation group
• `seqcluster.json`: json file containing all information
• `log/run.log`: all messages at debug level
• `log/trace.log`: to keep trace of algorithm decisions

### 3.2 Interactive HTML Report

This will create html report using the following command assuming the output of `seqcluster cluster` is at `res`:

```
seqcluster report -j res/seqcluster.json -o report -r $GENOME_FASTA_PATH
```

where `$GENOME_FASTA_PATH` is the path to the genome fasta file used in the alignment.

**Note:** you can try our new visualization tool!

- `report/html/index.html`: table with all clusters and the annotation with sorting option
- `report/html/[0-9]/maps.html`: summary of the cluster with expression profile, annotation, and all sequences inside
- `report/html/[0-9]/maps.fa`: putative precursor

An example of the output is below:
3.3 Easy start with bcbio-nextgen.py

Note: If you already are using bcbio, visit bcbio to run the pipeline there.

To install the small RNA data:

```
bcbio_nextgen.py upgrade --tools --datatarget smallrna
```

Options to run in a cluster

It uses ipython-cluster-helper to send jobs to nodes in the cluster

- `--parallel` should set to `ipython`
- `--scheduler` should be set to `sge,lsf,slurm`
- `--num-jobs` indicates how much jobs to launch. It will run samples independently. If you have 4 samples, and set this to 4, 4 jobs will be launch to the cluster
- `--queue` the queue to use
- `--resources` allows to set any special parameter for the cluster, such as, email in sge system: `M=my@email.com`

Read complete usability here: [https://github.com/roryk/ipython-cluster-helper](https://github.com/roryk/ipython-cluster-helper) An examples in slurm system is:

```
--parallel ipython --scheduler slurm --num-jobs 4 --queue general
```

Output

- one folder for each analysys, and inside one per sample
- adapter: *clean.fastq* is the file after adapter removal, *clean_trimmed.fastq* is the collapse *clean.fastq*, *fragments.fastq* is file without adapter, *short.fastq* is file with reads < 16 nt.
- align: BAM file results from align *trimmed.fastq*
- mirbase: file with miRNA anotation and novel miRNA discovery with mirdeep2
- tRNA: analysis done with tdmapper [citation needed]
- qc: *fastqc.html* is the fastqc results from the uncollapse fastq file
- seqcluster: is the result of running seqcluster. See its documentation for further information.
- `report/srna-report.Rmd`: template to create a quick html report with exploration and differential expression analysis. See example here
4.1 seqcluster

- **counts.tsv**: count matrix that can be input of downstream analyses. *nloci* will be 0 always that the meta-cluster has been resolved successfully. For instance, it can happen that you got sequences you have a bunch of sequences mapping to hundreds of different places on the genome, then seqcluster doesn’t resolve that, and put everything under the larger region covered by those sequences. So, mainly, 0 all are good rows. The *ann* column is just where the meta-clusters overlap with. It can happen that one name appears many times if different locations of the meta-cluster map to different copies of that feature. OR if the annotation file used had multiple lines for that.

- **read_stats.tsv**: number of reads for each sample after each step in the analysis. Meant to give a hint if we lose a lot of information or not.

- **size_counts.tsv**: size distribution of the small RNA by annotation group. (position, reads, cluster)

- **seqcluster.json**: json file containing all information. This file is used as the input of the report suit.

- **log/run.log**: all messages at debug level

- **log/trace.log**: to keep trace of algorithm decisions

4.2 Report

Beside the static HTML report that you can get using report subcommand, you can download this HTML. (watch the repository to get notifications of new releases.)

- Go inside seqclusterViz folder

- Open reader.html

- Upload the seqcluster.db file generated by report subcommand.

- Start browsing your data!

Meaning of different sections:
• Top-left table shows list of meta-clusters, user can filter by number ID or keywords.
• Top-right table shows positions where this meta-cluster has been detected.
• Expression profile along precursor: Lines are number of reads in that position of the precursor. It is sum of the log2 RPM of the expression for each sample.
• Table: raw counts for each sample and sequence. Only top 100 are shown.
• secondary structure: The region with more sequences inside meta-cluster is used to plot the secondary structure. Colors refers to abundance in each position. Darker means more abundance.

An example of the HTML code: _ ..examples
Examples of small RNA analysis

5.1 miRQC data

About

mirQC project

samples overview:

Universal Human miRNA reference RNA (Agilent Technologies, #750700), human brain total RNA (Life Technologies, #AM6050), human liver total RNA (Life Technologies, #AM7960) and MS2-phage RNA (Roche, #10165948001) were diluted to a platform-specific concentration. RNA integrity and purity were evaluated using the Experion automated gel electrophoresis system (Bio-Rad) and Nanodrop spectrophotometer. All RNA samples were of high quality (mirQC A: RNA quality index (RQI, scale from 0 to 10) = 9.0; mirQC B: RQI = 8.7; human liver RNA: RQI = 9.2) and high purity (data not shown). RNA was isolated from serum prepared from three healthy donors using the miRNeasy mini kit (Qiagen) according to the manufacturer’s instructions, and RNA samples were pooled. Informed consent was obtained from all donors (Ghent University Ethical Committee). Different kits for isolation of serum RNA are available; addressing their impact was outside the scope of this work. Synthetic miRNA templates for let-7a-5p, let-7b-5p, let-7c, let-7d-5p, miR-302a-3p, miR-302b-3p, miR-302c-3p, miR-302d-3p, miR-133a and miR-10a-5p were synthesized by Integrated DNA Technologies and 5 phosphorylated. Synthetic let-7 and miR-302 miRNAs were spiked into MS2-phage RNA and total human liver RNA, respectively, at 5 x 10^6 copies/μg RNA. These samples do not contain endogenous miR-302 or let-7 miRNAs, which allowed unbiased analysis of cross-reactivity between the individual miR-302 and let-7 miRNAs measured by the platform and the different miR-302 and let-7 synthetic templates in a complex RNA background. Synthetic miRNA templates for miR-10a-5p, let-7a-5p, miR-302a-3p and miR-133a were spiked in human serum RNA at 6 x 10^3 copies per microliter of serum RNA or at 5-times higher, 2-times higher, 2-times lower and 5-times lower concentrations, respectively. All vendors received 10 μl of each serum RNA sample.

Commands

Data was download from GEO web with this script. The following 2 configs were used for the two sets: mirqc samples and non mirqc samples. Samples were analyzed with bcbio with the following commands

report

Report showing part of the output report of bcbio pipelines together with some validations are here.
miRNA annotation is running inside bcbio small RNAseq pipeline together with other tools to do a complete small RNA analysis.

For some comparison with other tools go here.

You can run samples after processing the reads as shown below. Currently there are two version: JAVA

**Naming**

See always up to date information here in mirtop open project.

It is a working process, but since 10-21-2015 isomiR naming has changed to:

- **Nucleotide substitution:** NUMBER|NUCLEOTIDE_ISOMIR|NUCLEOTIDE_REFERENCE means at the position giving by the number the nucleotide in the sequence has substituted the nucleotide in the reference. This, as well, is a post-transcriptional modification.

- **Additions at 3’ end:** 0/NA means no modification. UPPER CASE LETTER means addition at the end. Note these nucleotides don’t match the precursor. So they are post-transcriptional modification.

- **Changes at 5’ end:** 0/NA means no modification. UPPER CASE LETTER means nucleotide insertions (sequence starts before miRBase mature position). LOWER CASE LETTER means nucleotide deletions (sequence starts after miRBase mature position).

- **Changes at 3’ end:** 0/NA means no modification. UPPER CASE LETTER means nucleotide insertions (sequence ends after miRBase mature position). LOWER CASE LETTER means nucleotide deletions (sequence ends before miRBase mature position).

### 6.1 Processing of reads

**REMOVE ADAPTER**

I am currently using cutadapt.

```bash
  cutadapt --adapter=$ADAPTER --minimum-length=8 --untrimmed-output=sample1_notfound.
  fastq -o sample1_clean.fastq -m 17 --overlap=8 sample1.fastq
```

(continues on next page)
COLLAPSE READS

To reduce computational time, I recommend to collapse sequences, also it would help to apply filters based on abundances. Like removing sequences that appear only once.

```bash
seqcluster collapse -f sample1_clean.fastq -o collapse
```

Here I am only using sequences that had the adapter, meaning that for sure are small fragments. The output will be named as `sample1_clean_trimmed.fastq`

### 6.2 Prepare databases

For human or mouse, follows this instruction to download easily miRBase files. In general you only need `hairpin.fa` and `miRNA.str` from miRBase site. mirGeneDB is also supported, download the needed files here.

Highly recommended to filter `hairpin.fa` to contain only the desired species.

### 6.3 miRNA/isomiR annotation with JAVA

**MIRALIGNER**

Download the tool from [miraligner repository](http://miraligner.readthedocs.io/en/dev/quick_start.html#from-seqbuster-miraligner-files-to-gff3).

Download the mirbase files (`hairpin` and `miRNA`) from the ftp and save it to `DB` folder.

You can map the miRNAs with.

```bash
java -jar miraligner.jar -sub 1 -trim 3 -add 3 -s hsa -i sample1_clean_trimmed.fastq -d DB -o output_prefix
```

Cite


**NOTE:** Check comparison of multiple tools for miRNA annotation.

### 6.4 Convert to GFF3-srna

Use mirtop to convert to GFF3-srna format. This is the desired format to share the isomiR information and can be used to join multiple projects together easily.

See this [http://mirtop.readthedocs.io/en/dev/quick_start.html#from-seqbuster-miraligner-files-to-gff3](http://mirtop.readthedocs.io/en/dev/quick_start.html#from-seqbuster-miraligner-files-to-gff3) to know how to convert all the output into a single file and share easily with collaborators:

```bash
mirtop gff --format seqbuster --sps hsa --hairpin database/hairpin.fa --gtf database/hsa.gff3 -o test_out out_folder/*/*.mirna
```
6.5 Post-analysis with R

Use the outputs to do differential expression, clustering and descriptive analysis with this package: isomiRs.

To load the data you can use IsomirDataSeqFromFiles function and get the count data with isoCounts to move to DESeq2 or similar packages.

6.6 Manual of miraligner(JAVA)

**options**

Add `-freq` if you have your fasta/fastq file with this format and you want a third column with the frequency (normally value after x character):

```
>seq_1_x4
CACCGCTGTCGGGGAACCGCGCCAATTT
```

Add `-pre` if you want also sequences that map to the precursor but outside the mature miRNA

- Parameter `-sub`: mismatches allowed (0/1)
- Parameter `-trim`: nucleotides allowed for trimming (max 3)
- Parameter `-add`: nucleotides allowed for addition (max 3)
- Parameter `-s`: species (3 letter, human=>hsa)
- Parameter `-i`: fasta file
- Parameter `-db`: folder where miRBase files are(one copy at miraligner-1.0/DB folder)
- Parameter `-o`: prefix for the output files
- Parameter `-freq`: add frequency of the sequence to the output (just where input is fasta file with name matching this patter: >seq_3_x67)
- Parameter `-pre`: add sequences mapping to precursors as well

**input**

A fasta/fastq file reads:

```
>seq
CACCGCTGTCGGGGAACCGCGCCAATTT
```

or tabular file with counts information:

```
CACCGCTGTCGGGGAACCGCGCCAATTT 45
```

**output**

Track file `*.mirna.opt`: information about the process

Non mapped sequences will be on `*.nomap`

Header of the `*.mirna.out` file:

- seq: sequence
- freq/name: depending on the input this column contains counts (tabular input file) or name (fasta file)
- mir: miRNA name
• start: start of the sequence at the precursor
• end: end of the sequence at the precursor
• mism: nucleotide substitution position | nucleotide at sequence | nucleotide at precursor
• addition: nucleotides at 3 end added:

<table>
<thead>
<tr>
<th>precursor</th>
<th>=&gt; cctgtggttagctgttcgctatcc</th>
</tr>
</thead>
<tbody>
<tr>
<td>annotated miRNA</td>
<td>=&gt; TGTGGTTAGCTGGTTGCATAT</td>
</tr>
<tr>
<td>sequence add:</td>
<td>TT =&gt; TGTGGTTAGCTGGTTGCATATTT</td>
</tr>
</tbody>
</table>

• tr5: nucleotides at 5 end different from the annotated sequence in miRBase:

<table>
<thead>
<tr>
<th>precursor</th>
<th>=&gt; cctgtggttagctgttcgctatcc</th>
</tr>
</thead>
<tbody>
<tr>
<td>annotated miRNA</td>
<td>=&gt; TGTGGTTAGCTGGTTGCATAT</td>
</tr>
<tr>
<td>sequence tr5:</td>
<td>CC =&gt; CCTGTGGTTAGCTGGTTGCATAT</td>
</tr>
<tr>
<td>sequence tr5:</td>
<td>tg =&gt; TGGTTAGCTGGTTGCATAT</td>
</tr>
</tbody>
</table>

• tr3: nucleotides at 3 end different from the annotated sequence in miRBase:

<table>
<thead>
<tr>
<th>precursor</th>
<th>=&gt; cctgtggttagctgttcgctatcc</th>
</tr>
</thead>
<tbody>
<tr>
<td>annotated miRNA</td>
<td>=&gt; TGTGGTTAGCTGGTTGCATAT</td>
</tr>
<tr>
<td>sequence tr3:</td>
<td>cc =&gt; TGTGGTTAGCTGGTTGCATATCC</td>
</tr>
<tr>
<td>sequence tr3:</td>
<td>AT =&gt; TGTGGTTAGCTGGTTGCAT</td>
</tr>
</tbody>
</table>

• s5: offset nucleotides at the begining of the annotated miRNAs:

<table>
<thead>
<tr>
<th>precursor</th>
<th>=&gt; agcctgtggttagctgttcgctatcc</th>
</tr>
</thead>
<tbody>
<tr>
<td>annotated miRNA</td>
<td>=&gt; TGTGGTTAGCTGGTTGCATAT</td>
</tr>
<tr>
<td>s5</td>
<td>=&gt; AGCCTGTG</td>
</tr>
</tbody>
</table>

• s3: offset nucleotides at the ending of the annotated miRNAs:

<table>
<thead>
<tr>
<th>precursor</th>
<th>=&gt; cctgtggttagctgttcgctatcc</th>
</tr>
</thead>
<tbody>
<tr>
<td>annotated miRNA</td>
<td>=&gt; TGTGGTTAGCTGGTTGCATAT</td>
</tr>
<tr>
<td>s3</td>
<td>=&gt; ATATCCGC</td>
</tr>
</tbody>
</table>

• type: mapped on precursor or miRNA sequences
• ambiguity: number of different detected precursors

Example:

<table>
<thead>
<tr>
<th>seq</th>
<th>miRNA</th>
<th>start</th>
<th>end</th>
<th>mism</th>
<th>tr5</th>
<th>tr3</th>
<th>add</th>
</tr>
</thead>
<tbody>
<tr>
<td>s5</td>
<td>s3</td>
<td>DB</td>
<td>amb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGGCTCAGTCAGCAGGACC hsa-mir-24-2</td>
<td>50</td>
<td>67</td>
<td>0</td>
<td>qCC</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ATGCCCTAAGTGCTCCTTCTG hsa-miR-18a*</td>
<td>47</td>
<td>68</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>tG</td>
<td></td>
</tr>
<tr>
<td>ATCTACTG CTGGCA miRNA 1</td>
<td>47</td>
<td>68</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>tG</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 7

Collapse fastq(.gz) files

Definition
Normally quality values are lost in small RNA-seq pipelines due to collapsing after adapter recognition. This option allow to collapse reads after adapter removal with cutadapt or any other tool. This way the mapping can use quality values, allowing to map using bwa for instance, or any other alignment tool that doesn’t support FASTA files.

Methods
The new quality values are the average of each of the sequence collapse.

Example

```
seqcluster collapse -f sample_trimmed.fastq -o collapse
```

- `-f` is the fastq(.gz) file
- `-o` the folder where the output will be created. A new FASTQ file, where the name stand for:

```
@seq_\[0-9\]_x\[0-9\]
```

The number right after `_x` means the abundance of this sequence in the sample
CHAPTER 8

Handling multi-mapped reads

Definition

multi-mapped reads are the sequences that map more than one time on the genome, for instance, because there are multiple copies of a gene, like happens with tRNA precursors

Consequence

Many pipelines ignores these sequences as defaults, what means that you are losing at least 20-30% of the data. In this case it is difficult to decide where these sequences come from and currently there are three strategies:

• ignore them

• count as many times as they appear: for instance, if a sequences map twice, just count it two times in the two loci. This will due an over-representation of the loci abundances, and actually is against the assumption of all packages that perform differential expression in count data.

• weight them: divide the total count by the number of places it maps. In the previous example, each loci would get 1/2 * count. This produces weird dispersion values for packages that fit this value as part of the model.

Our implementation
We try to decide the origin of these sequences. The most common scenario is that a group of sequences map two three different regions, probably due to multi-copies on the genome of the precursor.

We introduce two options:

- **most-voting strategy**: In this case, we just count once all sequences, and we output this like one unit of transcription with multiple regions. This is the option by default.

- **bayes inference**: we give the same prior probability to all locations, and use the number of sequences starting in the same position than the one we are trying to predict its location as $P(B|A)$. With this we calculate the posterior that will be used to get the proportion of counts to the different locations. We apply the code from the book: “Think Bayes” (Allen B. Downey). This is still under development. To activate this option, the user just needs to add `--method babes`

The main advantage of this, it is that it can be the input of any downstream analysis that is applied to RNA-seq, like DESeq, edgeR . . . As well, there is less noise, because there is only one output coming from here, not three.
CHAPTER 9

Tools for downstream analysis

9.1 Web-servers

TFmiR: disease-specific miRNA/transcription factor co-regulatory networks v1.2. It uses results from UP/DOWN regulated miRNA/Genes and allows to focus in only one disease to create different type of relationships between miRNA/TF/Gene. Easy to use. Probably need to filter the output sometime due to the big networks that can result from an analysis.

Diana-TarBase v7.0: Database for validated miRNA targets. Many filter options. Good for small candidate miRNAs set studies.

StarScan: Database to browse the targets of miRNAs from degradome data. It has a fancy interface, and many species and data from GEO.

miRtex gives targets from literature. Good for finding validated targets to help discussion in papers or further functional experiment based on new hypothesis.

piRBase: Database for piRNA annotation and function. Published last year, for now the best I can find out there.

chimira: Web tool to analyze isomiR. It gives you a quick idea of you samples.

MicroCosm: MiRNA target database. Updated and download option.

IsomiR Bank: isomiR database from many species and tissues. For single queries is useful.

9.2 Command-lines

miRVaS: tools to predict the functional changed due to nt changes in the miRNA sequence.
Relevant papers about isomiRs and other novel small RNAs with functional relevance

10.1 Validation

- Our approach can be adapted to many polyadenylation-based RT-qPCR technologies already exiting, providing a convenient way to distinguish long and short 3'-isomiRs.

10.2 IsomiRs

Naturally existing isoforms of miR-222 have distinct functions: this work demonstrates the capacity for 3’ isomiRs to mediate differential functions, we contend more attention needs to be given to 3’ variance given the prevalence of this class of isomiR.

miR-142-3p isomiR: “We furthermore demonstrate that miRNA 5-end variation leads to differential targeting and can thus broaden the target range of miRNAs.”

A highly expressed miR-101 isomiR is a functional silencing small RNA.

A challenge for miRNA: multiple isomiRs in miRNAomics.

miR-183-5p isomiR changes in breast cancer. Validated target regulation of new genes different from the reference miRNA.

A comprehensive survey of 3’ animal miRNA modification events and a possible role for 3’ adenylation in modulating miRNA targeting effectiveness.

PAPD5-mediated 3 adenylation and subsequent degradation of miR-21 is disrupted in proliferative disease.

High-resolution analysis of the human retina miRNome reveals isomiR variations and novel microRNAs.

Sequence features of Drosha and Dicer cleavage sites affect the complexity of isomiRs.

Knowledge about the presence or absence of miRNA isoforms (isomiRs) can successfully discriminate amongst 32 TCGA cancer types.
10.3 General

A novel piRNA mechanism in regulating gene expression in highly differentiated somatic cells. Differential and coherent processing patterns from small RNAs to detect changes in profiles of processing small RNAs. Survey of 800+ datasets from human tissue and body fluid reveals XenomiRs are likely artifacts.

10.4 Targets

Identification of factors involved in target RNA-directed microRNA degradation.

10.5 Technology

miRQC: work studying the accuracy and specificity of different technologies to detect miRNAs. Important features affecting the detection of small RNA biomarkers: How the sample can affect the detection of biomarkers (like RIN value, concentration, . . . )

Comparison of alignment and normalization. I will take the message that TMM and DESeq/2 normalization are the best to avoid strong bias if we consider to have a small proportion of DE miRNAs. For the alignments, here you have another comparison for miRNAs annotation: https://rawgit.com/lpantano/tools-mixer/master/mirna/mirannotation/stats.html

review of tools for detect miRNA-disease network.
review of tools for miRNA de-novo and interaction analysis

Evaluation of microRNA alignment techniques

BIG meeting on Dec,3 2015: bcbio-srnaseq-BIG-20151203.pdf
CHAPTER 11

Documentation
CHAPTER 12

Classes

Visit GitHub code
I am in the process to document all classes and methods
CHAPTER 13

Indices and tables

- genindex
- modindex
- search