Plateforme Bioinformatique Midi-Pyrénées

small RNAseq data analysis

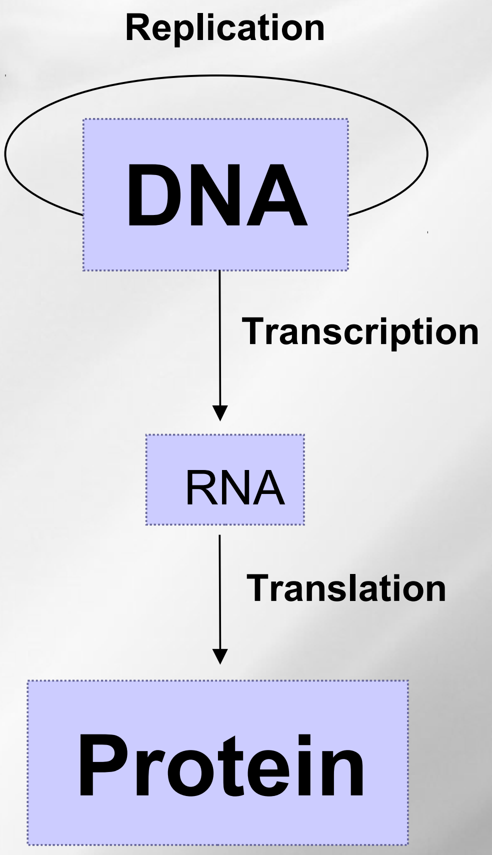
Philippe Bardou, Christine Gaspin
& Jérôme Mariette

Introduction to miRNA world and sRNAseq

Central dogma of molecular biology

- **Evolution of the dogma : 1950-1970**

DNA structure discovery.

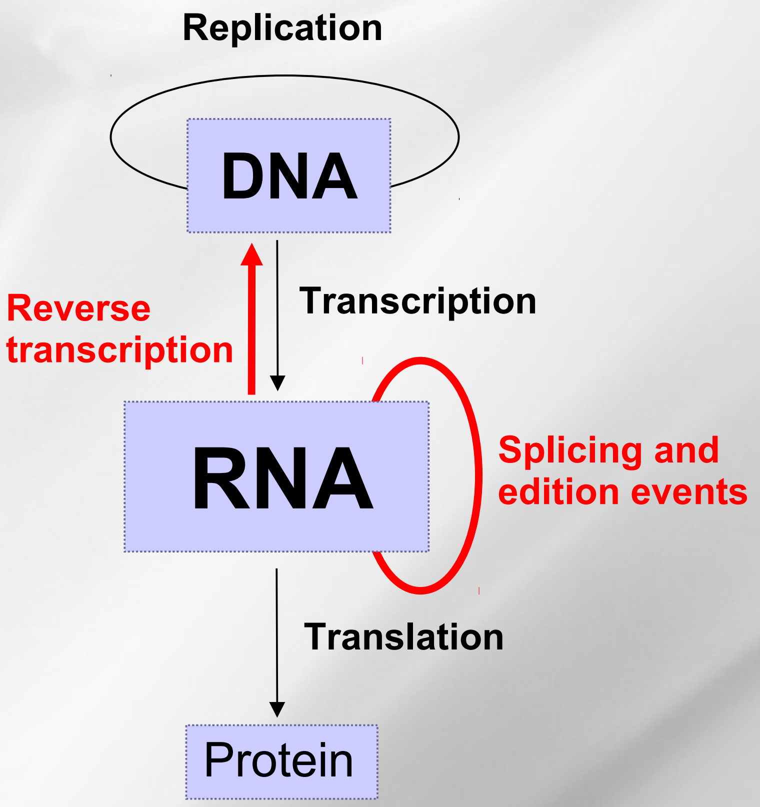


One gene = one function

Central dogma of molecular biology

- **Evolution of the dogma : 1970-1980**

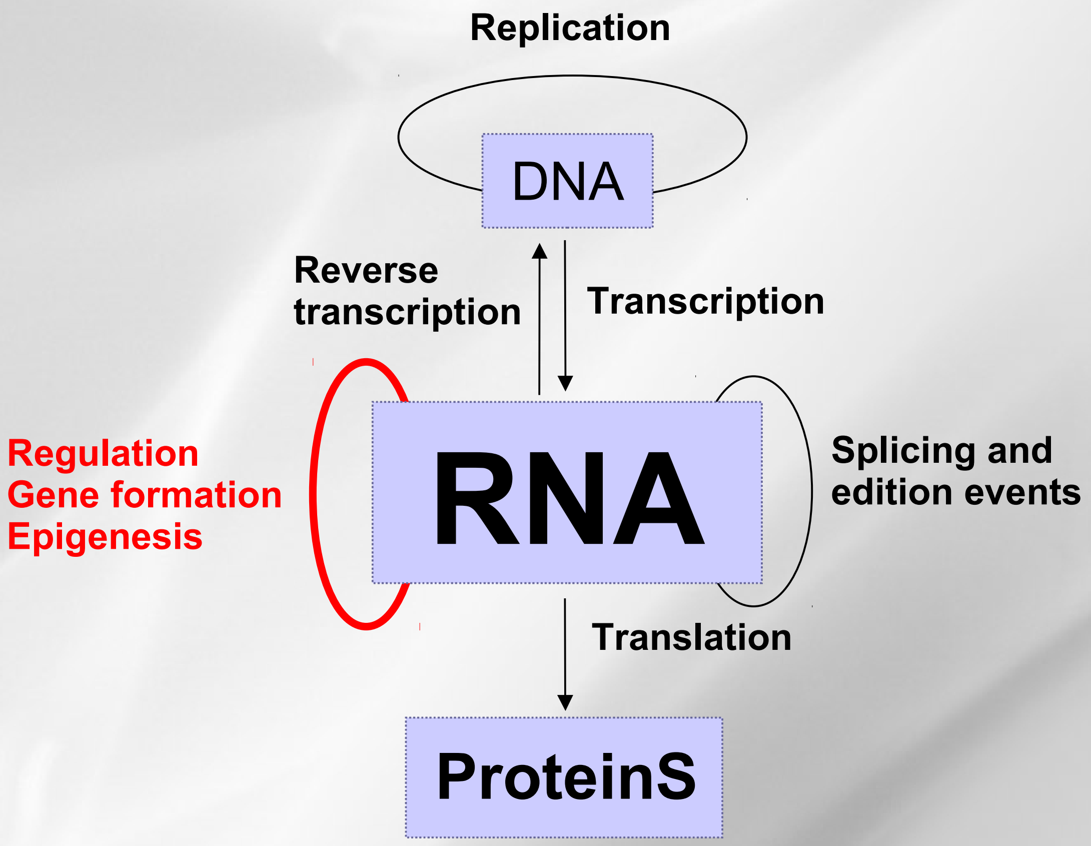
Genome analysis



Central dogma of molecular biology

- **Evolution of the dogma : today**

Genome analysis + Sequencing



Many genes = one functionnel complex

Central dogma of molecular biology

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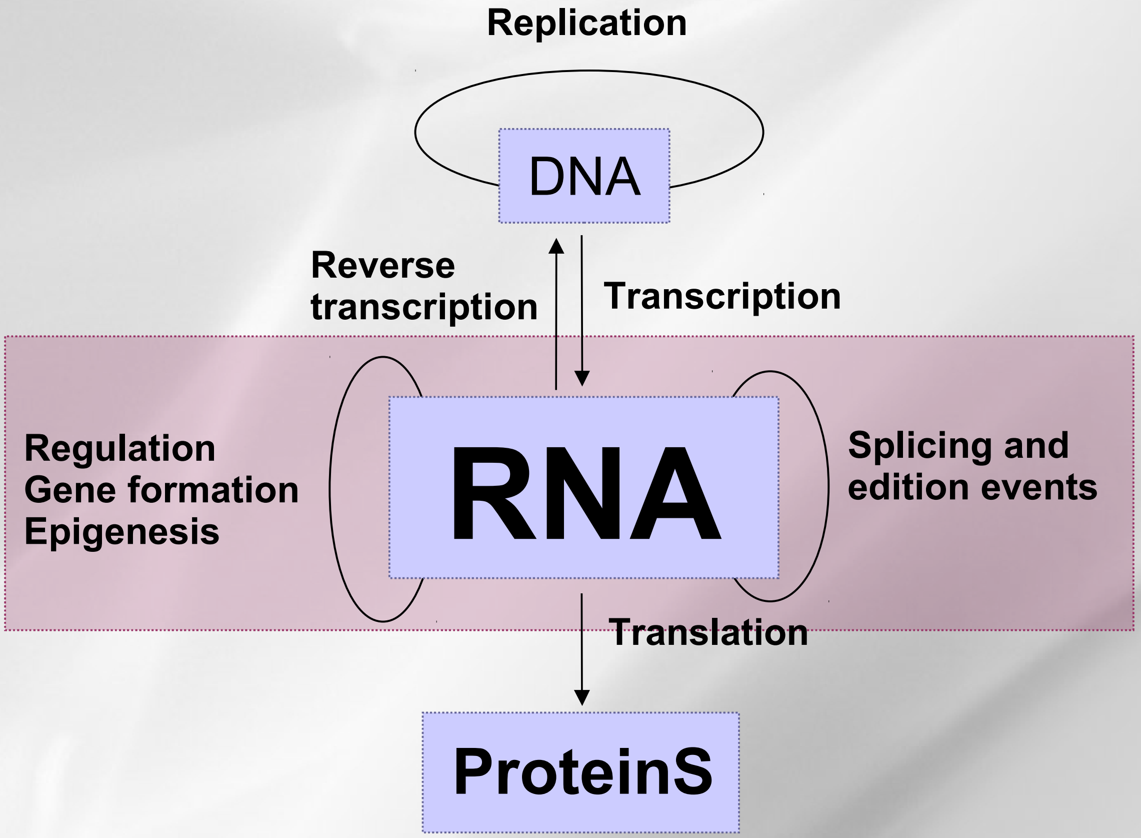
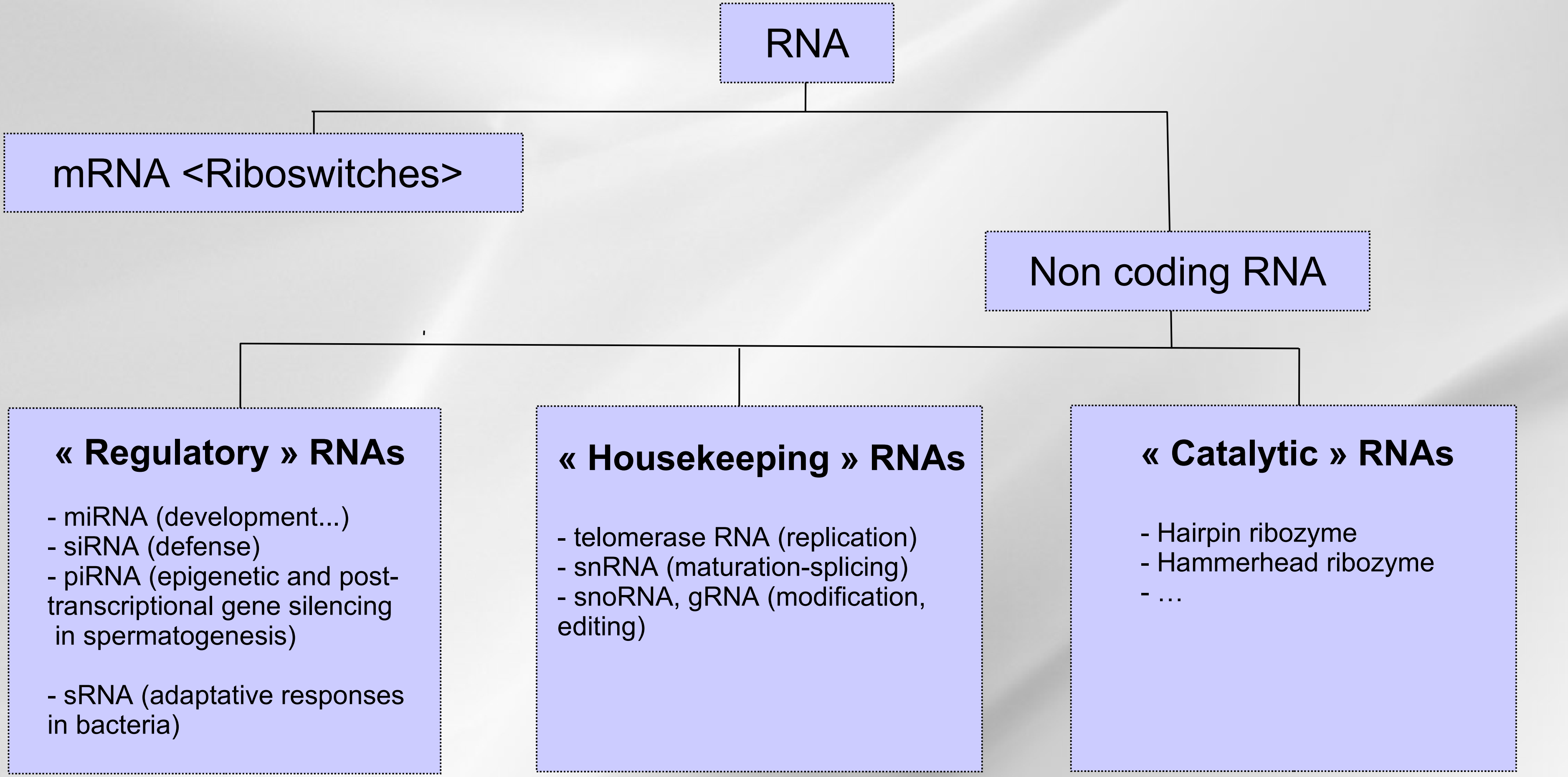


Many genes = one functionnel complex

The RNA world

- **An expending universe of RNA**



RNA

mRNA <Riboswitches>

Non coding RNA

« Regulatory » RNAs

- miRNA (development...)
- siRNA (defense)
- piRNA (epigenetic and post-transcriptional gene silencing in spermatogenesis)

- sRNA (adaptative responses in bacteria)

« Housekeeping » RNAs

- telomerase RNA (replication)
- snRNA (maturation-splicing)
- snoRNA, gRNA (modification, editing)

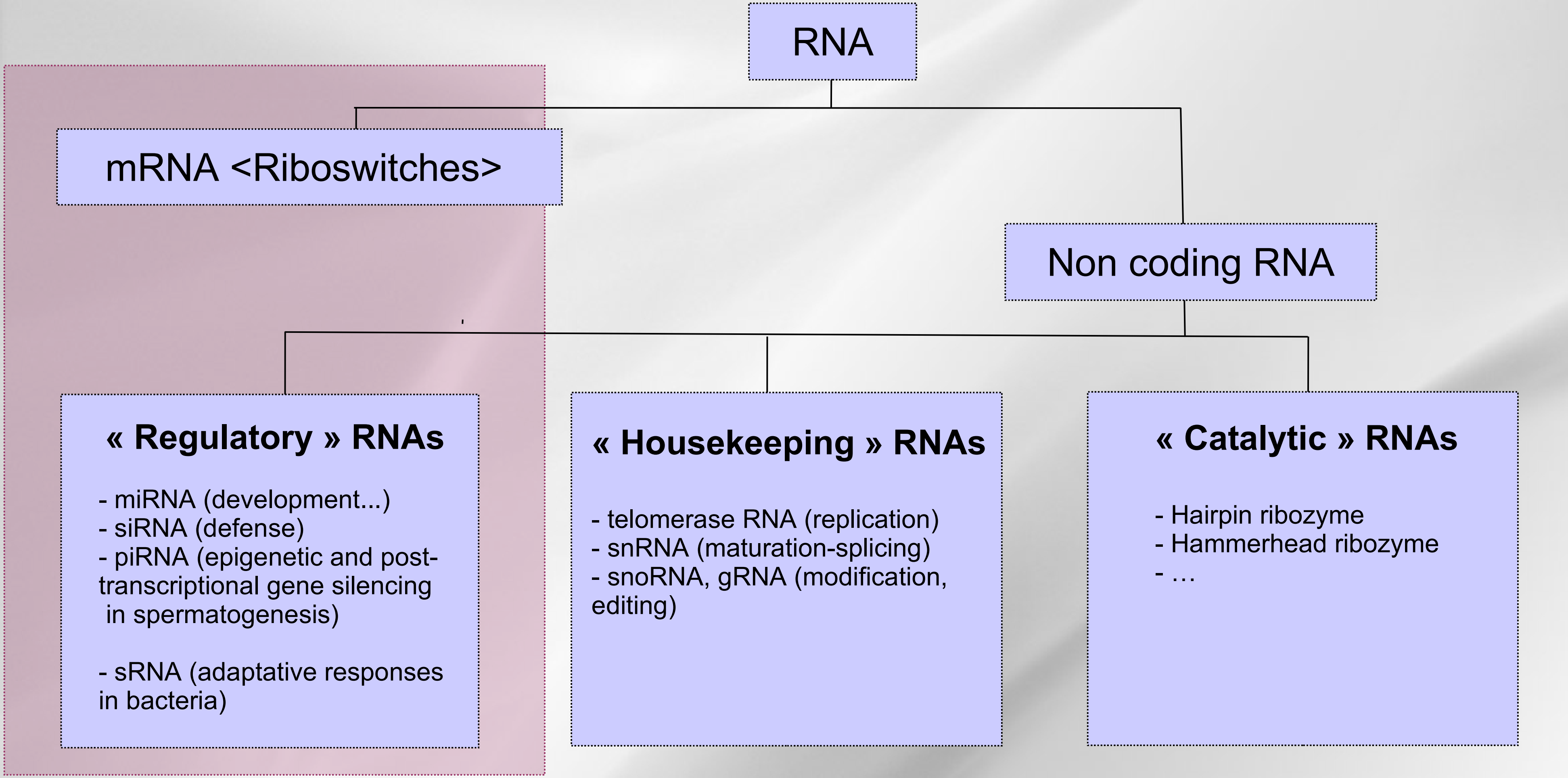
« Catalytic » RNAs

- Hairpin ribozyme
- Hammerhead ribozyme
- …

→ **Multiple roles of RNA in genes regulation**

The RNA world

- **An expending universe of RNA**



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- Hammerhead ribozyme
- …

→ **Multiple roles of RNA in genes regulation**

The non coding protein RNA world

- **Not predicted by gene prediction**
  - No specific signal (start, stop, splicing sites...)
  - Multiple location (intergenic, intronic, coding, antisens)
  - Variable size
  - No strong sequence conservation in general

- **A variety of existing approaches not always easy to integrate**
  - Known family: Homology prediction
  - New family: *De novo* prediction

The non coding protein RNA world

- **Large non coding protein RNA**
    - >300 nt
    - rRNA, Xist, H19, ...
    - Genome structure & expression
- **Small non coding protein RNA**
    - >30 nt
    - tRNA, snoRNA, snRNA...
    - mRNA maturation, translation
- **Micro non coding protein RNA**
    - 18-30 nt
    - miRNA, hc-siRNA, ta-siRNA, nat-siRNA, piRNA...
    - PTGS, TGS, Genome stability, defense...

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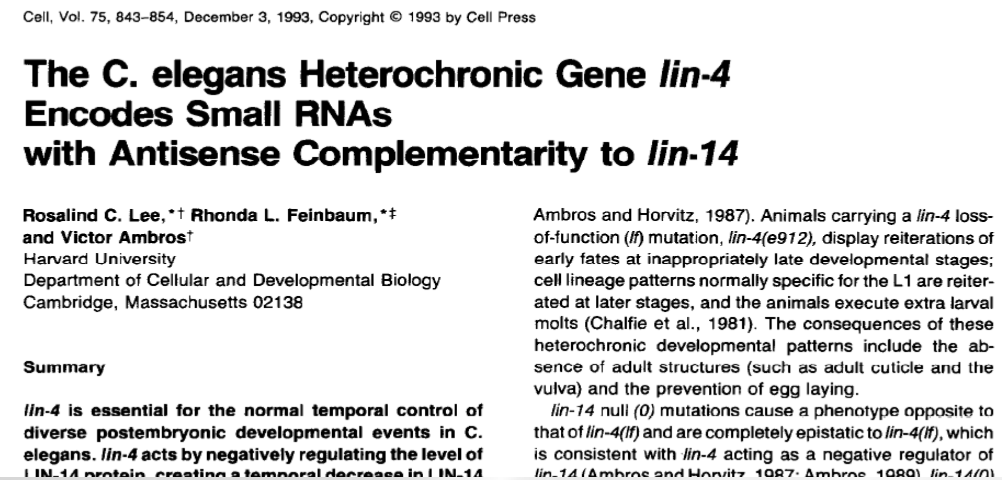
  - **miRNA**, hc-siRNA, ta-siRNA, nat-siRNA, piRNA...
  - Genome stability, defense...

The miRNA world

- **Discovery of lin-4 in C. elegans in 1993**



Cell, Vol. 75, 843–854, December 3, 1993, Copyright © 1993 by Cell Press

The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

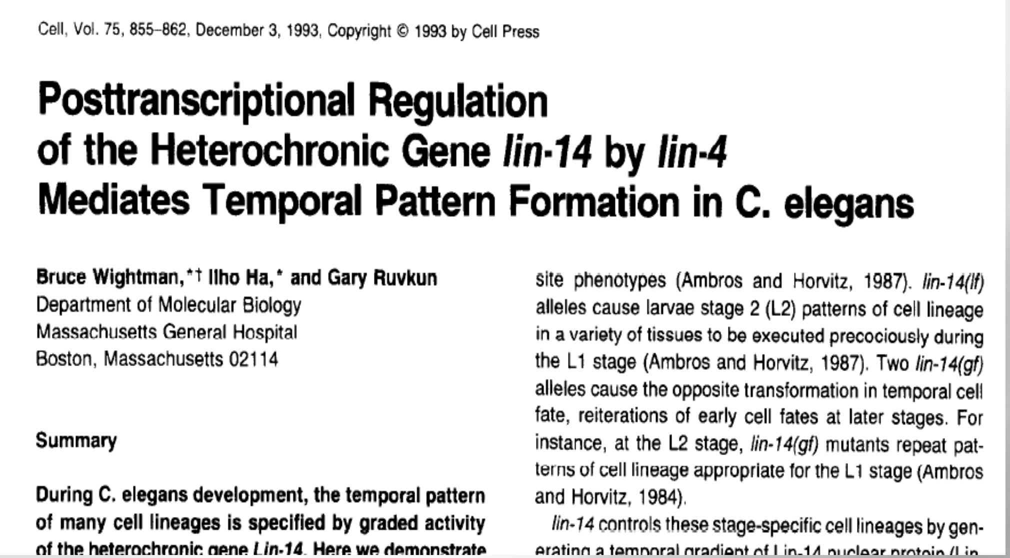
Rosalind C. Lee,[*†] **Rhonda L. Feinbaum,**[*‡] **and Victor Ambros**[†]
Harvard University
Department of Cellular and Developmental Biology
Cambridge, Massachusetts 02138

Summary

***lin-4* is essential for the normal temporal control of diverse postembryonic developmental events in C. elegans. *lin-4* acts by negatively regulating the level of LIN-14 protein, creating a temporal decrease in LIN-14**

Ambros and Horvitz, 1987). Animals carrying a *lin-4* loss-of-function (*lf*) mutation, *lin-4(e912),* display reiterations of early fates at inappropriately late developmental stages; cell lineage patterns normally specific for the L1 are reiterated at later stages, and the animals execute extra larval molts (Chalfie et al., 1981). The consequences of these heterochronic developmental patterns include the absence of adult structures (such as adult cuticle and the vulva) and the prevention of egg laying.

lin-14 null *(0)* mutations cause a phenotype opposite to that of *lin-4(lf)* and are completely epistatic to *lin-4(lf)*, which is consistent with *lin-4* acting as a negative regulator of *lin-14* (Ambros and Horvitz, 1987; Ambros, 1989). *lin-14(0)*



Cell, Vol. 75, 855–862, December 3, 1993, Copyright © 1993 by Cell Press

Posttranscriptional Regulation of the Heterochronic Gene *lin-14* by *lin-4* Mediates Temporal Pattern Formation in C. elegans

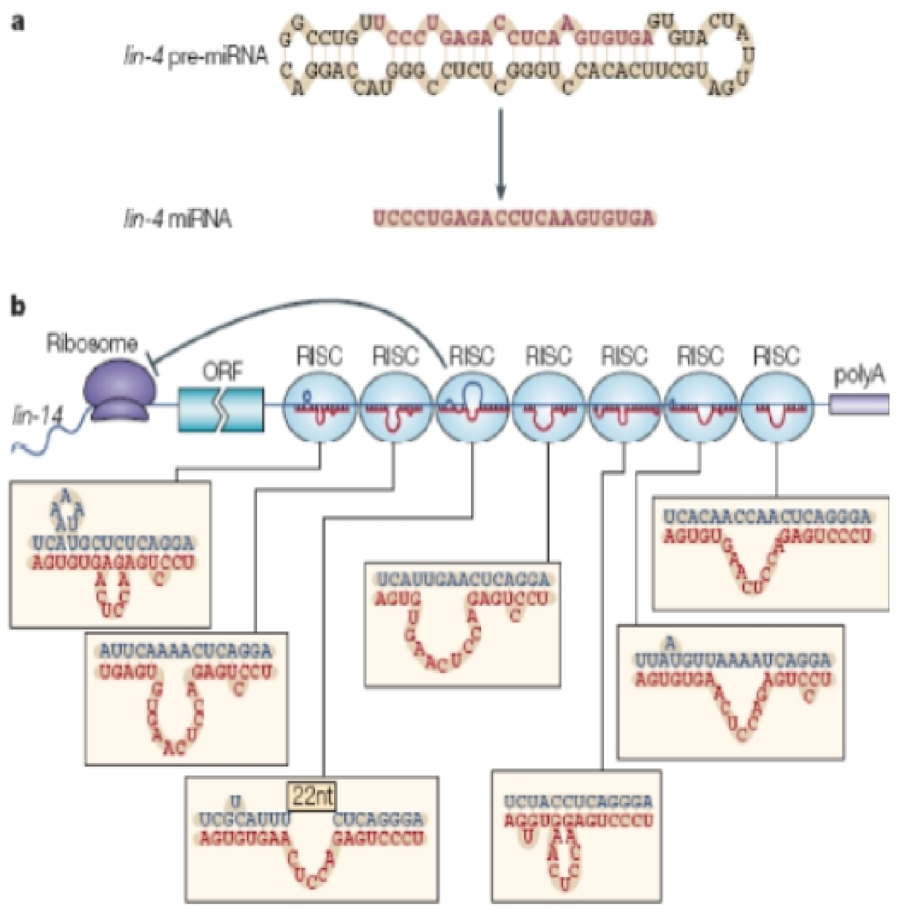
Bruce Wightman,[*†] **Ilho Ha,**[*] **and Gary Ruvkun**
Department of Molecular Biology
Massachusetts General Hospital
Boston, Massachusetts 02114

Summary

During C. elegans development, the temporal pattern of many cell lineages is specified by graded activity of the heterochronic gene *Lin-14*. Here we demonstrate

site phenotypes (Ambros and Horvitz, 1987). *lin-14(lf)* alleles cause larvae stage 2 (L2) patterns of cell lineage in a variety of tissues to be executed precociously during the L1 stage (Ambros and Horvitz, 1987). Two *lin-14(gf)* alleles cause the opposite transformation in temporal cell fate, reiterations of early cell fates at later stages. For instance, at the L2 stage, *lin-14(gf)* mutants repeat patterns of cell lineage appropriate for the L1 stage (Ambros and Horvitz, 1984).

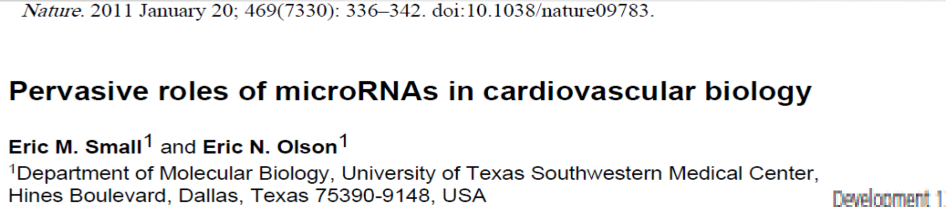
lin-14 controls these stage-specific cell lineages by generating a temporal gradient of Lin-14 nuclear protein (Lin-



(He & Hannon, Nature reviews, 2004)

The miRNA world

- **A key regulation function**

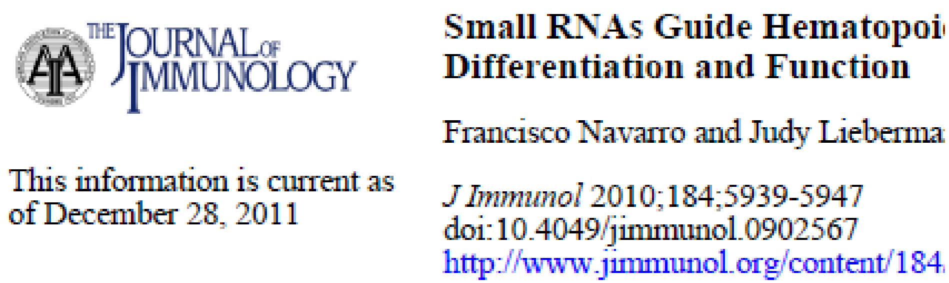


Nature. 2011 January 20; 469(7330): 336–342. doi:10.1038/nature09783.

Pervasive roles of microRNAs in cardiovascular biology

Eric M. Small[1] and **Eric N. Olson**[1]

[1]Department of Molecular Biology, University of Texas Southwestern Medical Center, Hines Boulevard, Dallas, Texas 75390-9148, USA



THE JOURNAL OF IMMUNOLOGY

Small RNAs Guide Hematopoi Differentiation and Function

Francisco Navarro and Judy Lieberma

This information is current as of December 28, 2011

J Immunol 2010;184;5939-5947
doi:10.4049/jimmunol.0902567
http://www.jimmunol.org/content/184

Development 138, 1081-1086 (2011) doi:10.1242/dev.056317
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Regulation of mouse stomach development and Barx1 expression by specific microRNAs

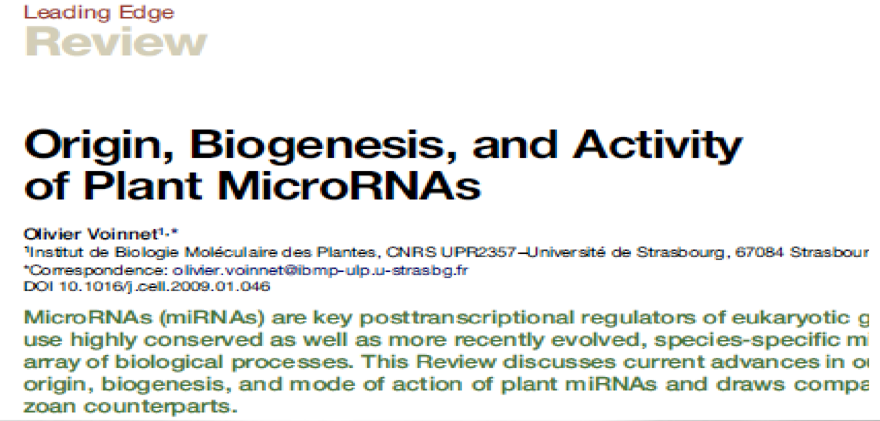
Byeong-Moo Kim[1,2,*,†], Janghee Woo[1,3,†], Chryssa Kanellopoulou[4] and Ramesh A. Shivdasani[1,2,‡]

Developmental Cell *11*, 441–450, October, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.devcel.2006.09.009

The Diverse Functions of MicroRNAs in Animal Development and Disease

Wigard P. Kloosterman[1] and **Ronald H.A. Plasterk**[1,2,*]
[1]Hubrecht Laboratory
Centre for Biomedical Genetics

Since then, several g RNA-cloning strategies to vertebrates and invertebra

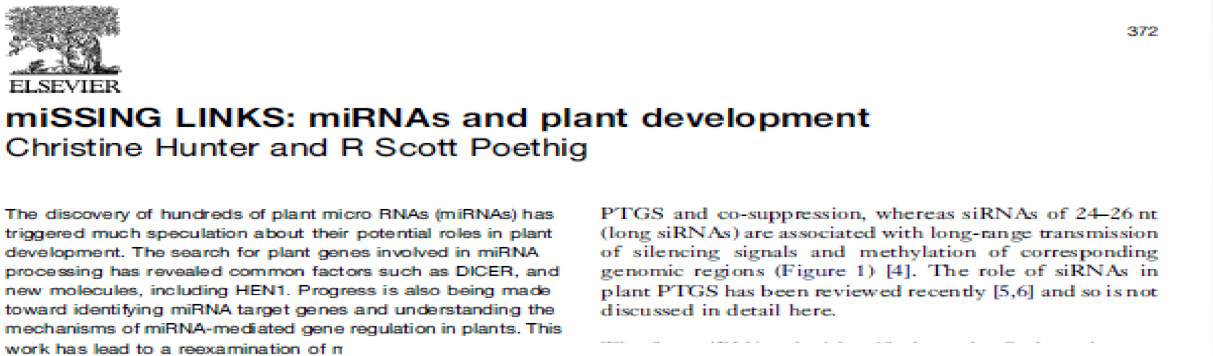


Leading Edge
Review

Origin, Biogenesis, and Activity of Plant MicroRNAs

Olivier Voinnet[1,*]
[1]Institut de Biologie Moléculaire des Plantes, CNRS UPR2357–Université de Strasbourg, 67084 Strasbour
*Correspondence: olivier.voinnet@ibmp-ulp.u-strasbg.fr
DOI 10.1016/j.cell.2009.01.046

MicroRNAs (miRNAs) are key posttranscriptional regulators of eukaryotic g use highly conserved as well as more recently evolved, species-specific mi array of biological processes. This Review discusses current advances in o origin, biogenesis, and mode of action of plant miRNAs and draws compa zoan counterparts.



ELSEVIER

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miSSING LINKS: miRNAs and plant development
Christine Hunter and R Scott Poethig

The discovery of hundreds of plant micro RNAs (miRNAs) has triggered much speculation about their potential roles in plant development. The search for plant genes involved in miRNA processing has revealed common factors such as DICER, and new molecules, including HEN1. Progress is also being made toward identifying miRNA target genes and understanding the mechanisms of miRNA-mediated gene regulation in plants. This work has lead to a reexamination of m characterized mutations that are now components or targets of miRNA-med

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Current Opinion in Genetics & Develo

This review comes from a themed issue Pattern formation and developmental m Edited by Anne Ephrussi and Olivier Po

0959-437X/$ – see front matter
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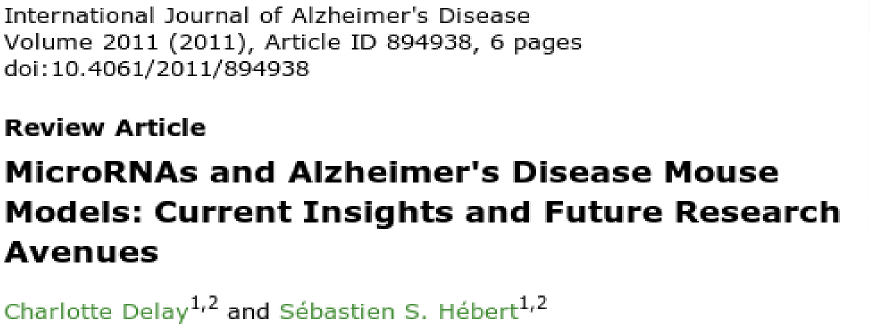
DOI 10.1016/S0959-437X(03)00081-9

PTGS and co-suppression, whereas siRNAs of 24–26 nt (long siRNAs) are associated with long-range transmission of silencing signals and methylation of corresponding genomic regions (Figure 1) [4]. The role of siRNAs in plant PTGS has been reviewed recently [5,6] and so is not discussed in detail here.



International Journal of Alzheimer's Disease
Volume 2011 (2011), Article ID 894938, 6 pages
doi:10.4061/2011/894938

Review Article

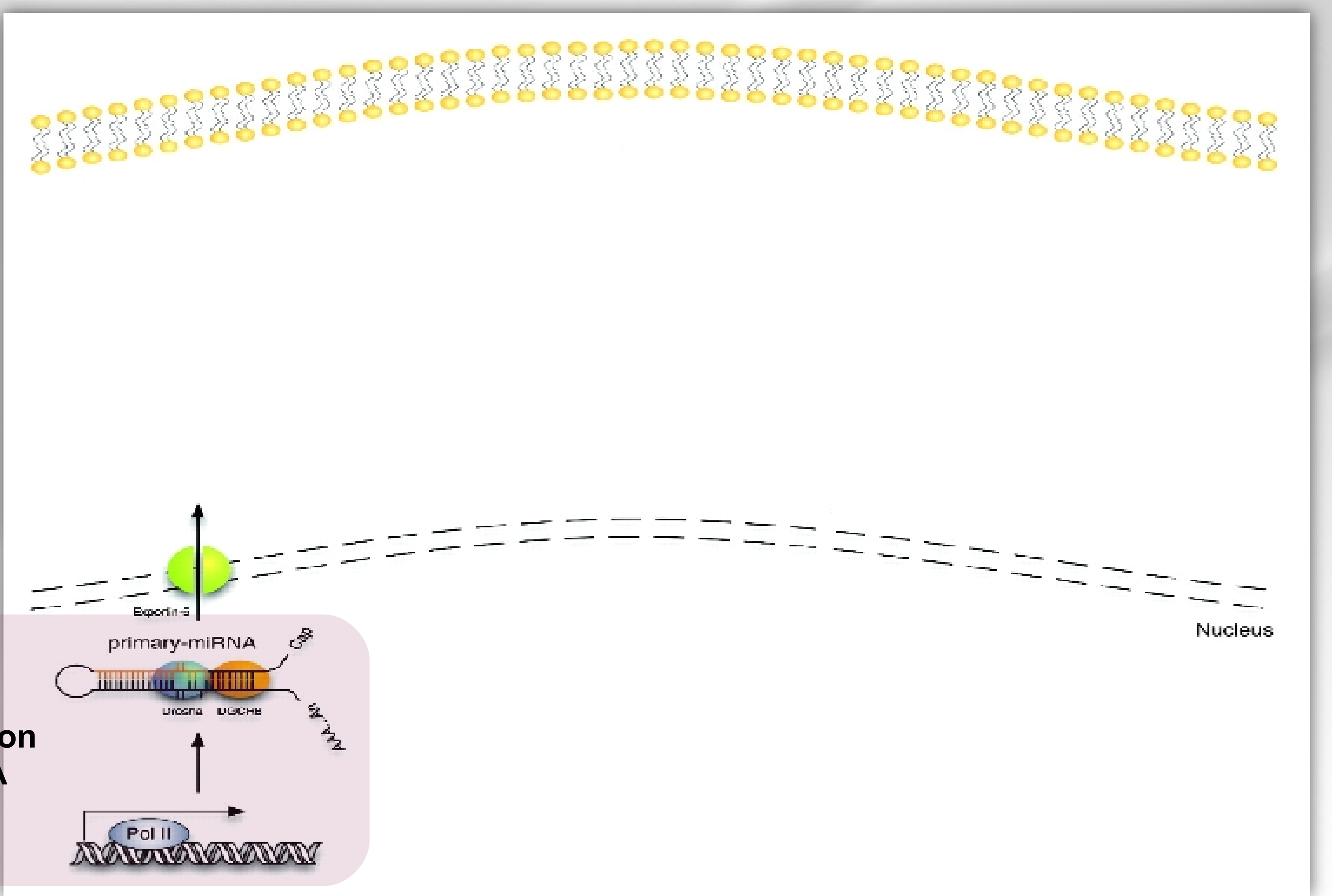
MicroRNAs and Alzheimer's Disease Mouse Models: Current Insights and Future Research Avenues

Charlotte Delay[1,2] and Sébastien S. Hébert[1,2]

The miRNA world

- Animals
  - Developmental timing (C. elegans): lin-4, let-7
  - Neuronal left/right asymetry (C. elegans): Lys-6, mir-273
  - Programmed cell death/fat metabolism (D. melanogaster): mir-14
  - Notch signaling (D. malanogaster): mir-7
  - Brain morphogenesis (Zebrafish): mir-430
  - Myogeneses and cardiogenesis: mir-1, miR-181, miR-133
  - Insulin secretion: miR-375
  - …
- Plants
  - Floral timing and leaf development: miR-156
  - Organ polarity, vascular and meristen development: mir-165, miR-166
  - Expression of auxin response genes: miR-160
  - ...

The miRNA biogenesis



**Pol II transcription
Into a pri-miRNA**

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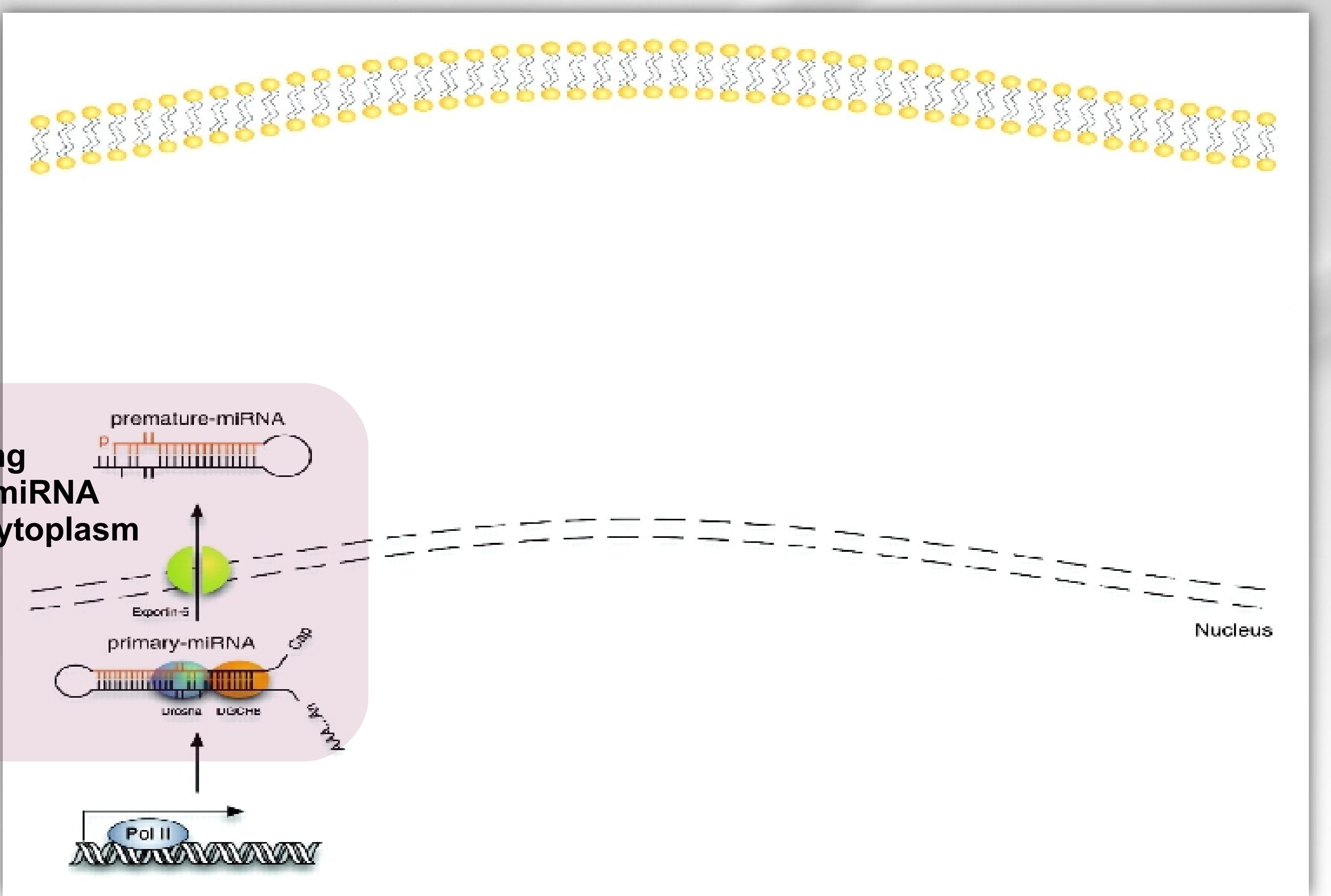
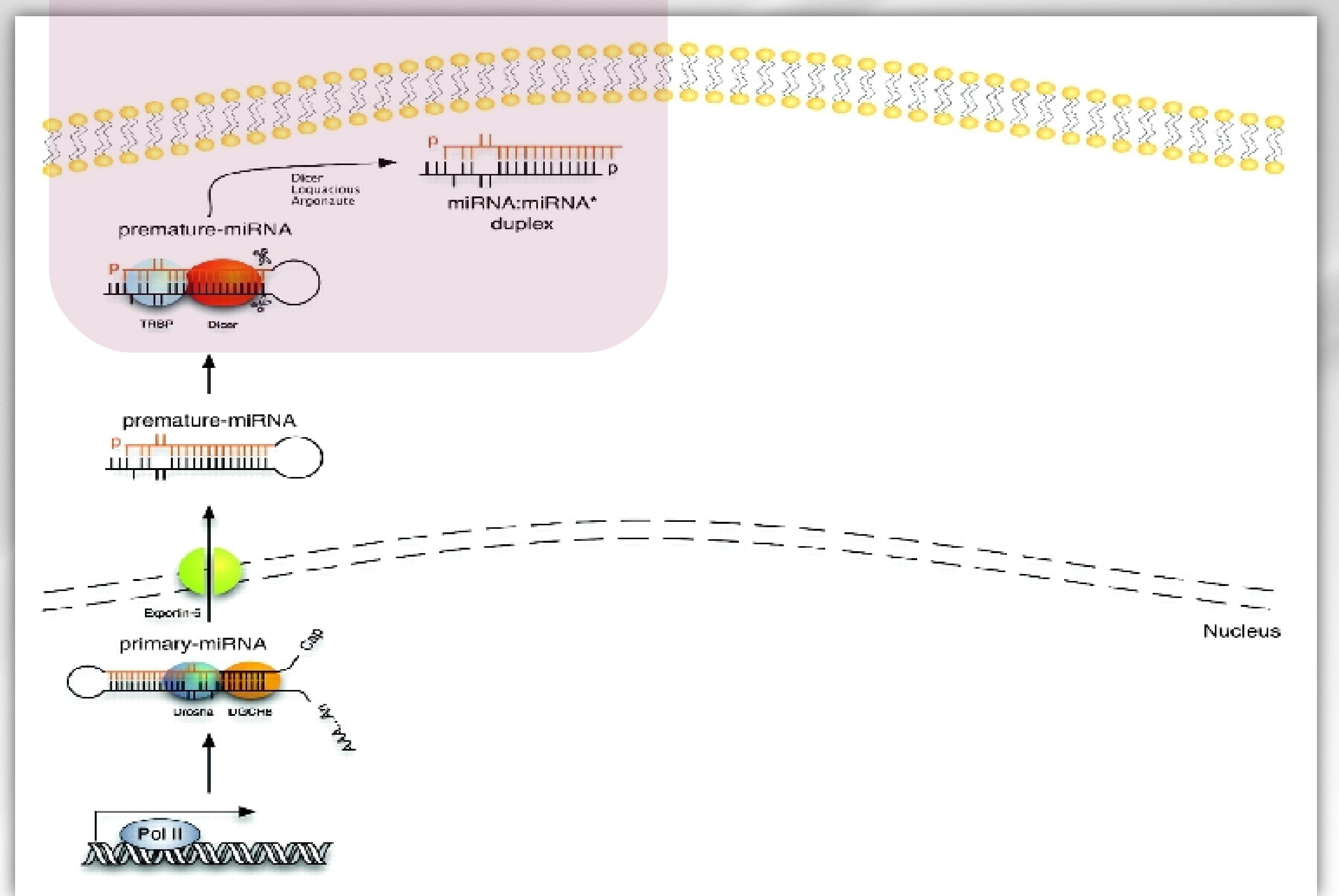
The miRNA biogenesis



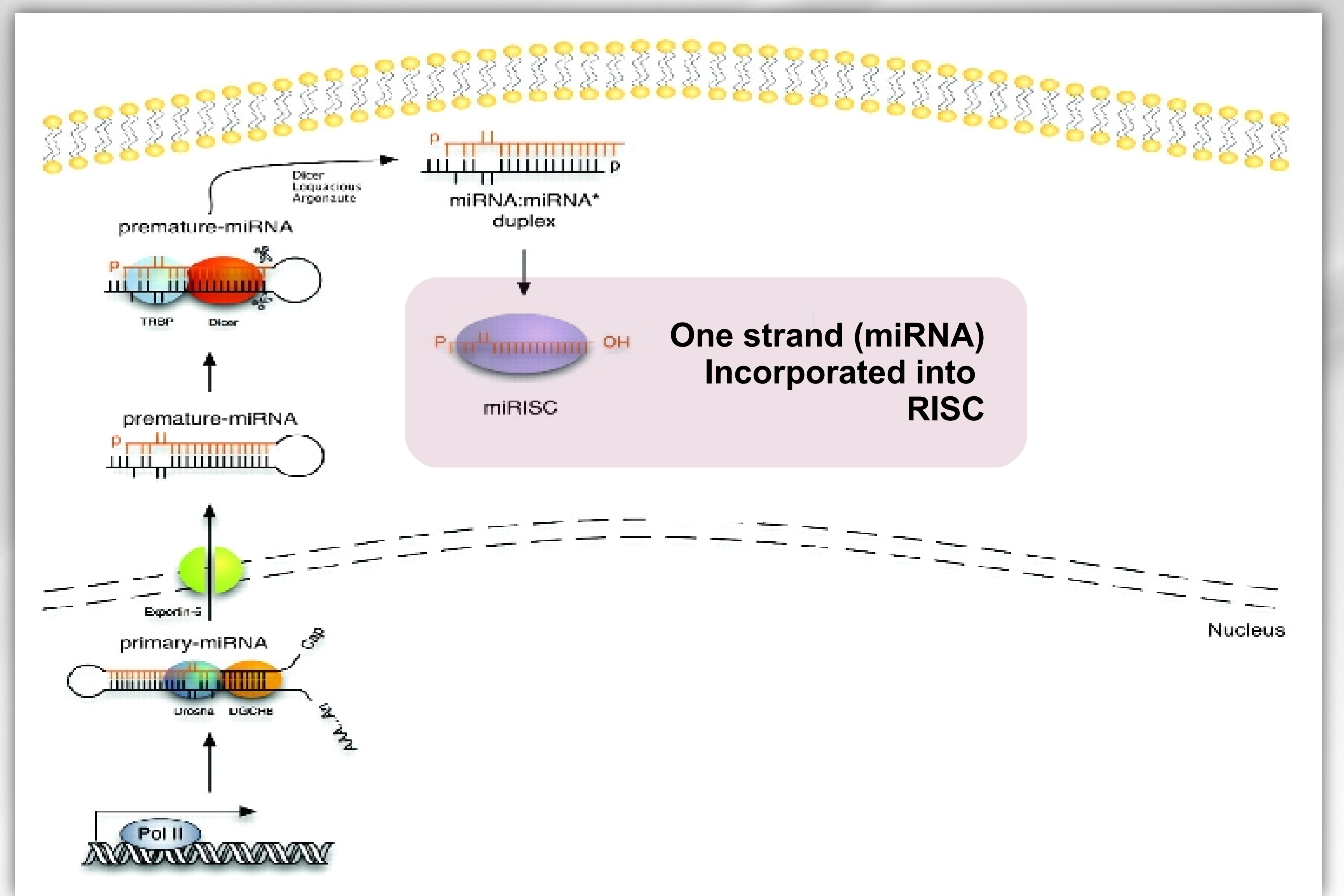
Drosha processing
one or more pre-miRNA
Exported in the cytoplasm

The miRNA biogenesis

**Dicer processing
Into a duplex miRNA
Structure**



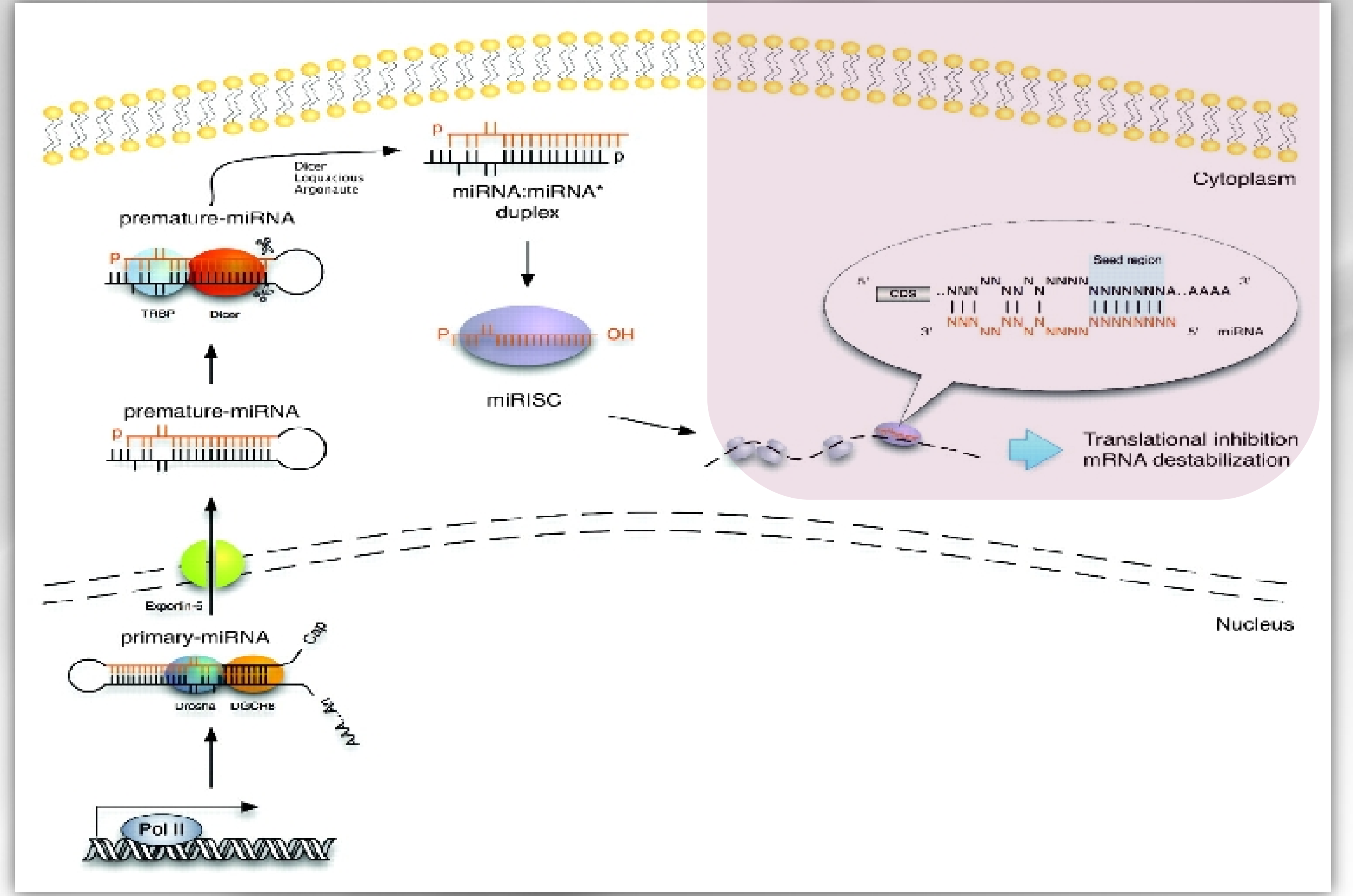
The miRNA biogenesis



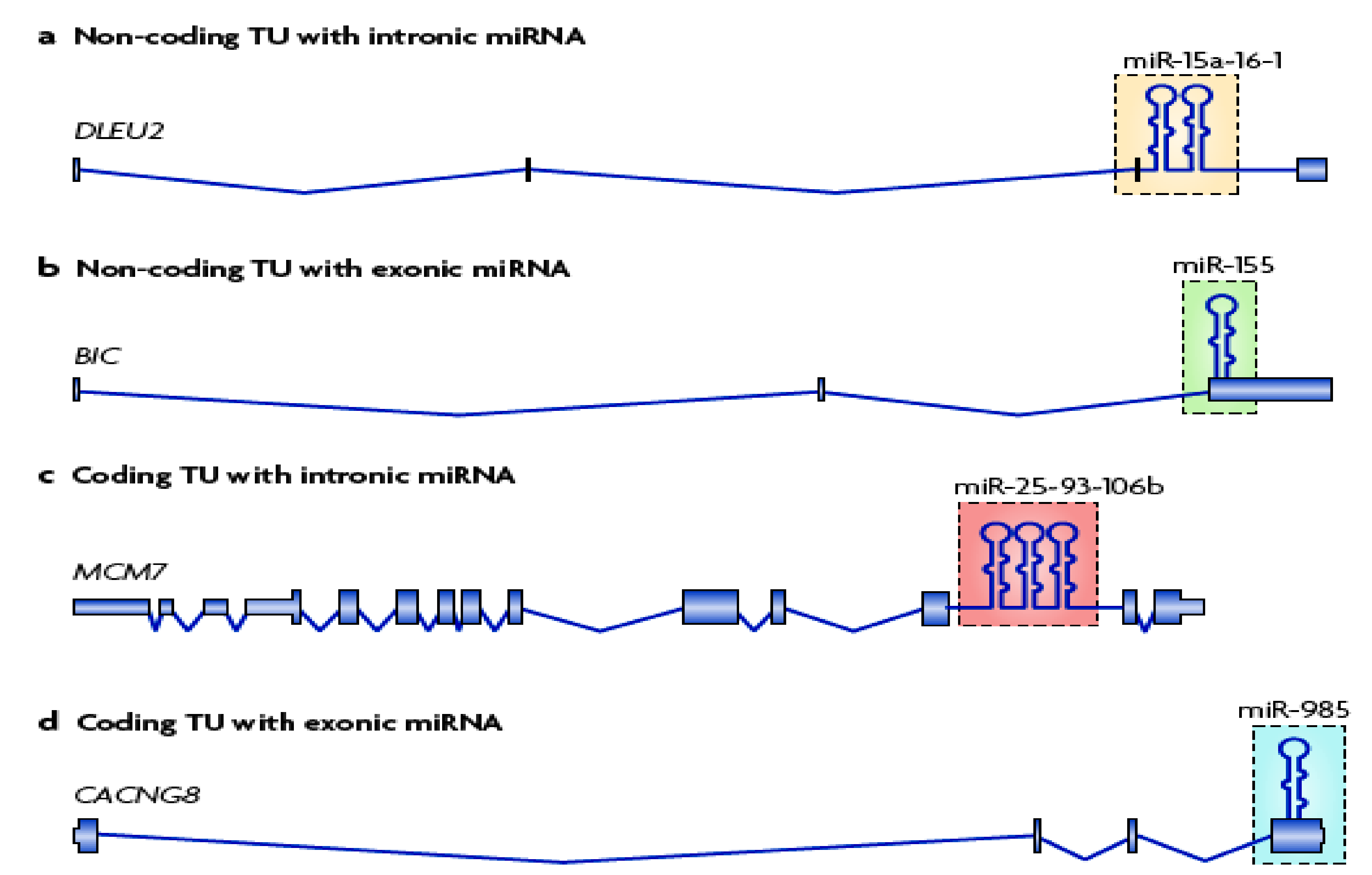
One strand (miRNA)
Incorporated into
RISC

The miRNA biogenesis

target mRNA translationally repressed

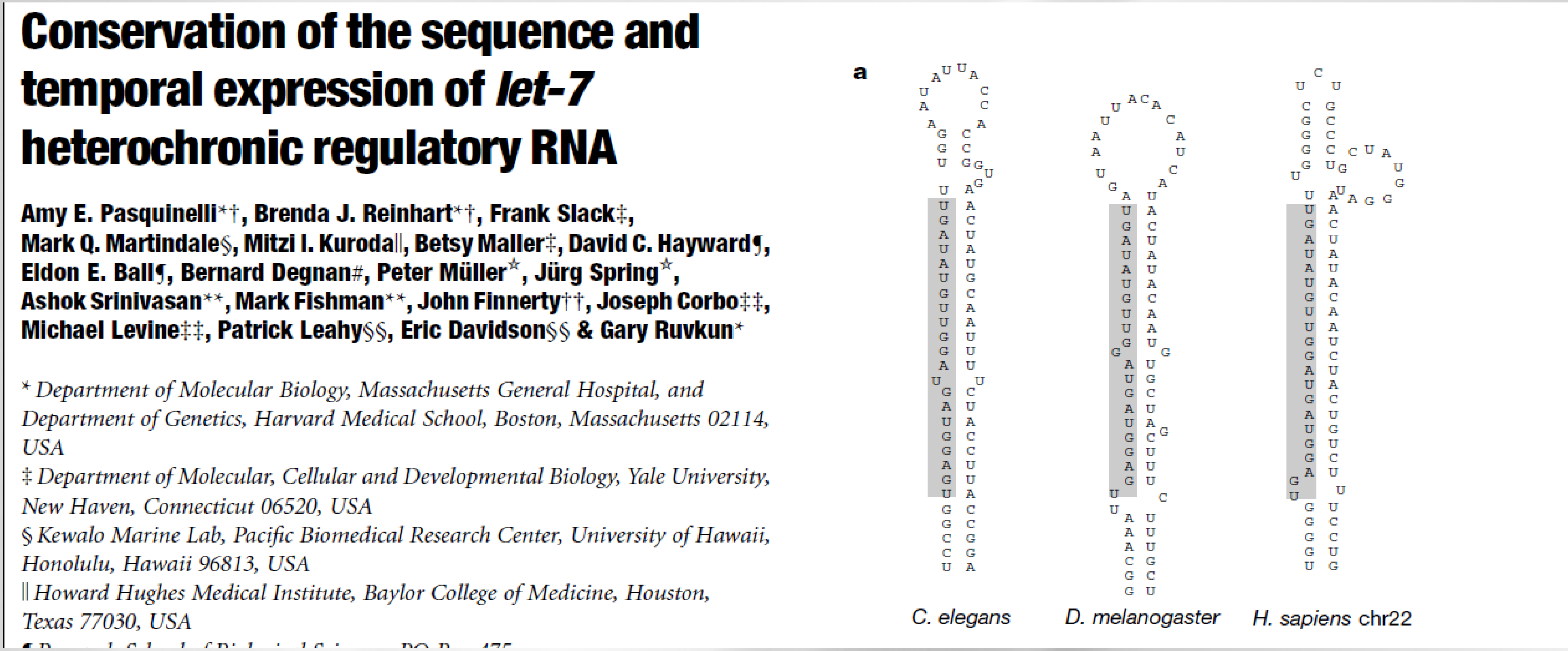


The miRNA location


a Non-coding TU with intronic miRNA
miR-15a-16-1
DLEU2
b Non-coding TU with exonic miRNA
miR-155
BIC
c Coding TU with intronic miRNA
miR-25-93-106b
MCM7
d Coding TU with exonic miRNA
miR-985
CACNG8

→ **Cluster organisation**

The miRNA conservation



Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA

Amy E. Pasquinelli*†, Brenda J. Reinhart*†, Frank Slack‡, Mark Q. Martindale§, Mitzi I. Kuroda||, Betsy Maller‡, David C. Hayward¶, Eldon E. Ball¶, Bernard Degnan#, Peter Müller☆, Jürg Spring☆, Ashok Srinivasan, Mark Fishman**, John Finnerty††, Joseph Corbo‡‡, Michael Levine‡‡, Patrick Leahy§§, Eric Davidson§§ & Gary Ruvkun***

** Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114, USA*

‡ Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520, USA

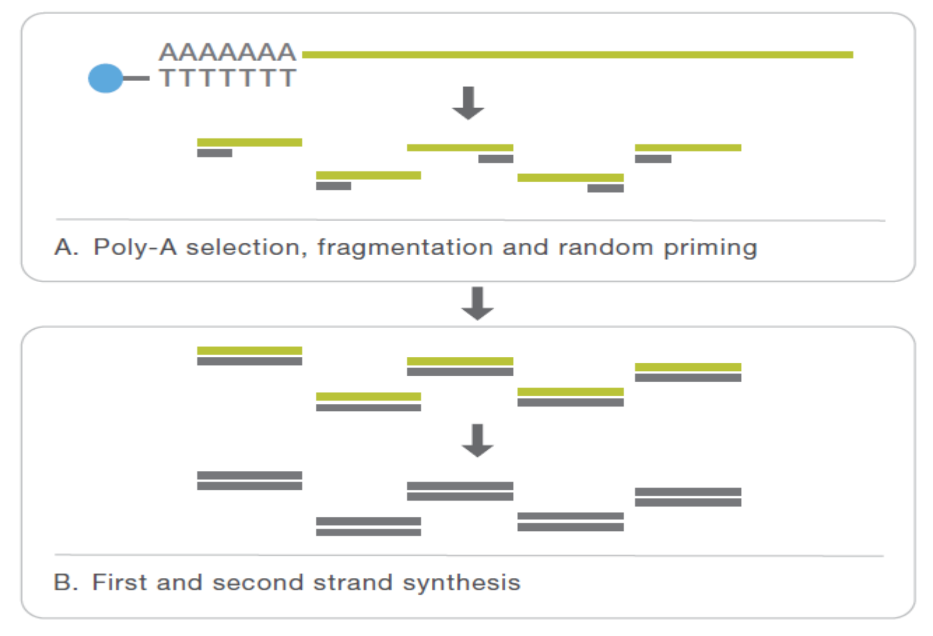
§ Kewalo Marine Lab, Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii 96813, USA

|| Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030, USA

A. E. Pasquinelli et al., Nature 408, 86-9 (2000)

How can we study miRNA ?

- RNAseq not suited for miRNA (protocol and size)


AAAAAAA
TTTTTTT
A. Poly-A selection, fragmentation and random priming
B. First and second strand synthesis

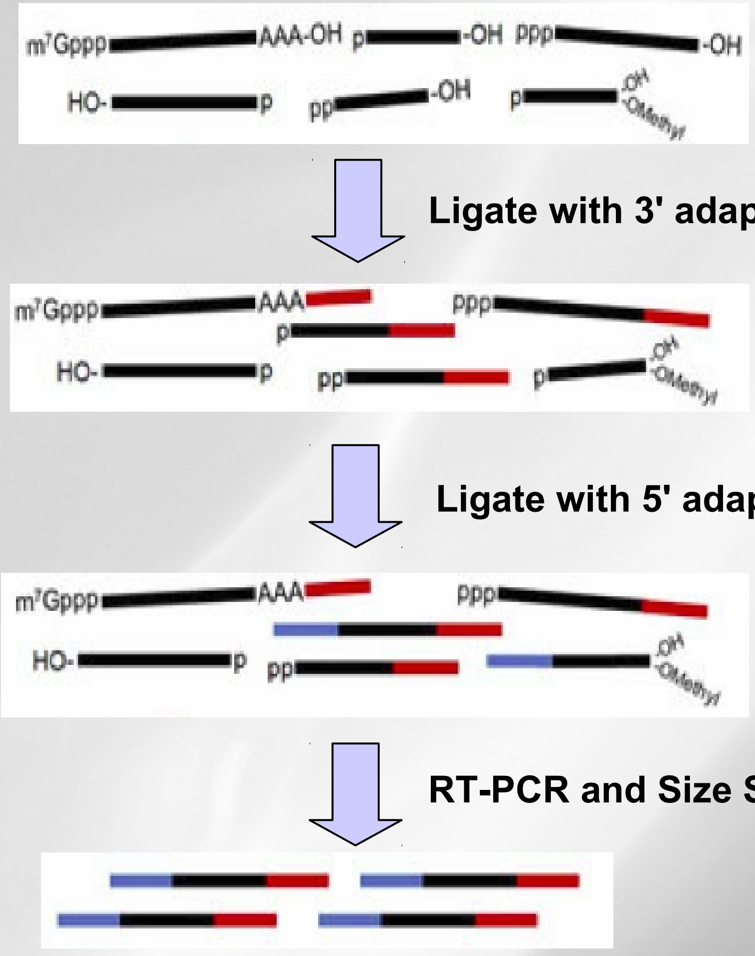
- small RNAseq: ability of high throughput sequencing to
  - Interrogate known and new small RNAs
  - Quantify them
  - Profile them on a large number of samples
  - Cost-effective

small RNAseq platforms comparisons

Platform	**454 Roche Titanium**	**HiSeq2000 Illumina**	**Solid 3+ Life Technologies**
Characteristics	-Titanium chemistry -Pyrosequencing -PCR amplification	- Polymerase-based sequence-by-synthesis -PCR amplification -Multiplexing	-ligation-base-sequencing -PCR amplification
Applications	-De novo sequencing -Small genomes -Transcriptome	-Resequencing -Transcriptomic/RNAseq -Epigenomic -Small RNA -Allele specific sequencing	-De novo sequencing -Resequencing -Transcriptomic/RNAseq -Epigenomic -Small RNA
Paired end separation	Not used	200bp	200bp
Mb / run	800Mb	600Gb	60Gb
Read length	800 bp	100bp	50bp
Known Biases	- Long homopolymer - makes signal saturation - read duplication	- Rich GC or AT regions: under-representation during amplification - Most error in end of cycle	- read duplication ?

small RNA-Seq library preparation

- Monophosphate presence in 5' extremity and OH presence in 3' extremity



Total RNA: contain all kinds of RNA species including miRNA, mRNA, tRNA, rRNA...

Ligate with 3' adapter

RNA with modified 3'-end will not ligate with 3' adapters. Only RNA with OH in 3'-end will ligate.

Ligate with 5' adapter

Only RNA with monophosphate in 5'-end will ligate with 5' adapters.

RT-PCR and Size Selection

CDNA containing both adapter sequences will be amplified. MicroRNA will be enriched from PCR and gel size selection.

MicroRNA sequencing library

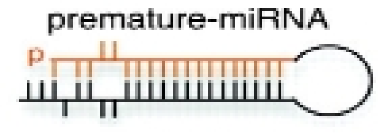
What are we looking for ?

- List of known miRNA
- List of new miRNA
- miRNA target(s)
- miRNA quantification
- Differential expression

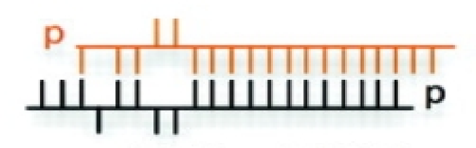
small RNAseq data analysis

What should we retain for data analysis ?

- Pre-miRNA information:



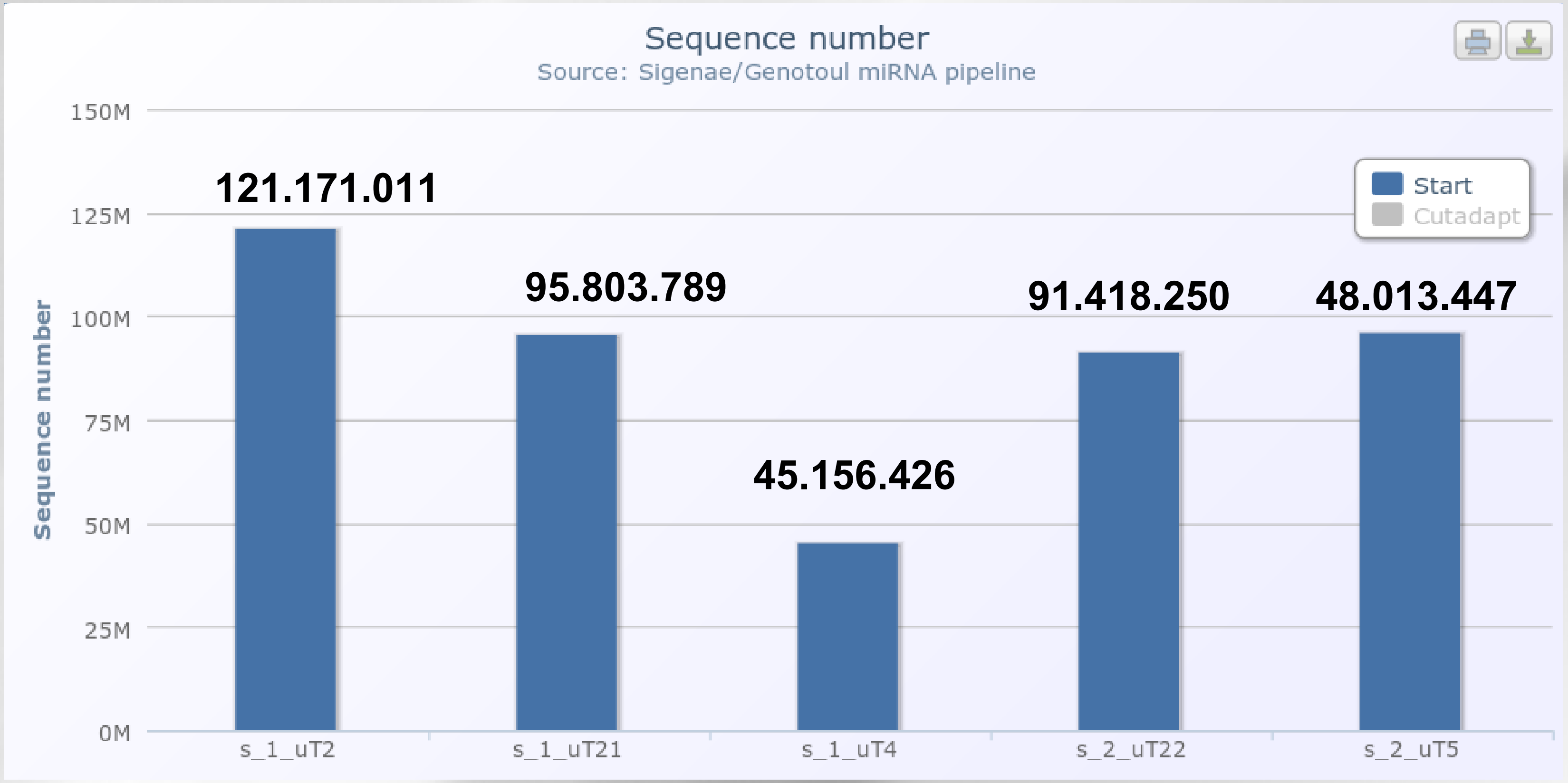
  - Hairpin structure of the pre-miRNA
  - Pre-miRNA localisation (coding/non coding TU intronic/exonic)
  - Presence of cluster
  - Size of the pre-miRNA
- miRNA and miRNA* information:



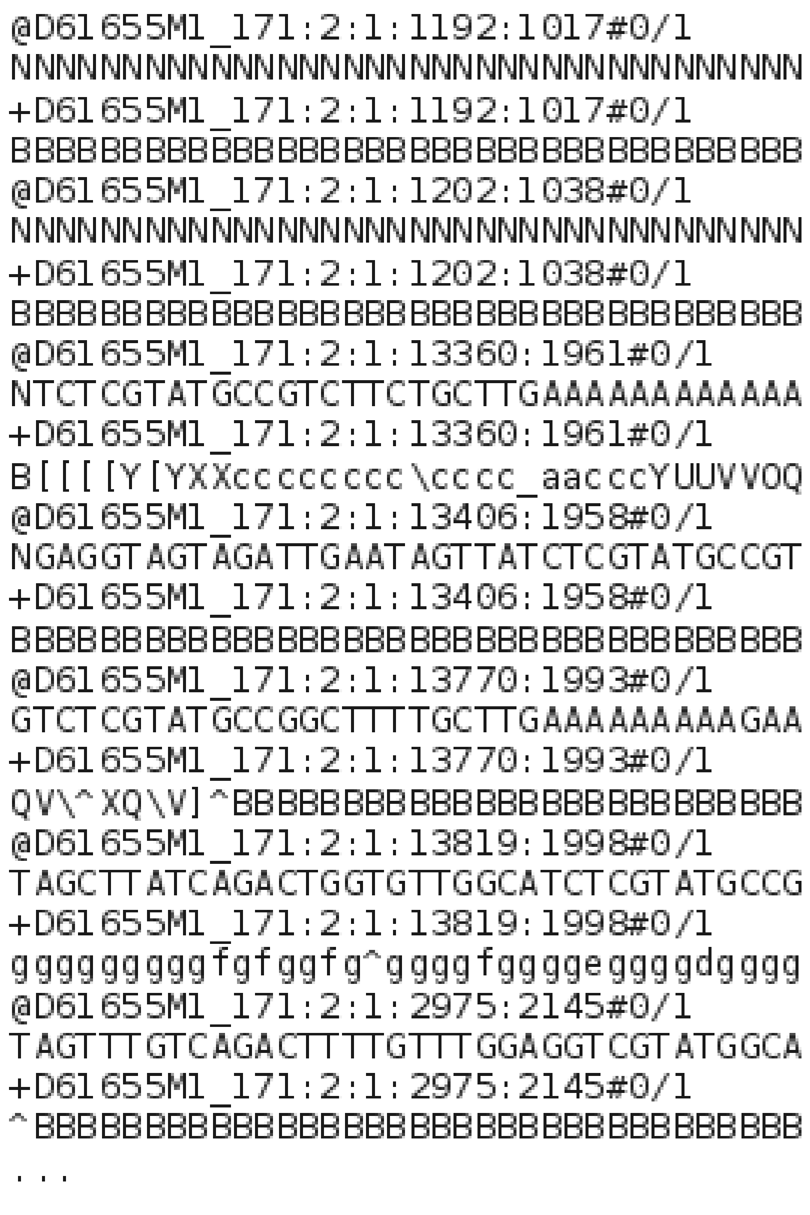
  - Existence of both miRNA and miRNA*
  - Sequence conservation
  - Overhang (around 2 nt) related to drosha and Dicer cuts
  - Size of miRNA and miRNA*
  - Overexpression of the miRNA compared to the miRNA*
- Existence of other products in sRNAseq data

Description of the dataset

- 5 experiments (5 lanes, no multiplexing)
  - Different tissues, different stages
- No reference genome
  - Only scaffolds



Fastq format



```
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+D61655M1_171:2:1:1192:1017#0/1
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@D61655M1_171:2:1:1202:1038#0/1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
+D61655M1_171:2:1:1202:1038#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13360:1961#0/1
NTCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
+D61655M1_171:2:1:13360:1961#0/1
B[[[[Y[YXXcccccccc\cccc_aacccYUUVVOQ
@D61655M1_171:2:1:13406:1958#0/1
NGAGGTAGTAGATTGAATAGTTATCTCGTATGCCGT
+D61655M1_171:2:1:13406:1958#0/1
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@D61655M1_171:2:1:13770:1993#0/1
GTCTCGTATGCCGGCTTTTGCTTGAAAAAAAAAGAA
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QV\^XQ\V]^BBBBBBBBBBBBBBBBBBBBBBBBBB
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+D61655M1_171:2:1:13819:1998#0/1
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@D61655M1_171:2:1:2975:2145#0/1
TAGTTTGTCAGACTTTTGTTTGGAGGTCGTATGGCA
+D61655M1_171:2:1:2975:2145#0/1
^BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
...
```
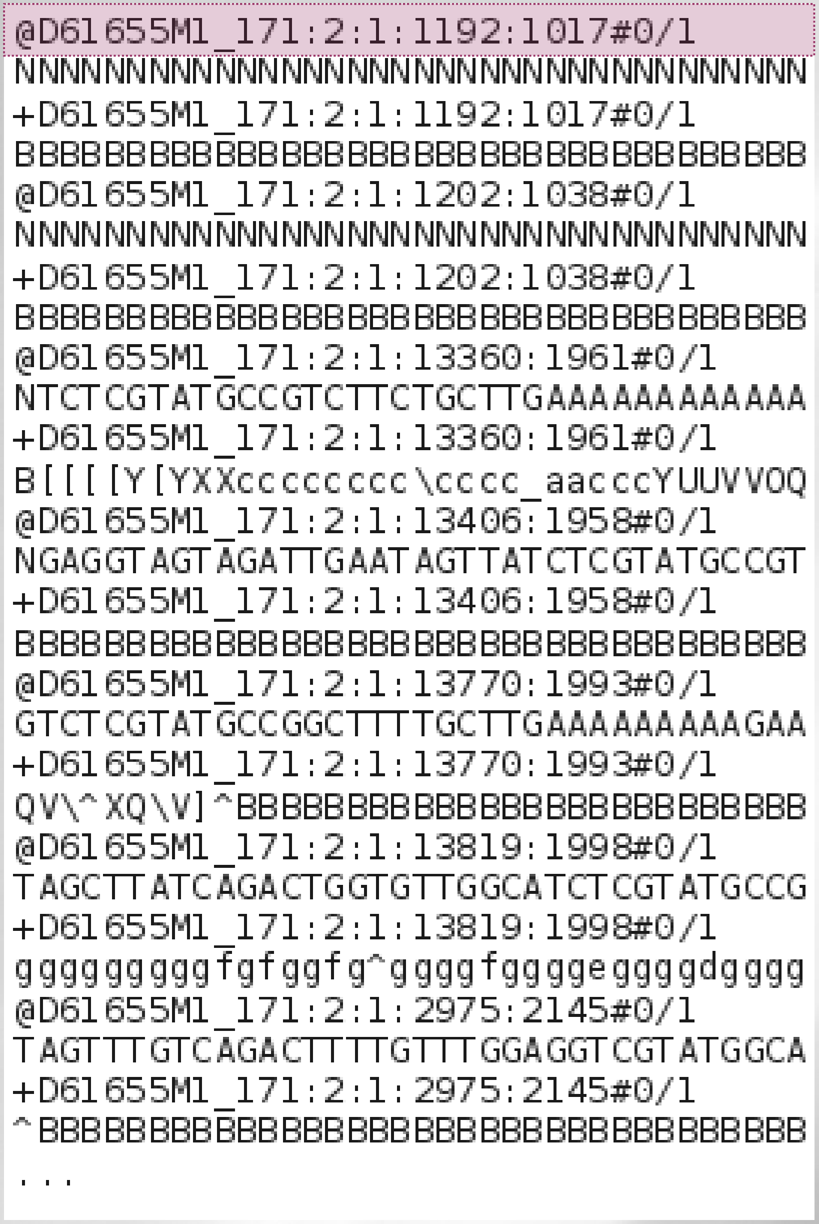

Fastq format



```
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@D61655M1_171:2:1:1202:1038#0/1
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+D61655M1_171:2:1:13770:1993#0/1
QV\^XQ\V]^BBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13819:1998#0/1
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
+D61655M1_171:2:1:13819:1998#0/1
gggggggggfgfggfg^ggggfggggeggggdgggg
@D61655M1_171:2:1:2975:2145#0/1
TAGTTTGTCAGACTTTTGTTTGGAGGTCGTATGGCA
+D61655M1_171:2:1:2975:2145#0/1
^BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
...
```
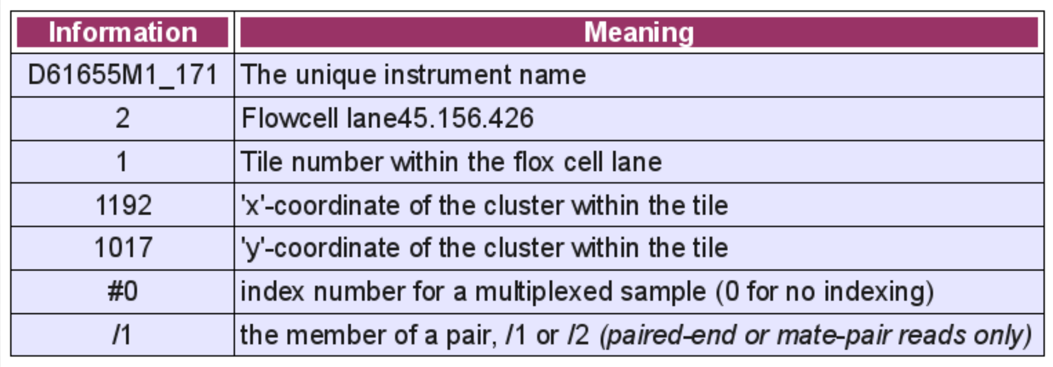
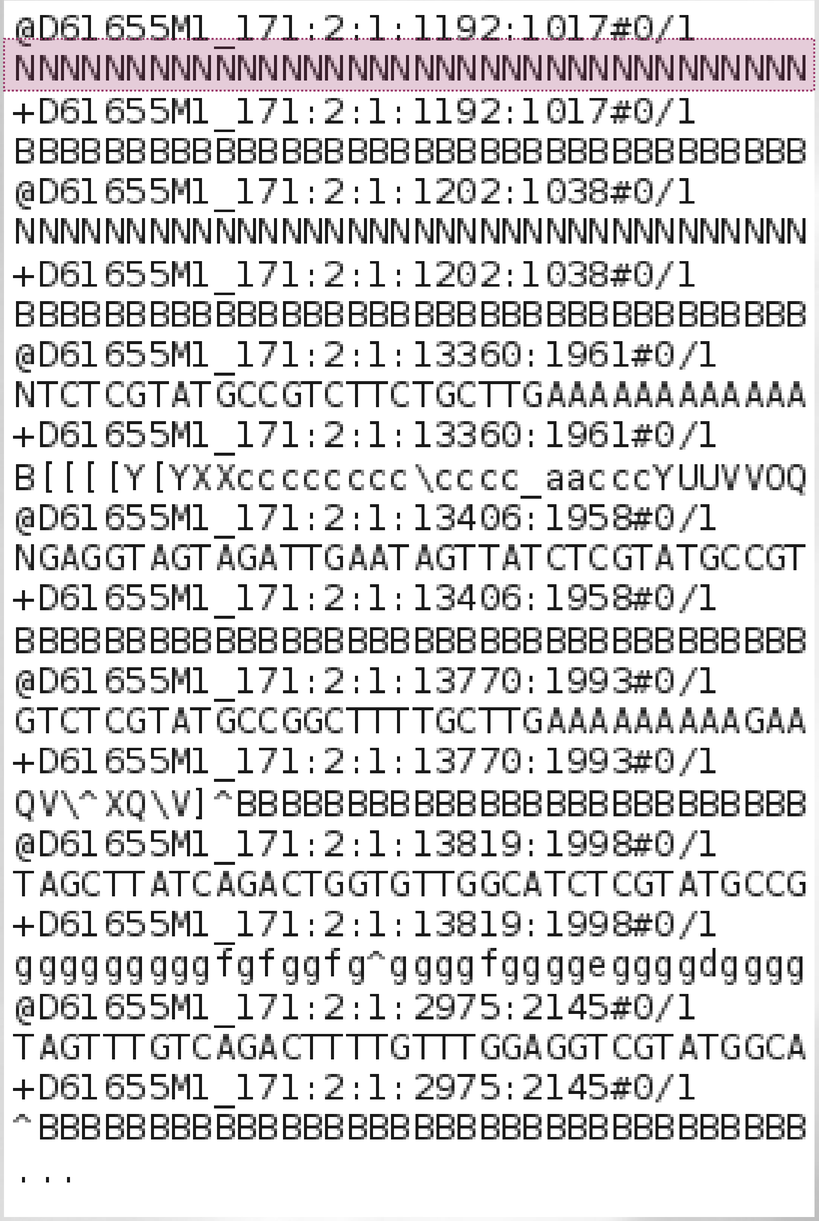

Line 1 starts with @



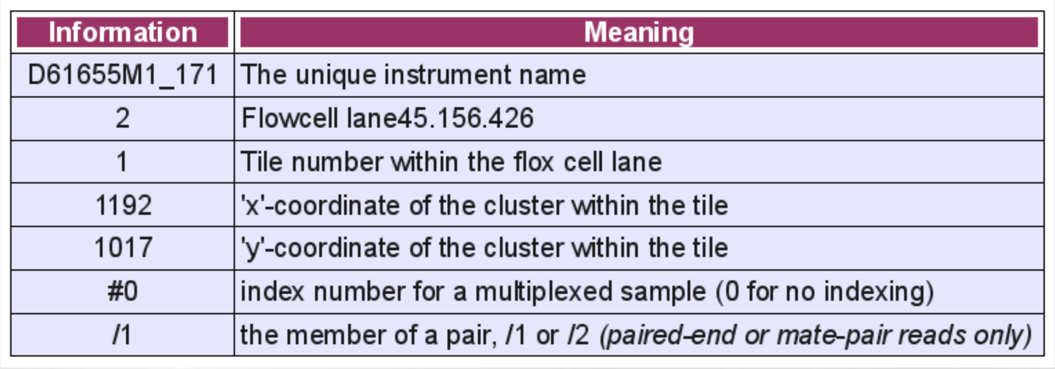
Information	Meaning
D61655M1_171	The unique instrument name
2	Flowcell lane45.156.426
1	Tile number within the flox cell lane
1192	'x'-coordinate of the cluster within the tile
1017	'y'-coordinate of the cluster within the tile
#0	index number for a multiplexed sample (0 for no indexing)
/1	the member of a pair, /1 or /2 *(paired-end or mate-pair reads only)*

Fastq format



```
@D61655M1_171:2:1:1192:1017#0/1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
+D61655M1_171:2:1:1192:1017#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:1202:1038#0/1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
+D61655M1_171:2:1:1202:1038#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13360:1961#0/1
NTCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
+D61655M1_171:2:1:13360:1961#0/1
B[[[[Y[YXXcccccccc\cccc_aacccYUUVVOQ
@D61655M1_171:2:1:13406:1958#0/1
NGAGGTAGTAGATTGAATAGTTATCTCGTATGCCGT
+D61655M1_171:2:1:13406:1958#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13770:1993#0/1
GTCTCGTATGCCGGCTTTTGCTTGAAAAAAAAAGAA
+D61655M1_171:2:1:13770:1993#0/1
QV\^XQ\V]^BBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13819:1998#0/1
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
+D61655M1_171:2:1:13819:1998#0/1
gggggggggfgfggfg^ggggfggggeggggdgggg
@D61655M1_171:2:1:2975:2145#0/1
TAGTTTGTCAGACTTTTGTTTGGAGGTCGTATGGCA
+D61655M1_171:2:1:2975:2145#0/1
^BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
...
```

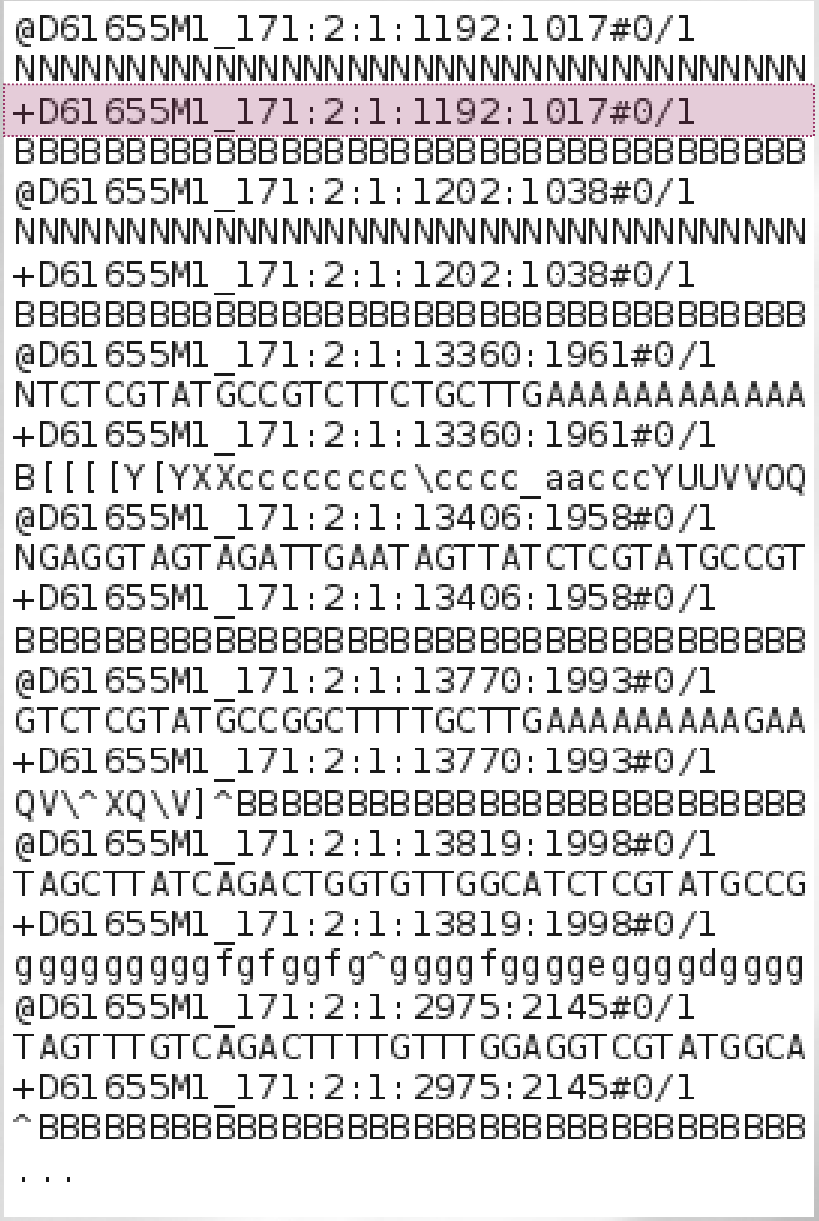
Line 1 starts with @



Information	Meaning
D61655M1_171	The unique instrument name
2	Flowcell lane45.156.426
1	Tile number within the flox cell lane
1192	'x'-coordinate of the cluster within the tile
1017	'y'-coordinate of the cluster within the tile
#0	index number for a multiplexed sample (0 for no indexing)
/1	the member of a pair, /1 or /2 *(paired-end or mate-pair reads only)*

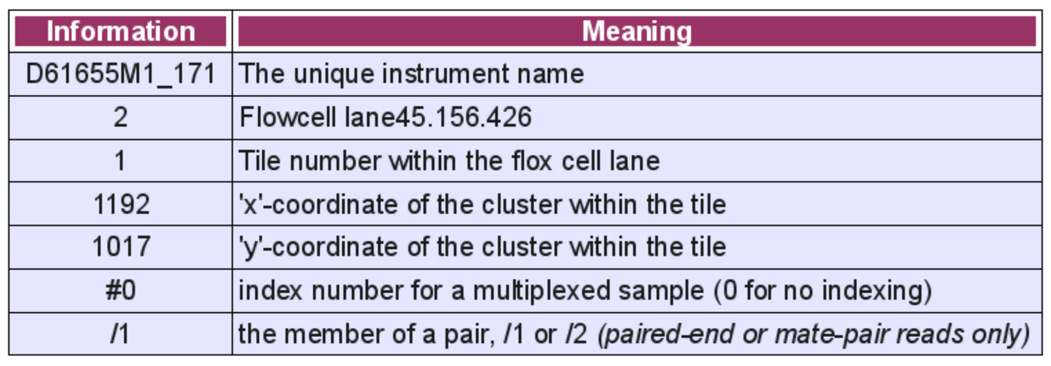
Line 2 Raw sequence of 36 nt (36 cycles in sequencing)

Fastq format



```
@D61655M1_171:2:1:1192:1017#0/1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
+D61655M1_171:2:1:1192:1017#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:1202:1038#0/1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
+D61655M1_171:2:1:1202:1038#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13360:1961#0/1
NTCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
+D61655M1_171:2:1:13360:1961#0/1
B[[[[Y[YXXcccccccc\cccc_aacccYUUVVOQ
@D61655M1_171:2:1:13406:1958#0/1
NGAGGTAGTAGATTGAATAGTTATCTCGTATGCCGT
+D61655M1_171:2:1:13406:1958#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13770:1993#0/1
GTCTCGTATGCCGGCTTTTGCTTGAAAAAAAAAGAA
+D61655M1_171:2:1:13770:1993#0/1
QV\^XQ\V]^BBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13819:1998#0/1
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
+D61655M1_171:2:1:13819:1998#0/1
gggggggggfgfggfg^ggggfggggeggggdgggg
@D61655M1_171:2:1:2975:2145#0/1
TAGTTTGTCAGACTTTTGTTTGGAGGTCGTATGGCA
+D61655M1_171:2:1:2975:2145#0/1
^BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
...
```

Line 1 starts with @



Information	Meaning
D61655M1_171	The unique instrument name
2	Flowcell lane45.156.426
1	Tile number within the flox cell lane
1192	'x'-coordinate of the cluster within the tile
1017	'y'-coordinate of the cluster within the tile
#0	index number for a multiplexed sample (0 for no indexing)
/1	the member of a pair, /1 or /2 *(paired-end or mate-pair reads only)*

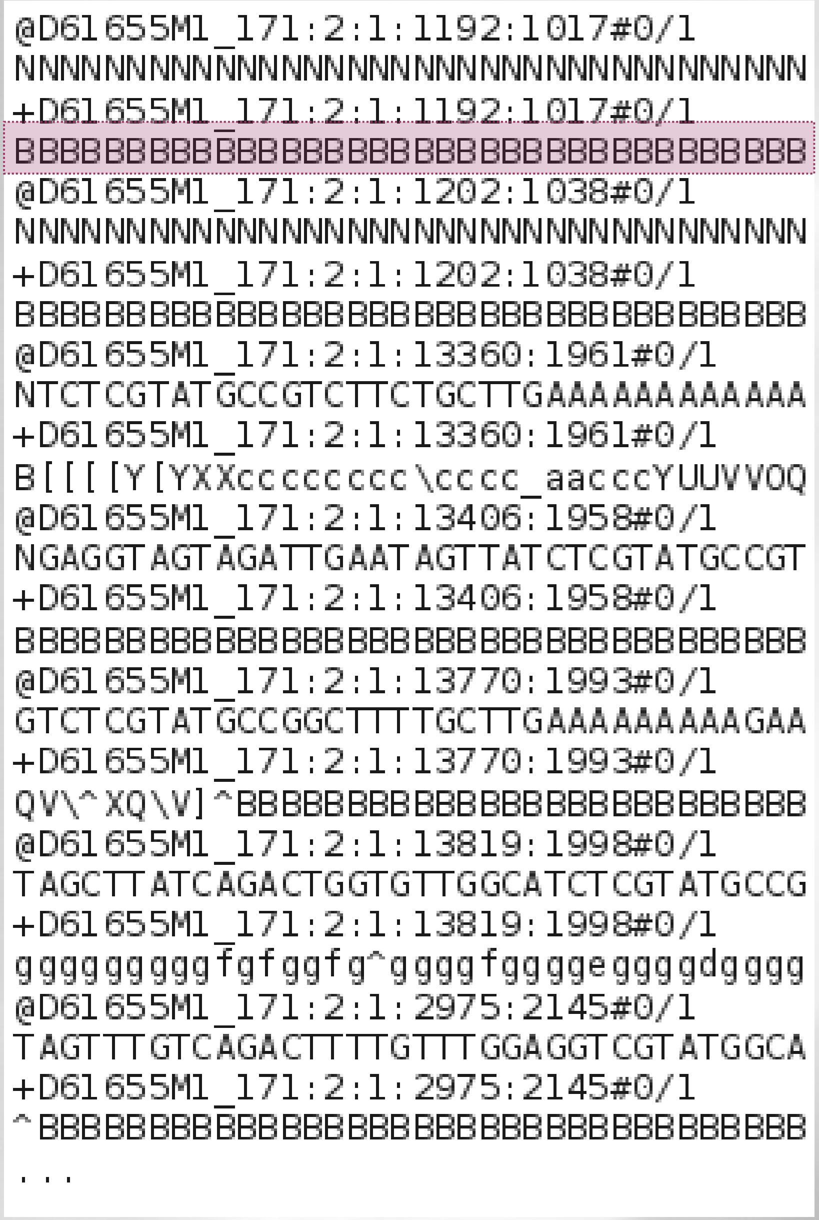
Line 2 Raw sequence of 36 nt (36 cycles in sequencing)

Line 3 starts with a '+' character and is optionally followed by the same sequence identifier (and any description) again.

Plateforme Bioinformatique Midi-Pyrénées

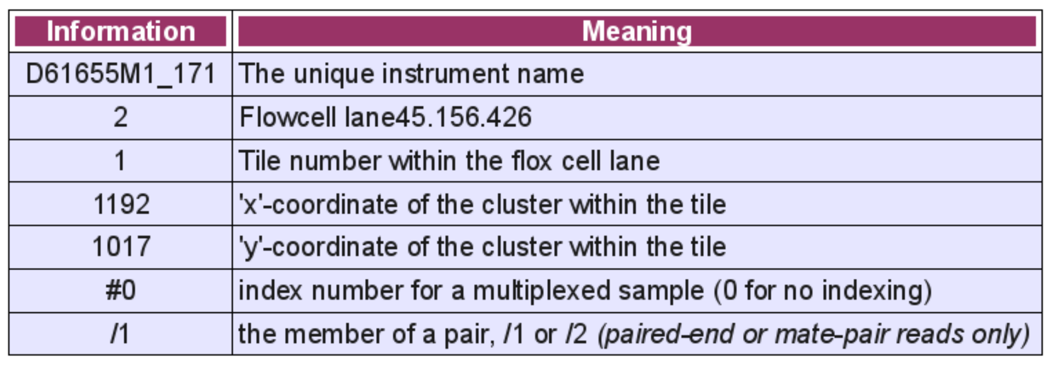
geno toul bioinfo

Fastq format



```
@D61655M1_171:2:1:1192:1017#0/1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
+D61655M1_171:2:1:1192:1017#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:1202:1038#0/1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
+D61655M1_171:2:1:1202:1038#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13360:1961#0/1
NTCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
+D61655M1_171:2:1:13360:1961#0/1
B[[[[Y[YXXcccccccc\cccc_aacccYUUVVOQ
@D61655M1_171:2:1:13406:1958#0/1
NGAGGTAGTAGATTGAATAGTTATCTCGTATGCCGT
+D61655M1_171:2:1:13406:1958#0/1
BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13770:1993#0/1
GTCTCGTATGCCGGCTTTTGCTTGAAAAAAAAAGAA
+D61655M1_171:2:1:13770:1993#0/1
QV\^XQ\V]^BBBBBBBBBBBBBBBBBBBBBBBBBB
@D61655M1_171:2:1:13819:1998#0/1
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
+D61655M1_171:2:1:13819:1998#0/1
gggggggggfgfggfg^ggggfggggeggggdgggg
@D61655M1_171:2:1:2975:2145#0/1
TAGTTTGTCAGACTTTTGTTTGGAGGTCGTATGGCA
+D61655M1_171:2:1:2975:2145#0/1
^BBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBBB
...
```

Line 1 starts with @



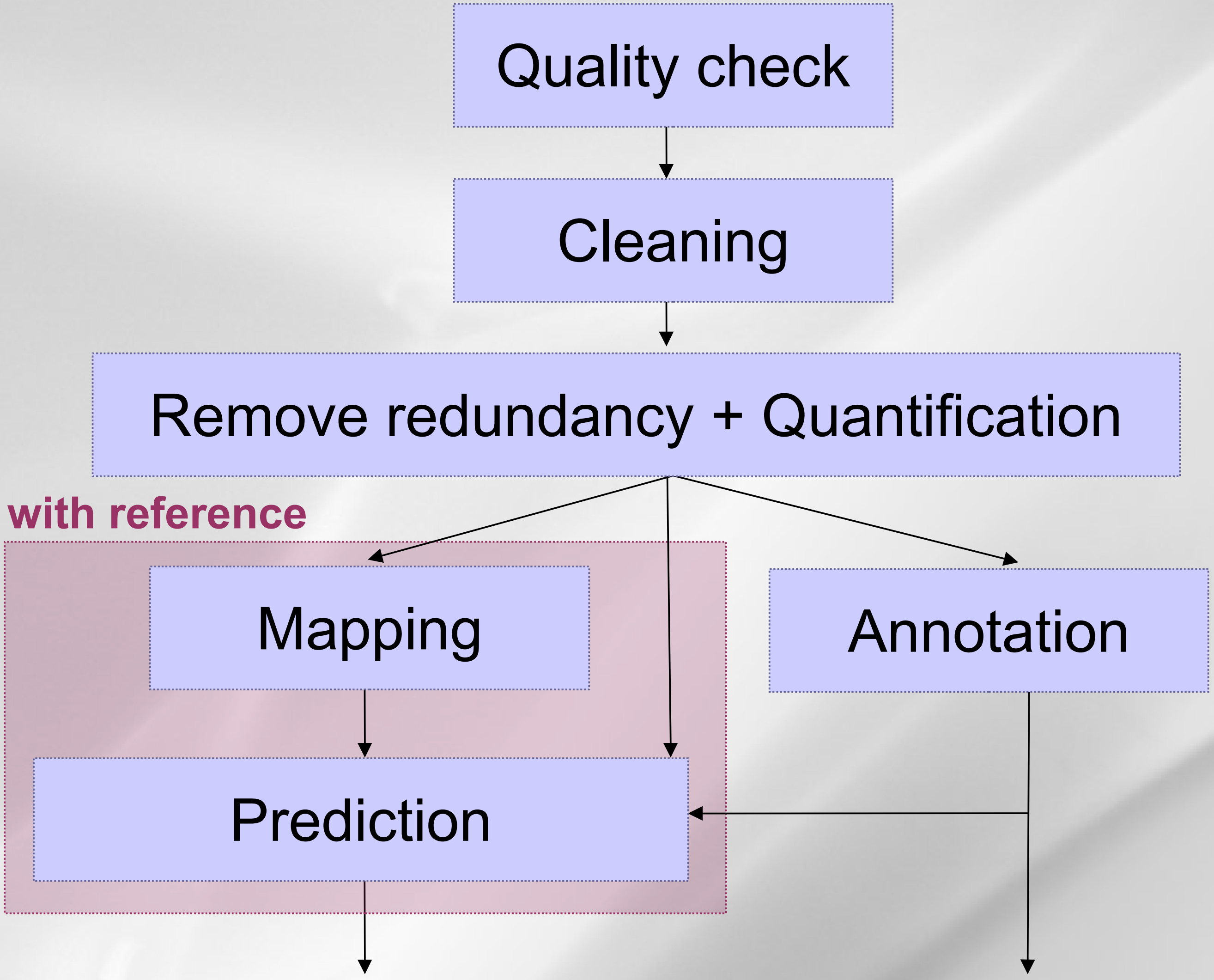
Information	Meaning
D61655M1_171	The unique instrument name
2	Flowcell lane45.156.426
1	Tile number within the flox cell lane
1192	'x'-coordinate of the cluster within the tile
1017	'y'-coordinate of the cluster within the tile
#0	index number for a multiplexed sample (0 for no indexing)
/1	the member of a pair, /1 or /2 *(paired-end or mate-pair reads only)*

Line 2 Raw sequence of 36 nt (36 cycles in sequencing)

Line 3 starts with a '+' character and is optionally followed by the same sequence identifier (and any description) again.

Line 4 Line 4 encodes the quality values for the sequence in Line 2, and must contain the same number of symbols as letters in the sequence.

small RNAseq pipeline



Quality check

Cleaning

Remove redundancy + Quantification

with reference

Mapping

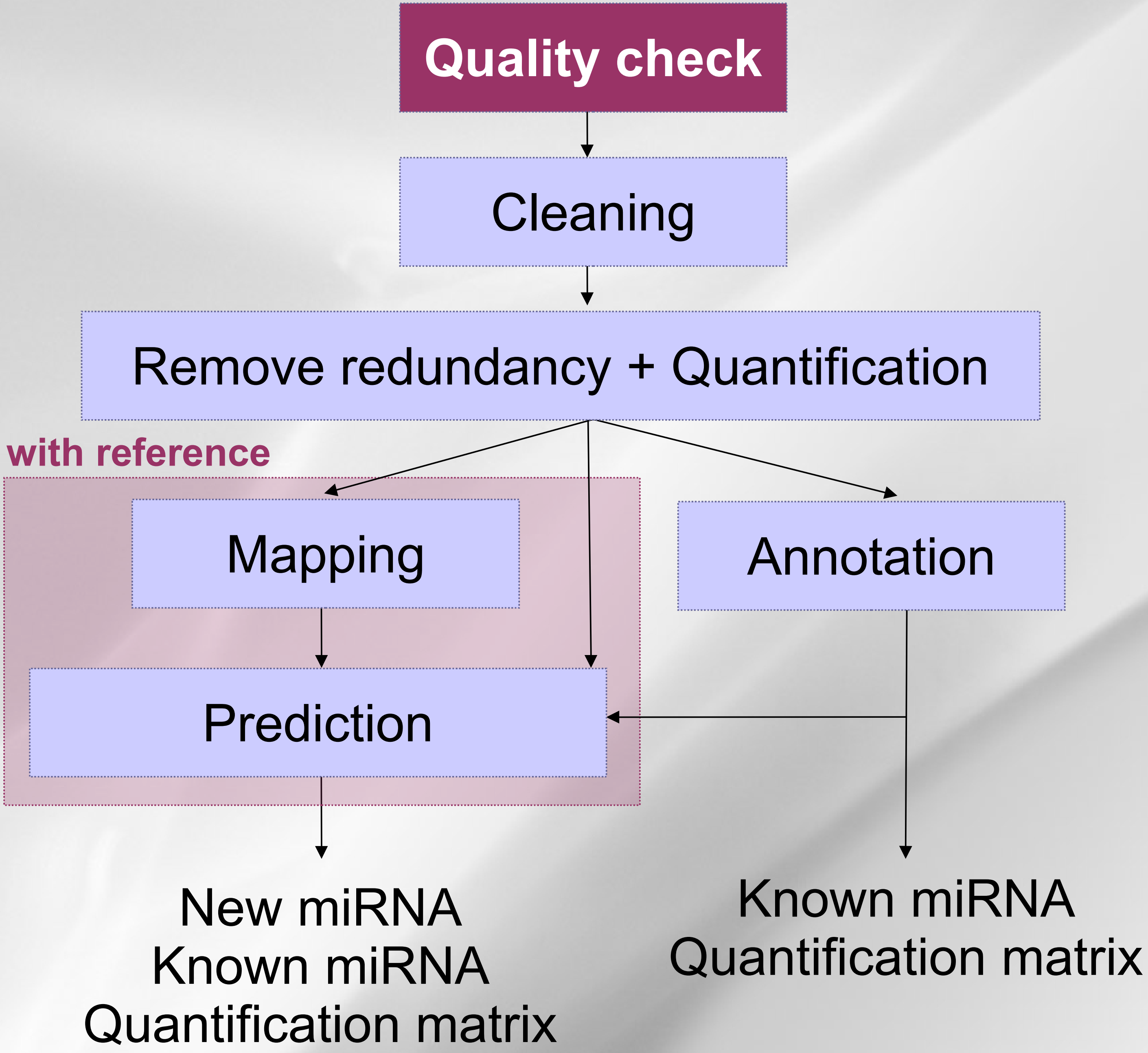
Annotation

Prediction

New miRNA
Known miRNA
Quantification matrix

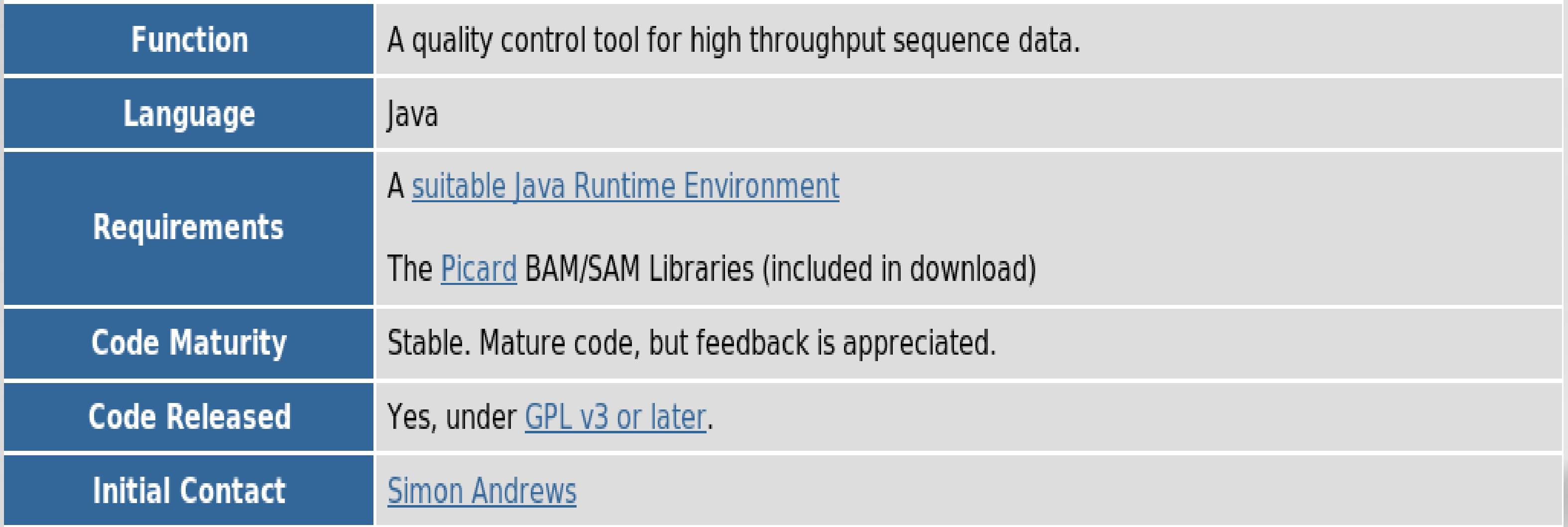
Known miRNA
Quantification matrix

small RNAseq pipeline



1. Quality control

- **FastQC** (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/)



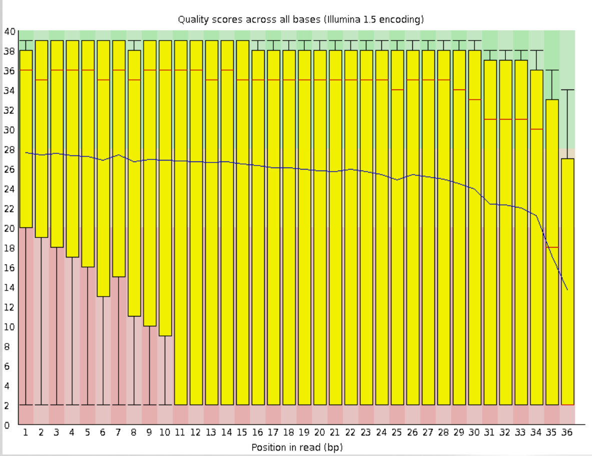
Function	A quality control tool for high throughput sequence data.
Language	Java
Requirements	A suitable Java Runtime Environment The Picard BAM/SAM Libraries (included in download)
Code Maturity	Stable. Mature code, but feedback is appreciated.
Code Released	Yes, under GPL v3 or later.
Initial Contact	Simon Andrews

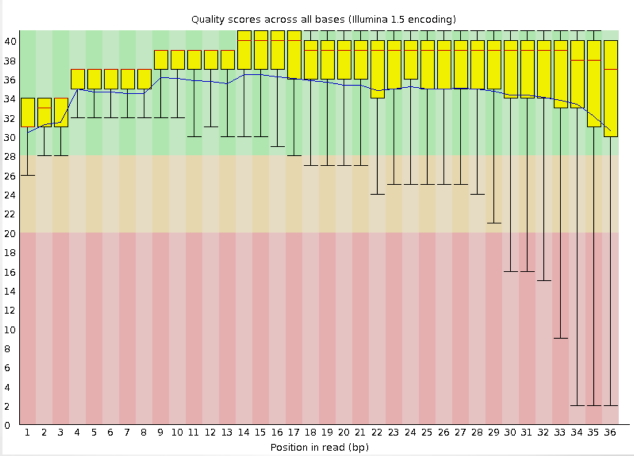
A simple way to do quality control. It provides a modular set of analyses to give a quick impression of whether data has any problems of which you should be aware before doing any further analysis. The main functions of FastQC are:
- Import of data from BAM, SAM or FastQ files (any variant)
- Provide a quick overview to tell you in which areas there may be problems
- Summary graphs and tables to quickly assess your data
- Export of results to an HTML based permanent report
- Offline operation to allow automated generation of reports without running the interactive application

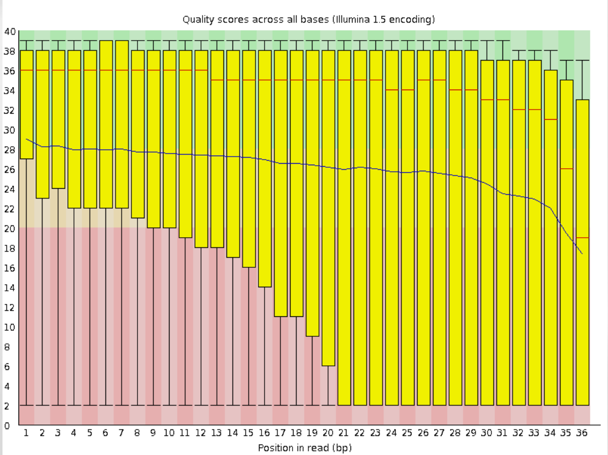
```
Fastqc -o nf.out nf_in.fastq
```

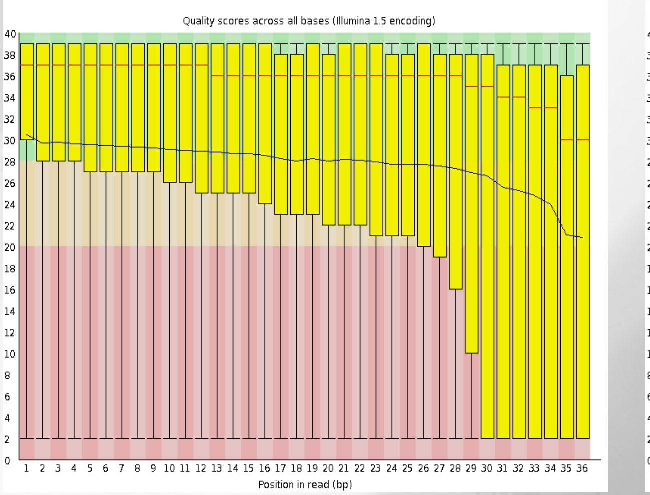
1. Quality control

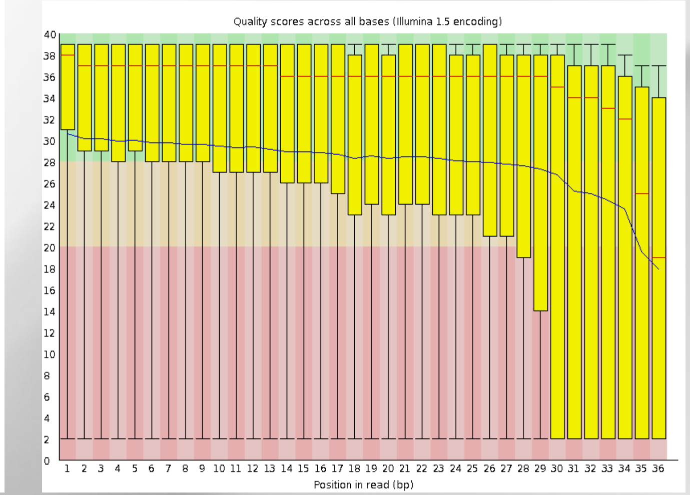
- **Per base quality**



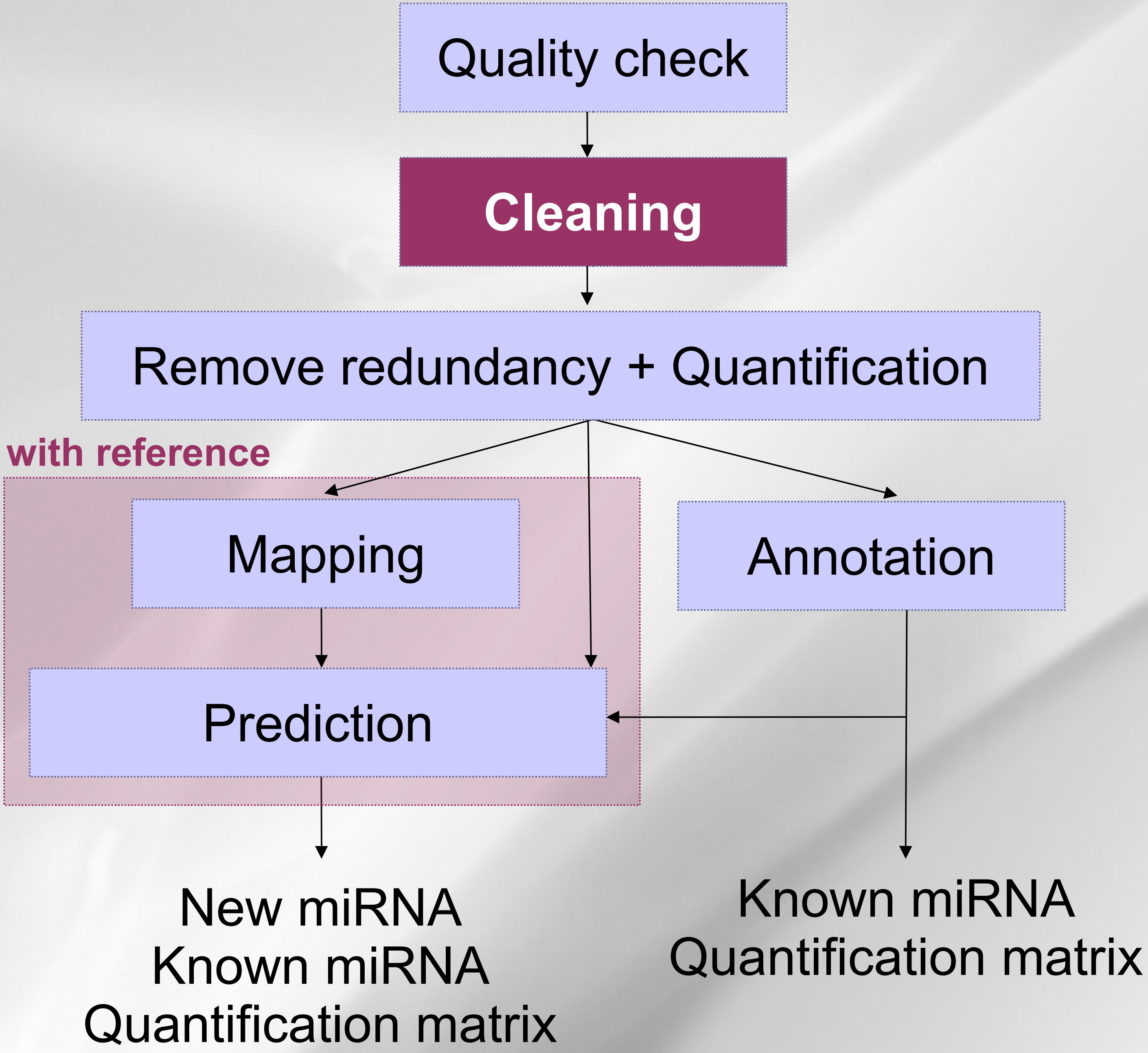








small RNAseq pipeline



Quality check

Cleaning

Remove redundancy + Quantification

with reference

Mapping

Annotation

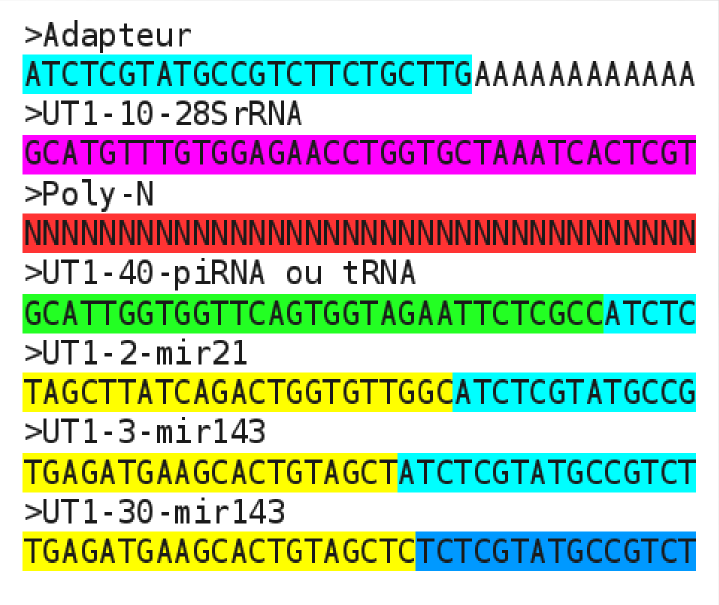
Prediction

New miRNA
Known miRNA
Quantification matrix

Known miRNA
Quantification matrix

2. Why cleaning ?

Outputed reads

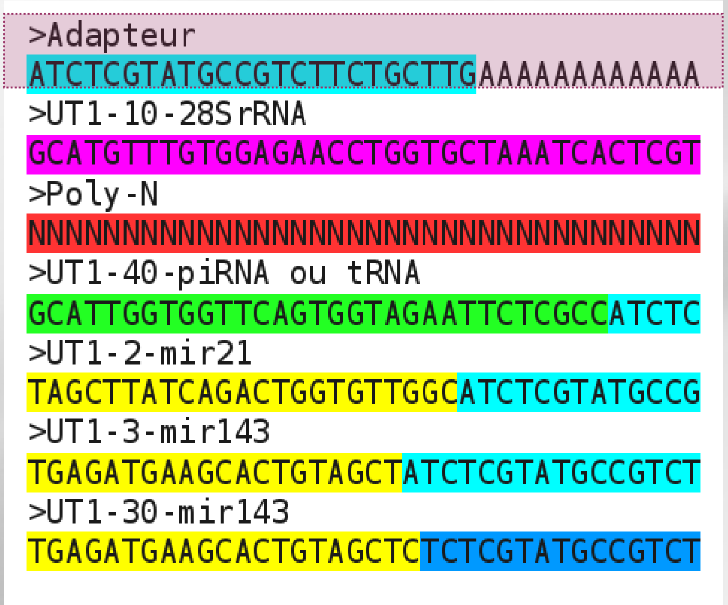


```
>Adapteur
ATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
>UT1-10-28SrRNA
GCATGTTTGTGGAGAACCTGGTGCTAAATCACTCGT
>Poly-N
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>UT1-40-piRNA ou tRNA
GCATTGGTGGTTCAGTGGTAGAATTCTCGCCATCTC
>UT1-2-mir21
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
>UT1-3-mir143
TGAGATGAAGCACTGTAGCTATCTCGTATGCCGTCT
>UT1-30-mir143
TGAGATGAAGCACTGTAGCTCTCTCGTATGCCGTCT
```

2. Why cleaning ?

Outputed reads

- Some sequences contain only adapters

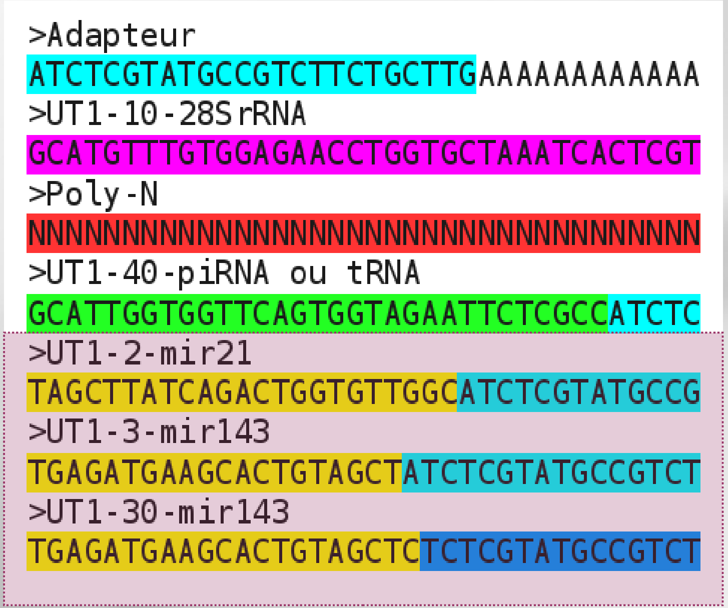


```
>Adapteur
ATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
>UT1-10-28SrRNA
GCATGTTTGTGGAGAACCTGGTGCTAAATCACTCGT
>Poly-N
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>UT1-40-piRNA ou tRNA
GCATTGGTGGTTCAGTGGTAGAATTCTCGCCATCTC
>UT1-2-mir21
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
>UT1-3-mir143
TGAGATGAAGCACTGTAGCTATCTCGTATGCCGTCT
>UT1-30-mir143
TGAGATGAAGCACTGTAGCTCTCTCGTATGCCGTCT
```

2. Why cleaning ?

Outputed reads

- Some sequences contain only adapters
- Some sequences contain sequences of interest flanked by the beginning of adapters:
    - Some of them are miRNA (yellow).

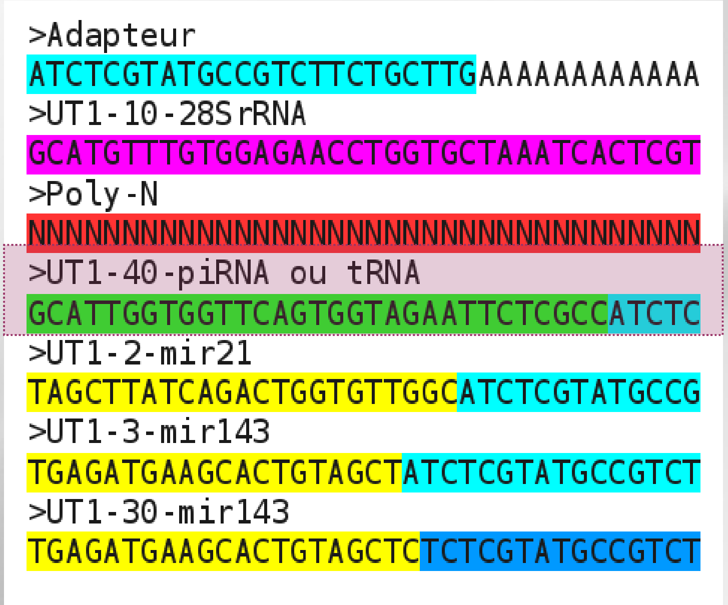


```
>Adapteur
ATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
>UT1-10-28S rRNA
GCATGTTTGTGGAGAACCTGGTGCTAAATCACTCGT
>Poly-N
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>UT1-40-piRNA ou tRNA
GCATTGGTGGTTCAGTGGTAGAATTCTCGCCATCTC
>UT1-2-mir21
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
>UT1-3-mir143
TGAGATGAAGCACTGTAGCTATCTCGTATGCCGTCT
>UT1-30-mir143
TGAGATGAAGCACTGTAGCTCTCTCGTATGCCGTCT
```

2. Why cleaning ?

Outputed reads

- Some sequences contain only adapters
- Some sequences contain sequences of interest flanked by the beginning of adapters:
  - Some of them are miRNA (yellow).
  - Some of them are other type of RNAs (green).

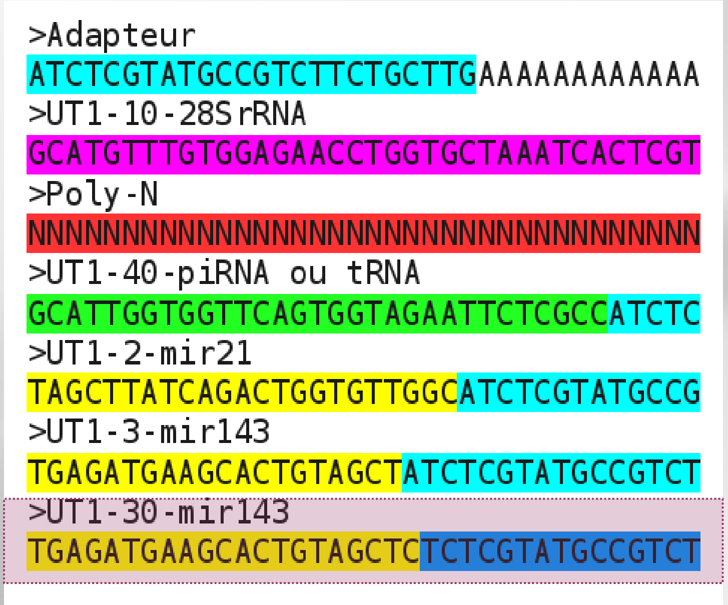


```
>Adapteur
ATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
>UT1-10-28S rRNA
GCATGTTTGTGGAGAACCTGGTGCTAAATCACTCGT
>Poly-N
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>UT1-40-piRNA ou tRNA
GCATTGGTGGTTCAGTGGTAGAATTCTCGCCATCTC
>UT1-2-mir21
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
>UT1-3-mir143
TGAGATGAAGCACTGTAGCTATCTCGTATGCCGTCT
>UT1-30-mir143
TGAGATGAAGCACTGTAGCTCTCTCGTATGCCGTCT
```

2. Why cleaning ?

Outputed reads

- Some sequences contain only adapters
- Some sequences contain sequences of interest flanked by the beginning of adapters:
    - Some of them are miRNA (yellow).
    - Some of them are other type of RNAs (green).
    - Some adapters contain errors (blue).

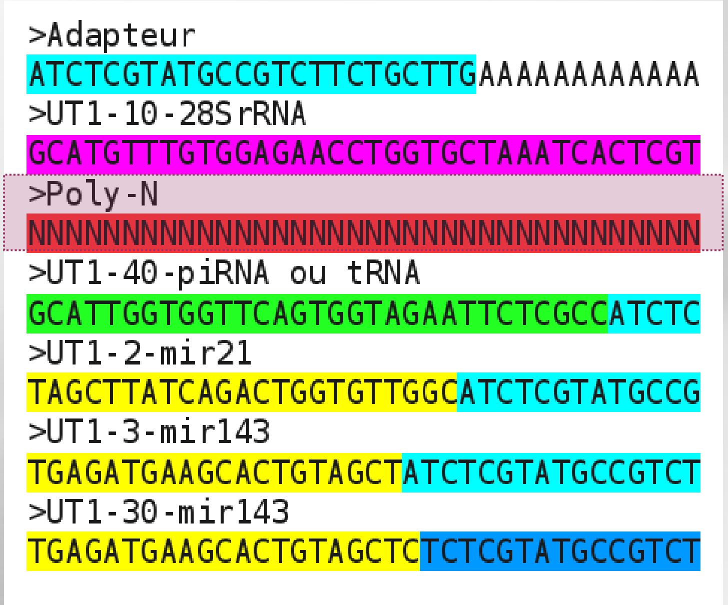


```
>Adapteur
ATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
>UT1-10-28S rRNA
GCATGTTTGTGGAGAACCTGGTGCTAAATCACTCGT
>Poly-N
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>UT1-40-piRNA ou tRNA
GCATTGGTGGTTCAGTGGTAGAATTCTCGCCATCTC
>UT1-2-mir21
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
>UT1-3-mir143
TGAGATGAAGCACTGTAGCTATCTCGTATGCCGTCT
>UT1-30-mir143
TGAGATGAAGCACTGTAGCTCTCTCGTATGCCGTCT
```

2. Why cleaning ?

Outputed reads

- Some sequences contain only adapters
- Some sequences contain sequences of interest flanked by the beginning of adapters:
    - Some of them are miRNA (yellow).
    - Some of them are other type of RNAs (green).
    - Some adapters contain errors (blue).
- Some sequences contain polyN (red)



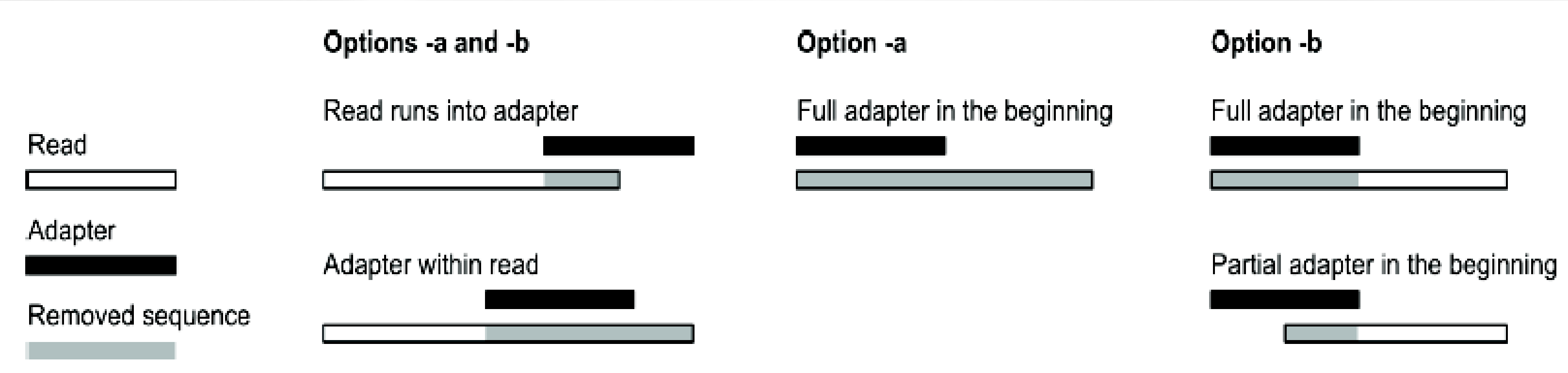
```
>Adapteur
ATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAA
>UT1-10-28S rRNA
GCATGTTTGTGGAGAACCTGGTGCTAAATCACTCGT
>Poly-N
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
>UT1-40-piRNA ou tRNA
GCATTGGTGGTTCAGTGGTAGAATTCTCGCCATCTC
>UT1-2-mir21
TAGCTTATCAGACTGGTGTTGGCATCTCGTATGCCG
>UT1-3-mir143
TGAGATGAAGCACTGTAGCTATCTCGTATGCCGTCT
>UT1-30-mir143
TGAGATGAAGCACTGTAGCTCTCTCGTATGCCGTCT
```

2. Cleaning

- **Adapters removing and length filtering**

Cutadapt http://code.google.com/p/cutadapt/.

Cutadapt removes adapter sequences from high-throughput sequencing data. Indeed, reads are usually longer than the RNA, and therefore contain parts of the 3’ adapter. It also allows to keep only sequences of desired length (15<length<29).

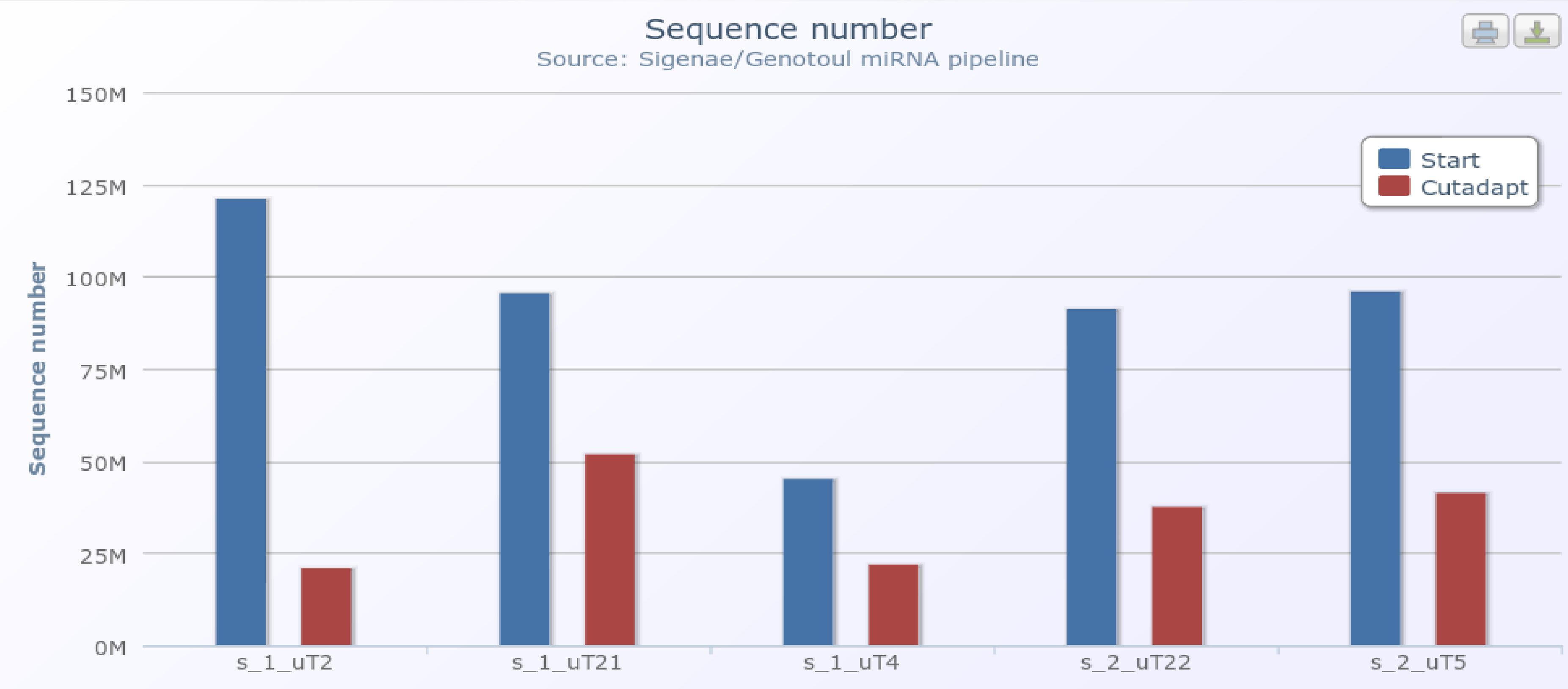


```
cutadapt -a ATCTCGTATGCCGTCTTCTGCTTG -m15  -M 29 -o nf_out.fg nf_in.fq
```

Plateforme Bioinformatique Midi-Pyrénées

2. Cleaning

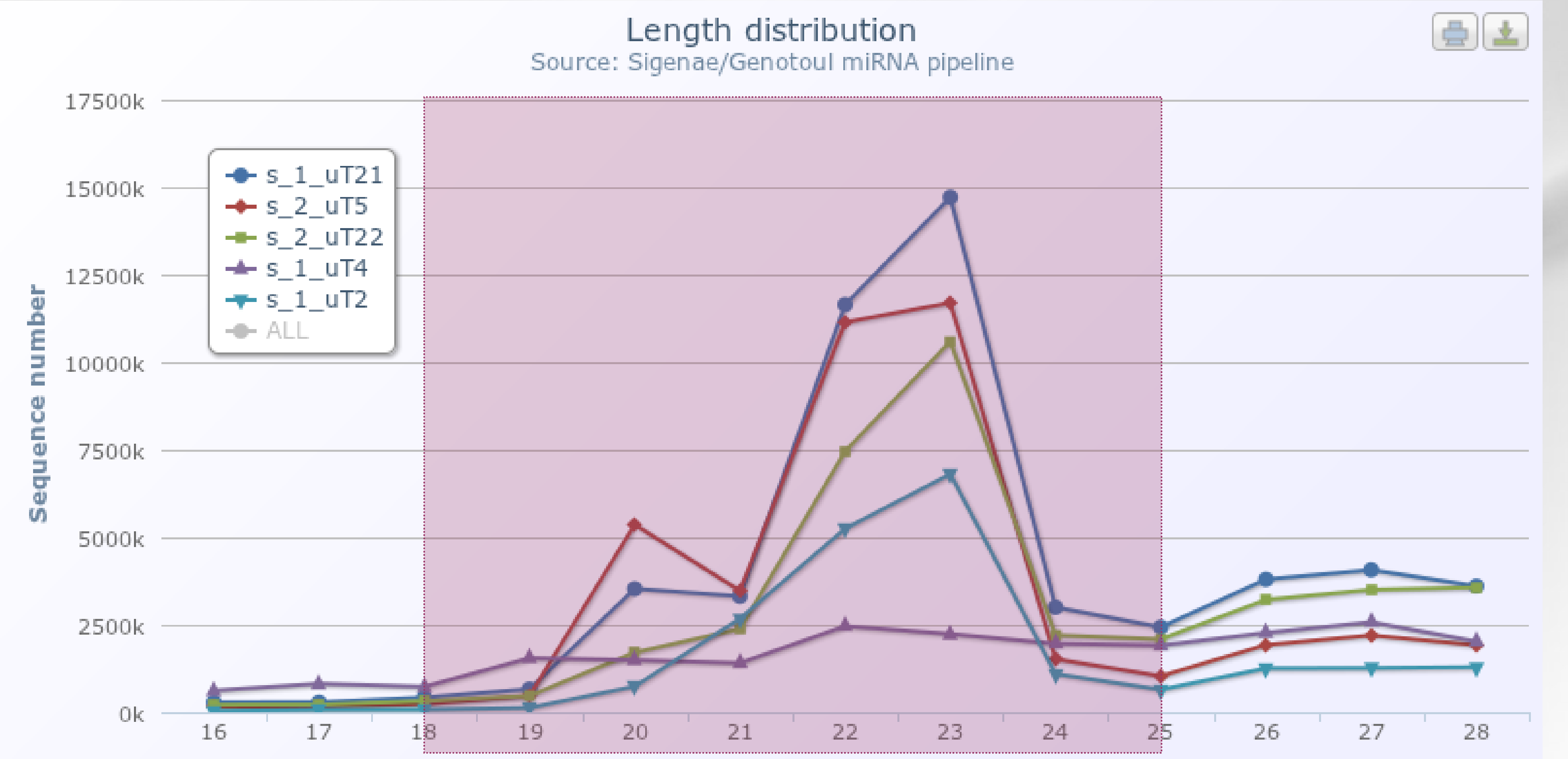
- **56 % of reads discarded**



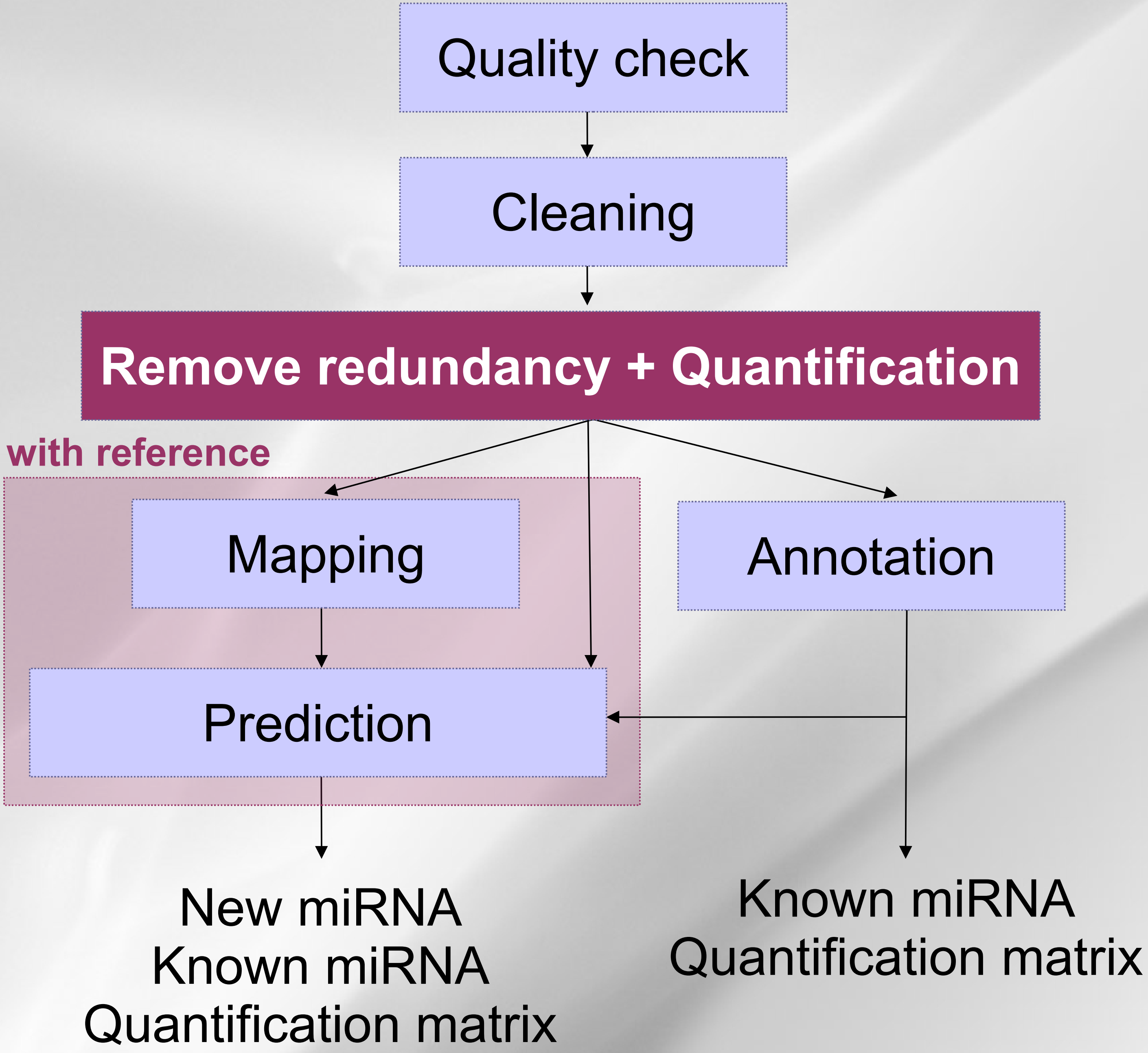
2. Cleaning

- **Size in between 18bp:24bp**

  → miRNA ?



small RNAseq pipeline



3. Remove redundancy

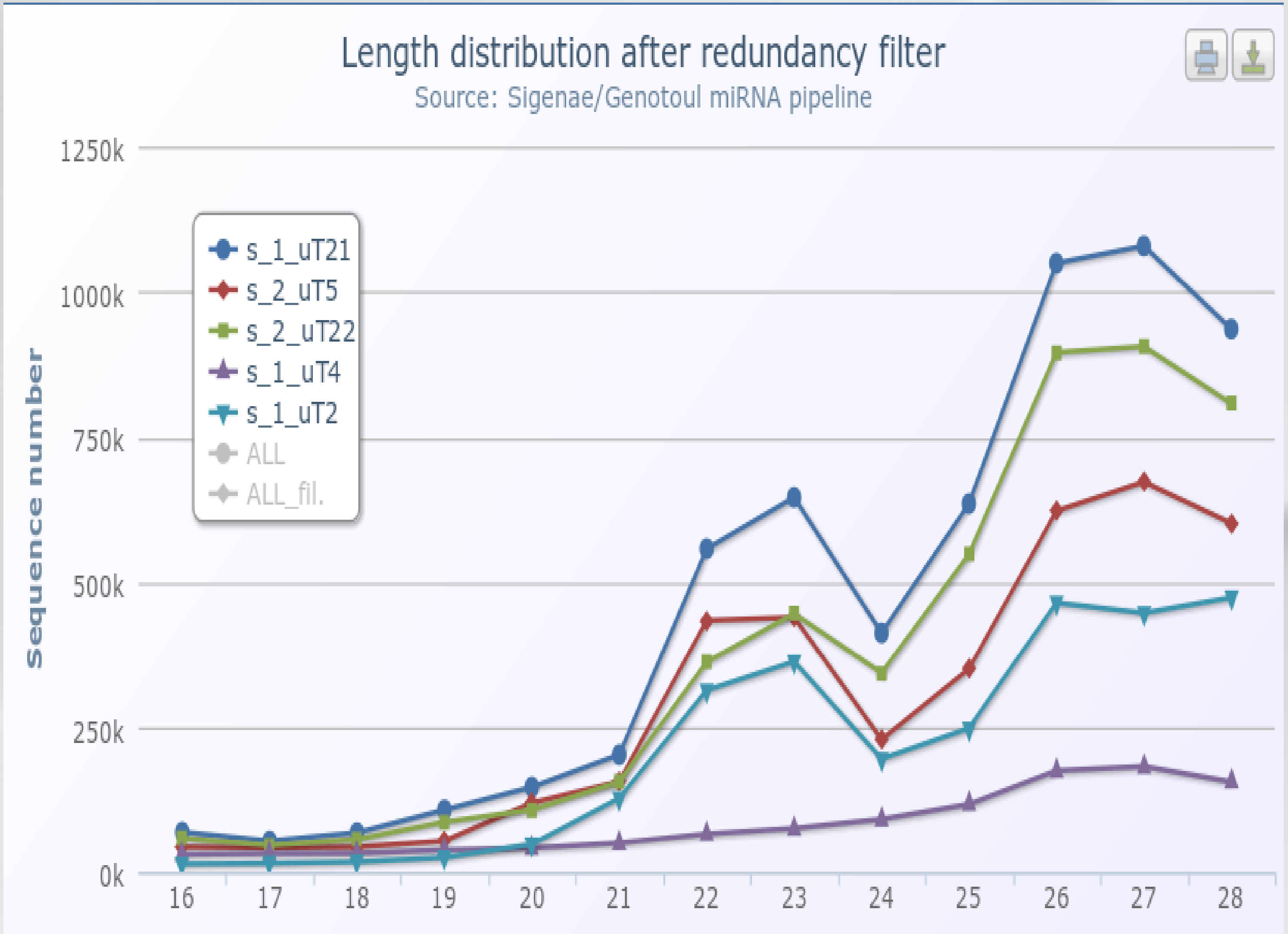
- **Removing identical reads**
  - **save computational time**
  - **useless to keep all the read**
  - **Keep the number of occurrence for each reads**

```
…
AAATGAATGATCTATGGACAGCA            2
AAATGAATGATCTATGGACAGCAG           38
AAATGAATGATCTATGGACAGCAGA          2
AAATGAATGATCTATGGACAGCAGAAAG       1
AAATGAATGATCTATGGACAGCAGC          51
AAATGAATGATCTATGGACAGCAGCA         82
AAATGAATGATCTATGGACAGCAGCAA        5
AAATGAATGATCTATGGACAGCAGCAAA       2
AAATGAATGATCTATGGACAGCAGCAAC       3
AAATGAATGATCTATGGACAGCAGCAAG       57
AAATGAATGATCTATGGACAGCAGCAG        2
AAATGAATGATCTATGGACAGCCGC          1
AAATGAATGATCTATGGACGGCAGCA         1
...
```

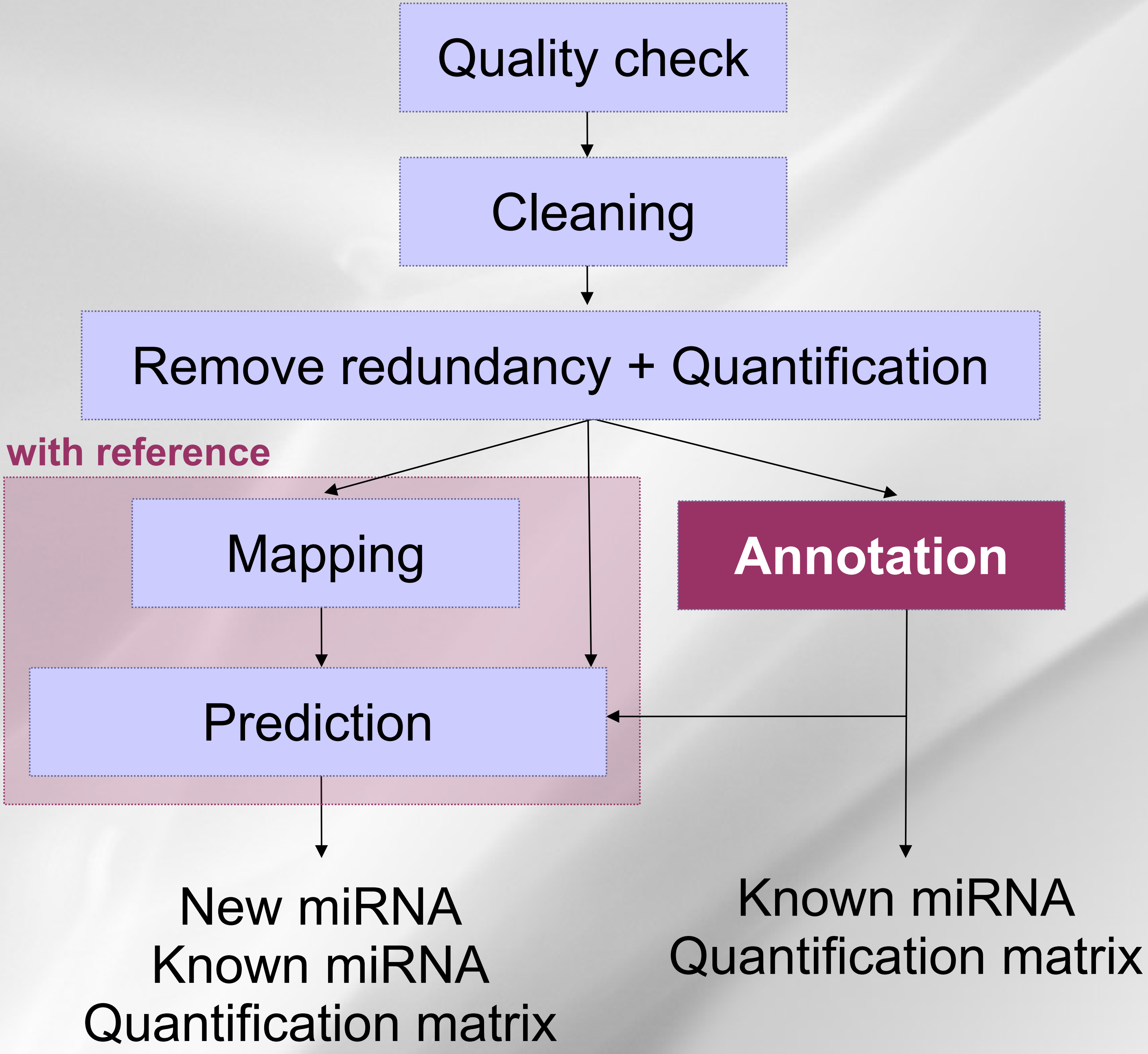
```
fastqnr.pl sample.fq | sort -k1,1 >  sample.matrix
```

Plateforme Bioinformatique Midi-Pyrénées

3. Remove redundancy



small RNAseq pipeline



Quality check

Cleaning

Remove redundancy + Quantification

with reference

Mapping

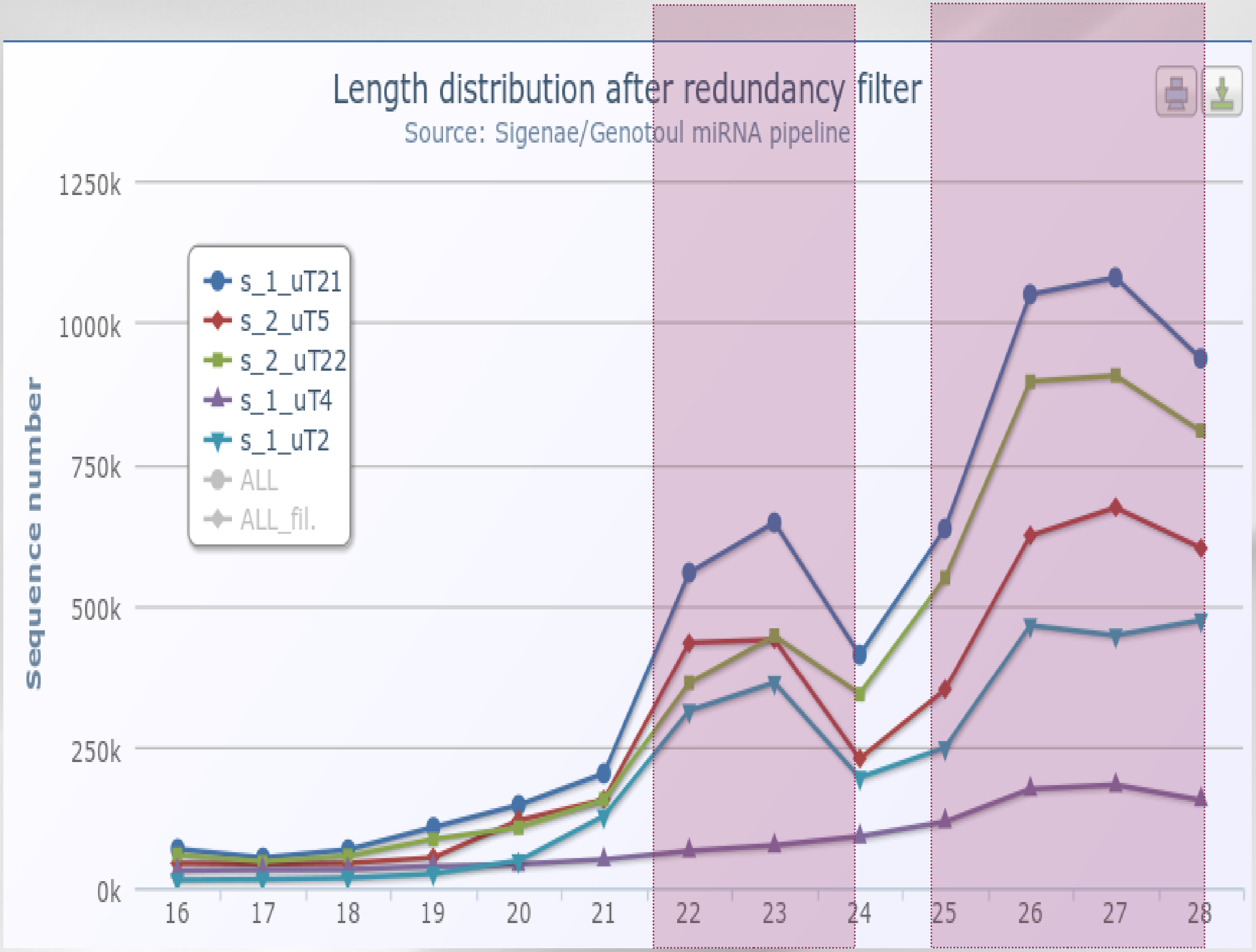
Annotation

Prediction

New miRNA
Known miRNA
Quantification matrix

Known miRNA
Quantification matrix

3. Remove redundancy



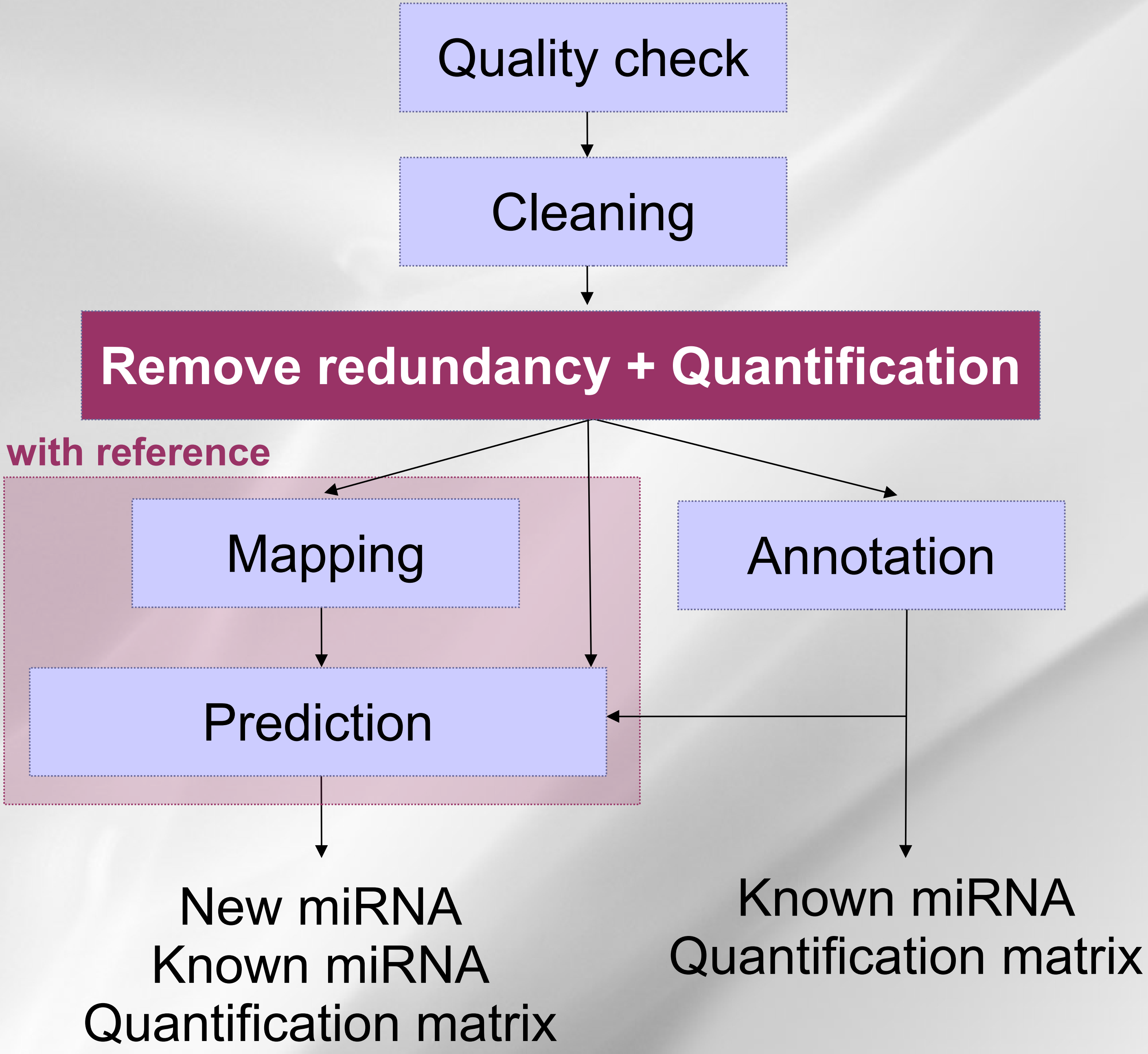
- **More differencies between piRNAs than with miRNAs ?**

Exercices

– Exercice 1: Nettoyage de séquences

– Pour les séquences contenues dans le fichier /work/gaspin/L3/TP0.fastq, utiliser cutadapt pour enlever les adaptateurs (adaptateur=ATCTCGTATGCCGTCTTCTGCTTG) et conserver uniquement les séquences comprises entre 20 et 26 nt. Combien de séquences avez vous éliminé ? Ecrire un programme permettant de compter le nombre de séquences de 20, 21 ...26 nt. Que déduisez-vous des résultats ?

small RNAseq pipeline



3. Quantification

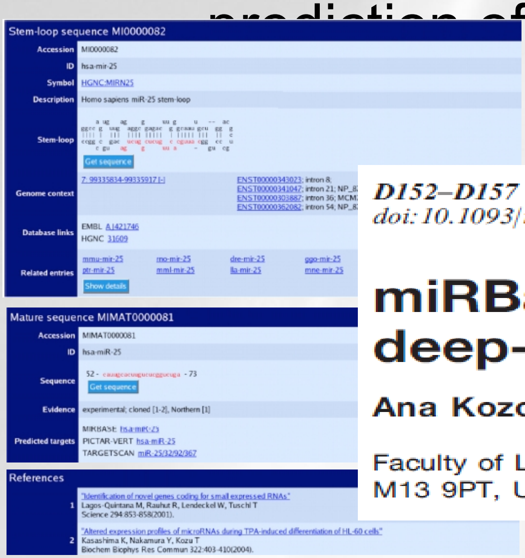
- Computes an expression matrix
  - Read must be at least in 2 samples if present less than 5 times

```
#seq                          s_1_uT21  s_1_uT2  s_1_uT4  s_2_uT22  s_2_uT5
...
AAAAGGGCTGTTTGTGCAGGCAG       87        14       0        85        5
AAAAGGGCTGTTTGTGCAGGCAGA      1         0        0        1         0
AAAAGGGCTGTTTGTGCAGGCAGG      1         0        0        2         0
AAAAGGGCTGTTTGTGCAGGCAGT      1         0        0        3         0
AAAAGGGCTGTTTGTGCAGGCAGTTT    0         0        0        0         1
AAAAGGGCTGTTTGTGCAGGCAT       1         2        0        3         0
AAAAGGGCTGTTTGTGCAGGCTA       0         0        0        1         0
AAAAGGGCTGTTTGTGCAGGCTG       1         0        0        1         0
AAAAGGGCTGTTTGTGCAGGCTT       1         0        0        0         0
AAAAGGGCTGTTTGTGCAGGG         6         1        0        4         2
AAAAGGGCTGTTTGTGCAGGGA        11        1        0        3         4
AAAAGGGCTGTTTGTGCAGGGAG       88        9        0        62        11
AAAAGGGCTGTTTGTGCAGGGAGC      1         0        0        0         0
AAAAGGGCTGTTTGTGCAGGGAGCTGA   0         0        0        1         0
AAAAGGGCTGTTTGTGCAGGGAGT      0         1        0        0         0
AAAAGGGCTGTTTGTGCAGGGAGTT     0         0        0        1         0
AAAAGGGCTGTTTGTGCAGGGAT       2         0        0        0         1
AAAAGGGCTGTTTGTGCAGGGATT      1         0        0        0         0
...
```

```
quatification.pl  -i 2 -a 5 sample1.matrix sample2.matrix ... > quantification.matrix
```

4. Annotation

- Useful databases:
  - miRbase (http://microrna.sanger.ac.uk/) miRBase
    - miRBase::Registry provides names to novel miRNA genes prior to their publication.
    - **miRBase::Sequences provides miRNA sequence data, annotation, references and links to other resources for all published miRNAs.**
    - miRBase::Targets provides an automated pipeline for the prediction of targets for all published animal miRNAs.



Stem-loop sequence MI0000082

Accession	MI0000082
ID	hsa-mir-25
Symbol	HGNC:MIRN25
Description	Homo sapiens miR-25 stem-loop
Stem-loop	(structure below) Get sequence
Genome context	7: 99335834-99335917 [-] ENST00000343023; intron 8; ENST00000341047; intron 21; NP_8 ENST00000303887; intron 36; MCM ENST00000362082; intron 54; NP_8
Database links	EMBL AJ421746 HGNC 31609
Related entries	mmu-mir-25 rno-mir-25 dre-mir-25 ggo-mir-25 ptr-mir-25 mml-mir-25 lla-mir-25 mne-mir-25 Show details

```
    a ug   ag    g     uu g    u   --  ac
ggcc g  uug   aggc gagac  g gcauu gcu  gg  g
|||| |  |||   |||| |||||  | ||||| |||  ||  c
ccgg c  gac   ucug cucug  c cguua cgg  cc  u
    c gu   ag    g     uu a    -   gu  cg
```

Mature sequence MIMAT0000081

Accession	MIMAT0000081
ID	hsa-miR-25
Sequence	52 - cauugcacuugucucggucuga - 73 Get sequence
Evidence	experimental; cloned [1-2], Northern [1]
Predicted targets	MIRBASE hsa-miR-25 PICTAR-VERT hsa-miR-25 TARGETSCAN miR-25/32/92/367

References

1. "Identification of novel genes coding for small expressed RNAs"
Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T
Science 294:853-858(2001).
2. "Altered expression profiles of microRNAs during TPA-induced differentiation of HL-60 cells"
Kasashima K, Nakamura Y, Kozu T
Biochem Biophys Res Commun 322:403-410(2004).

D152–D157 Nucleic Acids Research, 2011, Vol. 39, Database issue *Published online 30 October 2010*
doi:10.1093/nar/gkq1027

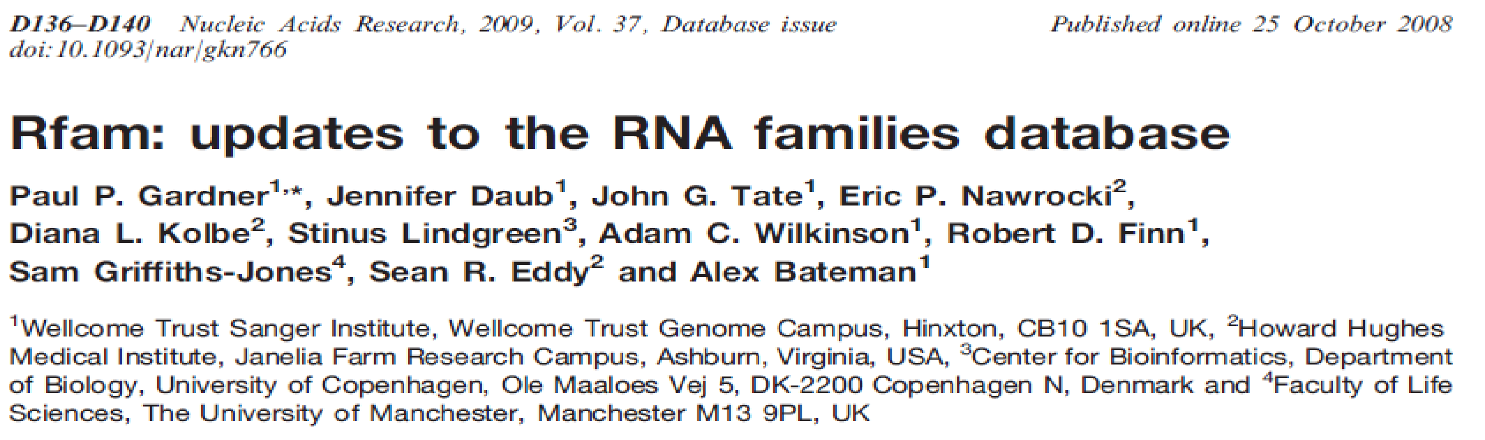
miRBase: integrating microRNA annotation and deep-sequencing data

Ana Kozomara and Sam Griffiths-Jones*

Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK

4. Annotation

- Useful databases:
  - miRbase (http://microrna.sanger.ac.uk/)
  - Rfam (http://rfam.sanger.ac.uk/)
    - A collection of RNA families
      - Rfam 10.1, June 2011, 1973 families
    - A track now included in the UCSC genome browser
    - Be careful: also contains (not all) miRNA families



D136–D140 Nucleic Acids Research, 2009, Vol. 37, Database issue *Published online 25 October 2008*
doi:10.1093/nar/gkn766

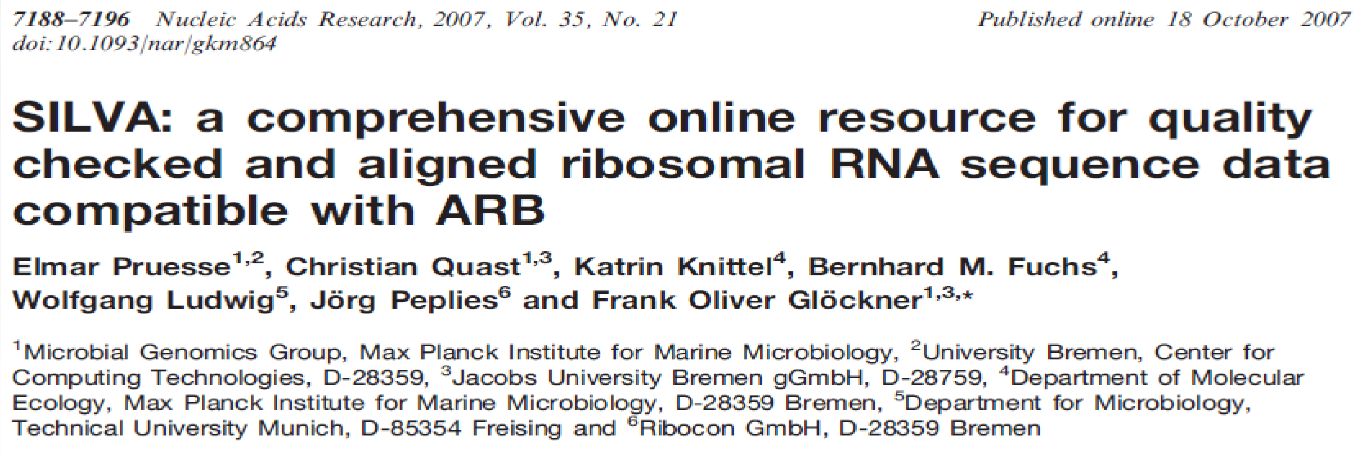
Rfam: updates to the RNA families database

Paul P. Gardner[1,*], Jennifer Daub[1], John G. Tate[1], Eric P. Nawrocki[2], Diana L. Kolbe[2], Stinus Lindgreen[3], Adam C. Wilkinson[1], Robert D. Finn[1], Sam Griffiths-Jones[4], Sean R. Eddy[2] and Alex Bateman[1]

[1]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, UK, [2]Howard Hughes Medical Institute, Janelia Farm Research Campus, Ashburn, Virginia, USA, [3]Center for Bioinformatics, Department of Biology, University of Copenhagen, Ole Maaloes Vej 5, DK-2200 Copenhagen N, Denmark and [4]Faculty of Life Sciences, The University of Manchester, Manchester M13 9PL, UK

4. Annotation

- Useful databases:
  - miRbase (http://microrna.sanger.ac.uk/) miRBase
  - Rfam (http://rfam.sanger.ac.uk/)
  - Silva (http://www.arb-silva.de/) silva comprehensive ribosomal RNA databases
    - A comprehensive on-line resource for quality checked and aligned ribosomal RNA sequence data.
      - SSU (16S rRNA, 18S rRNA)
      - LSU (23S rRNA, 28S rRNA)
      - Eukarya, bacteria, archaea



7188–7196 Nucleic Acids Research, 2007, Vol. 35, No. 21 *Published online 18 October 2007*
doi:10.1093/nar/gkm864

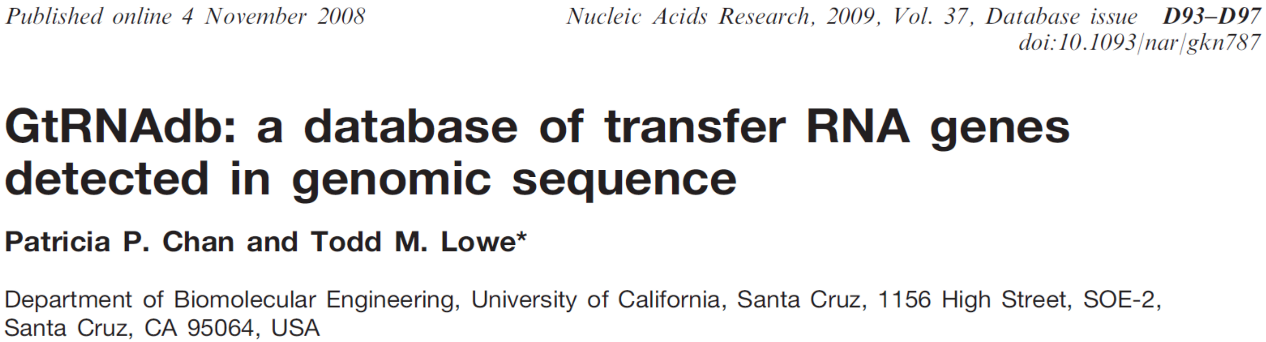
SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB

Elmar Pruesse[1,2], Christian Quast[1,3], Katrin Knittel[4], Bernhard M. Fuchs[4], Wolfgang Ludwig[5], Jörg Peplies[6] and Frank Oliver Glöckner[1,3,*]

[1]Microbial Genomics Group, Max Planck Institute for Marine Microbiology, [2]University Bremen, Center for Computing Technologies, D-28359, [3]Jacobs University Bremen gGmbH, D-28759, [4]Department of Molecular Ecology, Max Planck Institute for Marine Microbiology, D-28359 Bremen, [5]Department for Microbiology, Technical University Munich, D-85354 Freising and [6]Ribocon GmbH, D-28359 Bremen

4. Annotation

- Useful databases:
  - miRbase (http://microrna.sanger.ac.uk/)
  - Rfam (http://rfam.sanger.ac.uk/)
  - Silva (http://www.arb-silva.de/)
  - GtRNAdb(http://gtrnadb.ucsc.edu/)
    - Contains tRNA gene predictions made by the program tRNAscan-SE (Lowe & Eddy, Nucl Acids Res 25: 955-964, 1997) on complete or nearly complete genomes.
    - All annotation is automated and has not been inspected for agreement with published literature.



Published online 4 November 2008 *Nucleic Acids Research, 2009, Vol. 37, Database issue* **D93–D97**
doi:10.1093/nar/gkn787

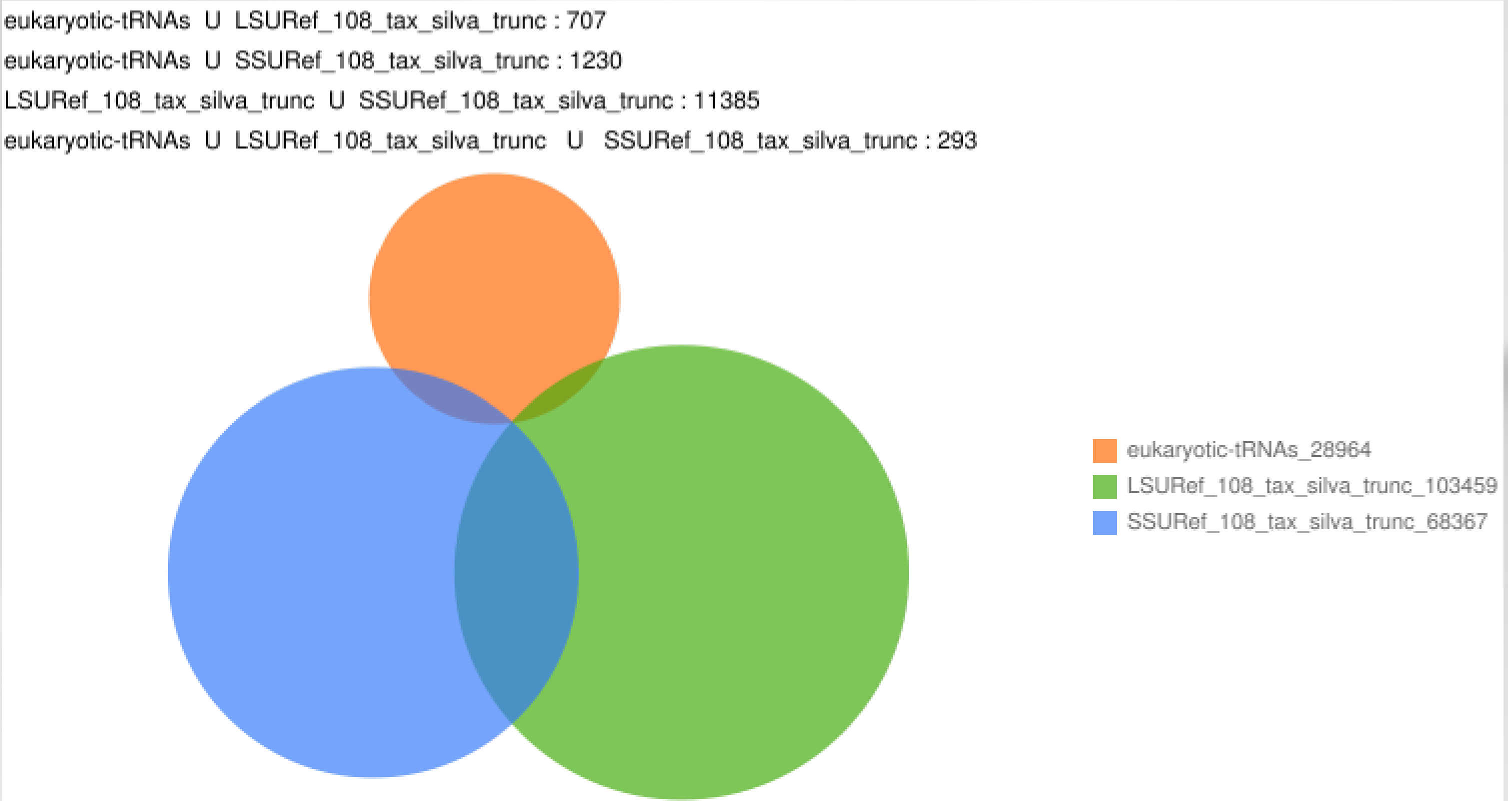
GtRNAdb: a database of transfer RNA genes detected in genomic sequence

Patricia P. Chan and Todd M. Lowe*

Department of Biomolecular Engineering, University of California, Santa Cruz, 1156 High Street, SOE-2, Santa Cruz, CA 95064, USA

4. Annotation

- **Reads with multiple annotation**



eukaryotic-tRNAs U LSURef_108_tax_silva_trunc : 707

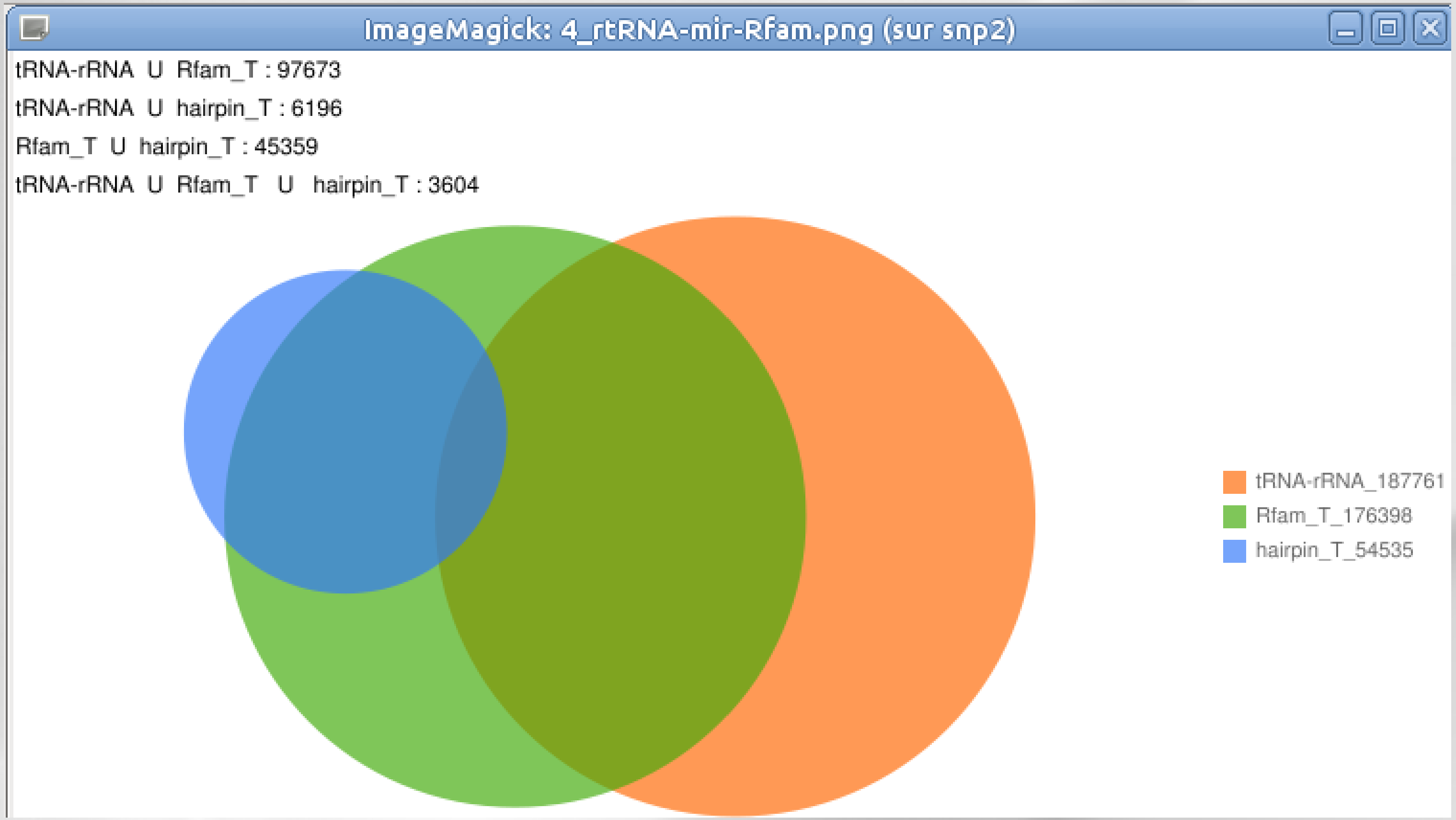
eukaryotic-tRNAs U SSURef_108_tax_silva_trunc : 1230

LSURef_108_tax_silva_trunc U SSURef_108_tax_silva_trunc : 11385

eukaryotic-tRNAs U LSURef_108_tax_silva_trunc U SSURef_108_tax_silva_trunc : 293

4. Annotation

- **Reads with multiple annotation**

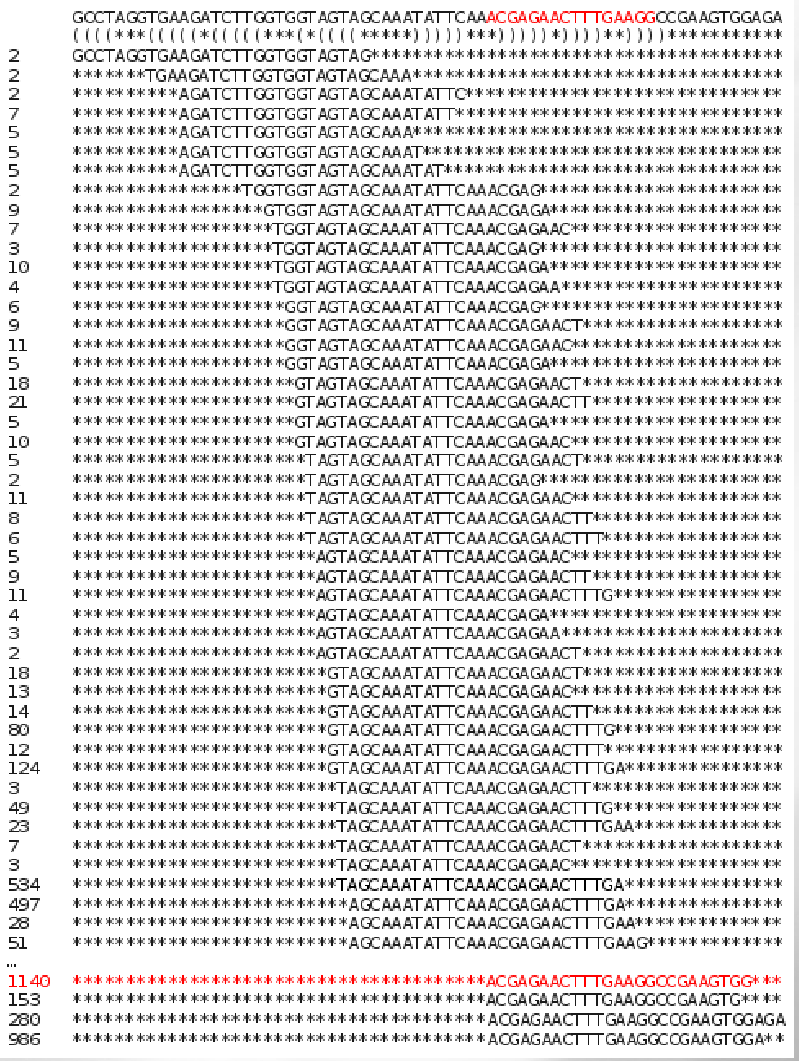


tRNA-rRNA U Rfam_T : 97673
tRNA-rRNA U hairpin_T : 6196
Rfam_T U hairpin_T : 45359
tRNA-rRNA U Rfam_T U hairpin_T : 3604

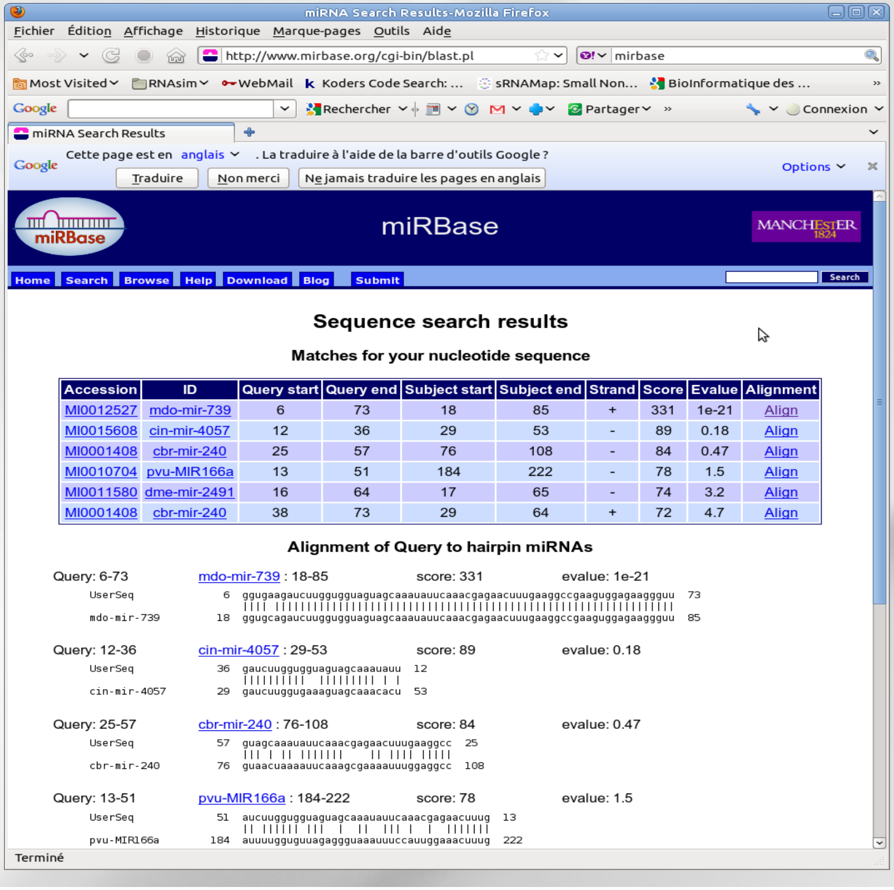
→ A lot of reads annotated with mirBase but also with tRNA and rRNA database

• rRNA present in miRBase

Mir-739 or 28S rRNA ?



```
      GCCTAGGTGAAGATCTTGGTGGTAGTAGCAAATATTCAAACGAGAACTTTGAAGGCCGAAGTGGAGA
      ((((***(((((*(((((***(*(((((*****)))))***)))))*))))**))))***********
2     GCCTAGGTGAAGATCTTGGTGGTAGTAG***************************************
2     *******TGAAGATCTTGGTGGTAGTAGCAAA***********************************
2     **********AGATCTTGGTGGTAGTAGCAAATATTC******************************
7     **********AGATCTTGGTGGTAGTAGCAAATATT*******************************
5     **********AGATCTTGGTGGTAGTAGCAAA***********************************
5     **********AGATCTTGGTGGTAGTAGCAAAT**********************************
5     **********AGATCTTGGTGGTAGTAGCAAATAT********************************
2     ****************TGGTGGTAGTAGCAAATATTCAAACGAG***********************
9     ******************GTGGTAGTAGCAAATATTCAAACGAGA**********************
7     *******************TGGTAGTAGCAAATATTCAAACGAGAAC********************
3     *******************TGGTAGTAGCAAATATTCAAACGAG***********************
10    *******************TGGTAGTAGCAAATATTCAAACGAGA**********************
4     *******************TGGTAGTAGCAAATATTCAAACGAGAA*********************
6     ********************GGTAGTAGCAAATATTCAAACGAG***********************
9     ********************GGTAGTAGCAAATATTCAAACGAGAACT*******************
11    ********************GGTAGTAGCAAATATTCAAACGAGAAC********************
5     ********************GGTAGTAGCAAATATTCAAACGAGA**********************
18    *********************GTAGTAGCAAATATTCAAACGAGAACT*******************
21    *********************GTAGTAGCAAATATTCAAACGAGAACTT******************
5     *********************GTAGTAGCAAATATTCAAACGAGA**********************
10    *********************GTAGTAGCAAATATTCAAACGAGAAC********************
5     **********************TAGTAGCAAATATTCAAACGAGAACT*******************
2     **********************TAGTAGCAAATATTCAAACGAG***********************
11    **********************TAGTAGCAAATATTCAAACGAGAAC********************
8     **********************TAGTAGCAAATATTCAAACGAGAACTT******************
6     **********************TAGTAGCAAATATTCAAACGAGAACTTT*****************
5     ***********************AGTAGCAAATATTCAAACGAGAAC********************
9     ***********************AGTAGCAAATATTCAAACGAGAACTT******************
11    ***********************AGTAGCAAATATTCAAACGAGAACTTTG****************
4     ***********************AGTAGCAAATATTCAAACGAGA**********************
3     ***********************AGTAGCAAATATTCAAACGAGAA*********************
2     ***********************AGTAGCAAATATTCAAACGAGAACT*******************
18    ************************GTAGCAAATATTCAAACGAGAACT*******************
13    ************************GTAGCAAATATTCAAACGAGAAC********************
14    ************************GTAGCAAATATTCAAACGAGAACTT******************
80    ************************GTAGCAAATATTCAAACGAGAACTTTG****************
12    ************************GTAGCAAATATTCAAACGAGAACTTT*****************
124   ************************GTAGCAAATATTCAAACGAGAACTTTGA***************
3     *************************TAGCAAATATTCAAACGAGAACTT******************
49    *************************TAGCAAATATTCAAACGAGAACTTTG****************
23    *************************TAGCAAATATTCAAACGAGAACTTTGAA**************
7     *************************TAGCAAATATTCAAACGAGAACT*******************
3     *************************TAGCAAATATTCAAACGAGAAC********************
534   *************************TAGCAAATATTCAAACGAGAACTTTGA***************
497   **************************AGCAAATATTCAAACGAGAACTTTGA***************
28    **************************AGCAAATATTCAAACGAGAACTTTGAA**************
51    **************************AGCAAATATTCAAACGAGAACTTTGAAG*************
…
1140  ***************************************ACGAGAACTTTGAAGGCCGAAGTGG***
153   ***************************************ACGAGAACTTTGAAGGCCGAAGTG****
280   ***************************************ACGAGAACTTTGAAGGCCGAAGTGGAGA
986   ***************************************ACGAGAACTTTGAAGGCCGAAGTGGA**
```



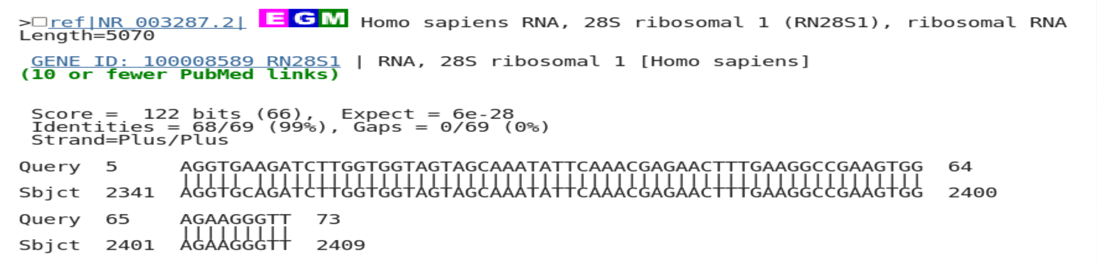
Sequence search results

Matches for your nucleotide sequence

Accession	ID	Query start	Query end	Subject start	Subject end	Strand	Score	Evalue	Alignment
MI0012527	mdo-mir-739	6	73	18	85	+	331	1e-21	Align
MI0015608	cin-mir-4057	12	36	29	53	-	89	0.18	Align
MI0001408	cbr-mir-240	25	57	76	108	-	84	0.47	Align
MI0010704	pvu-MIR166a	13	51	184	222	-	78	1.5	Align
MI0011580	dme-mir-2491	16	64	17	65	-	74	3.2	Align
MI0001408	cbr-mir-240	38	73	29	64	+	72	4.7	Align

Alignment of Query to hairpin miRNAs

```
Query: 6-73          mdo-mir-739 : 18-85          score: 331          evalue: 1e-21
      UserSeq          6   ggugaagaucuuggugguaguagcaaauauucaaacgagaacuuugaaggccgaaguggagaaggguu  73
                           |||| |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      mdo-mir-739     18   ggugcagaucuuggugguaguagcaaauauucaaacgagaacuuugaaggccgaaguggagaaggguu  85

Query: 12-36         cin-mir-4057 : 29-53         score: 89           evalue: 0.18
      UserSeq         36   gaucuugguggguaguagcaaauauu  12
                           ||||||||||  ||||||||| | |
      cin-mir-4057    29   gaucuuggugaaaguagcaaacacu  53

Query: 25-57         cbr-mir-240 : 76-108         score: 84           evalue: 0.47
      UserSeq         57   guagcaaauauucaaacgagaacuuugaaggcc  25
                           ||| | || |||||||    || |||| |||||
      cbr-mir-240     76   guaacuaaaauucaaagcgaaaauuuggaggcc  108

Query: 13-51         pvu-MIR166a : 184-222        score: 78           evalue: 1.5
      UserSeq         51   aucuugguggguaguagcaaauauucaaacgagaacuuug  13
                           || |||||| |||  |  ||  ||| |  |  |||||||
      pvu-MIR166a    184   auuuugguguuagaggguaaauuuccauuggaaacuuug  222
```

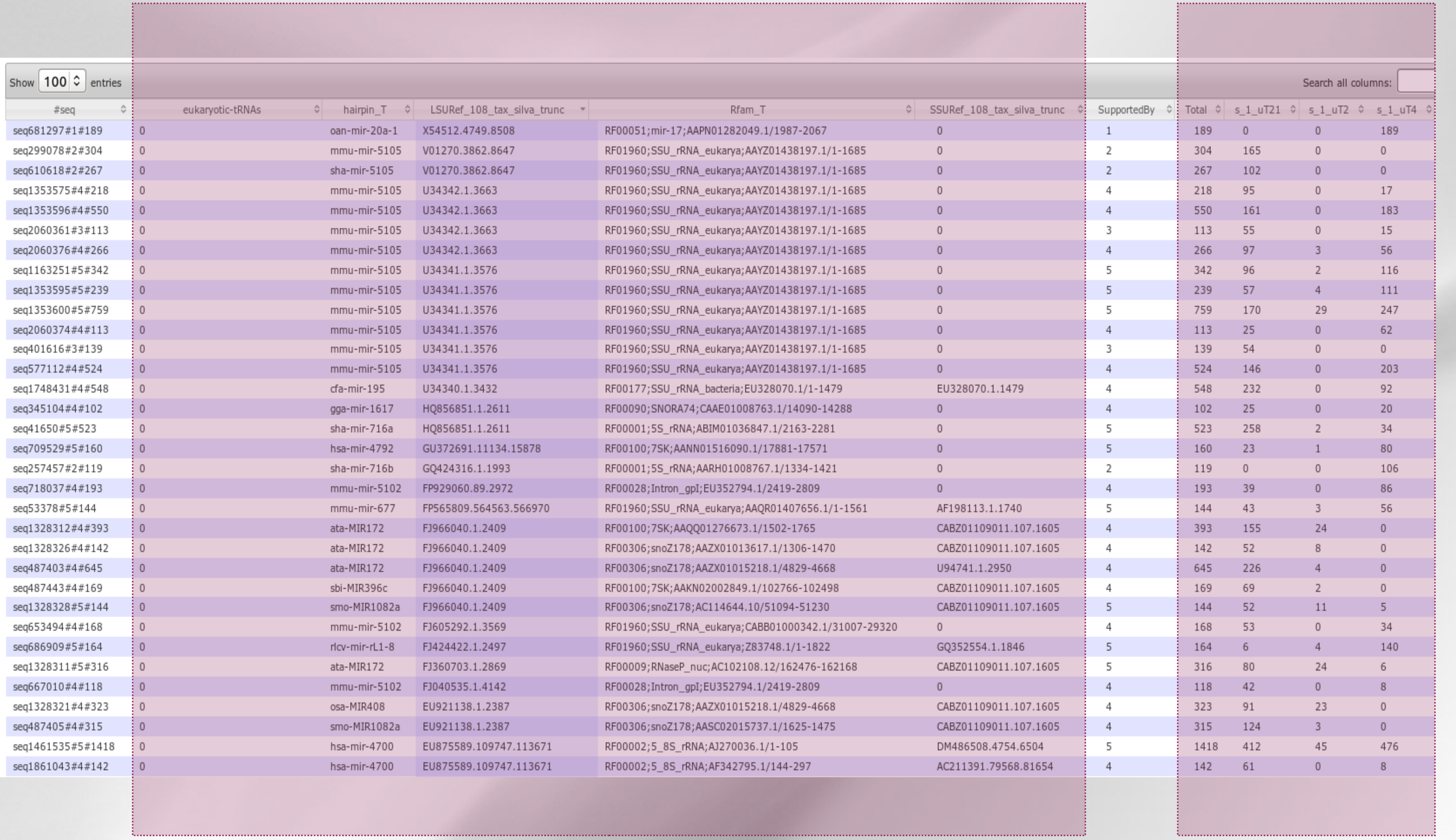


```
>□ref|NR_003287.2|  EGM  Homo sapiens RNA, 28S ribosomal 1 (RN28S1), ribosomal RNA
Length=5070

 GENE ID: 100008589 RN28S1 | RNA, 28S ribosomal 1 [Homo sapiens]
(10 or fewer PubMed links)

 Score =  122 bits (66),  Expect = 6e-28
 Identities = 68/69 (99%), Gaps = 0/69 (0%)
 Strand=Plus/Plus

Query  5     AGGTGAAGATCTTGGTGGTAGTAGCAAATATTCAAACGAGAACTTTGAAGGCCGAAGTGG  64
             ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2341  AGGTGCAGATCTTGGTGGTAGTAGCAAATATTCAAACGAGAACTTTGAAGGCCGAAGTGG  2400

Query  65    AGAAGGGTT  73
             |||||||||
Sbjct  2401  AGAAGGGTT  2409
```

4. Annotation

Annotation

occurences



Show 100 entries

Search all columns:

#seq	eukaryotic-tRNAs	hairpin_T	LSURef_108_tax_silva_trunc	Rfam_T	SSURef_108_tax_silva_trunc	SupportedBy	Total	s_1_uT21	s_1_uT2	s_1_uT4
seq681297#1#189	0	oan-mir-20a-1	X54512.4749.8508	RF00051;mir-17;AAPN01282049.1/1987-2067	0	1	189	0	0	189
seq299078#2#304	0	mmu-mir-5105	V01270.3862.8647	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	2	304	165	0	0
seq610618#2#267	0	sha-mir-5105	V01270.3862.8647	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	2	267	102	0	0
seq1353575#4#218	0	mmu-mir-5105	U34342.1.3663	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	4	218	95	0	17
seq1353596#4#550	0	mmu-mir-5105	U34342.1.3663	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	4	550	161	0	183
seq2060361#3#113	0	mmu-mir-5105	U34342.1.3663	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	3	113	55	0	15
seq2060376#4#266	0	mmu-mir-5105	U34342.1.3663	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	4	266	97	3	56
seq1163251#5#342	0	mmu-mir-5105	U34341.1.3576	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	5	342	96	2	116
seq1353595#5#239	0	mmu-mir-5105	U34341.1.3576	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	5	239	57	4	111
seq1353600#5#759	0	mmu-mir-5105	U34341.1.3576	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	5	759	170	29	247
seq2060374#4#113	0	mmu-mir-5105	U34341.1.3576	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	4	113	25	0	62
seq401616#3#139	0	mmu-mir-5105	U34341.1.3576	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	3	139	54	0	0
seq577112#4#524	0	mmu-mir-5105	U34341.1.3576	RF01960;SSU_rRNA_eukarya;AAYZ01438197.1/1-1685	0	4	524	146	0	203
seq1748431#4#548	0	cfa-mir-195	U34340.1.3432	RF00177;SSU_rRNA_bacteria;EU328070.1/1-1479	EU328070.1.1479	4	548	232	0	92
seq345104#4#102	0	gga-mir-1617	HQ856851.1.2611	RF00090;SNORA74;CAAE01008763.1/14090-14288	0	4	102	25	0	20
seq41650#5#523	0	sha-mir-716a	HQ856851.1.2611	RF00001;5S_rRNA;ABIM01036847.1/2163-2281	0	5	523	258	2	34
seq709529#5#160	0	hsa-mir-4792	GU372691.11134.15878	RF00100;7SK;AANN01516090.1/17881-17571	0	5	160	23	1	80
seq257457#2#119	0	sha-mir-716b	GQ424316.1.1993	RF00001;5S_rRNA;AARH01008767.1/1334-1421	0	2	119	0	0	106
seq718037#4#193	0	mmu-mir-5102	FP929060.89.2972	RF00028;Intron_gpI;EU352794.1/2419-2809	0	4	193	39	0	86
seq53378#5#144	0	mmu-mir-677	FP565809.564563.566970	RF01960;SSU_rRNA_eukarya;AAQR01407656.1/1-1561	AF198113.1.1740	5	144	43	3	56
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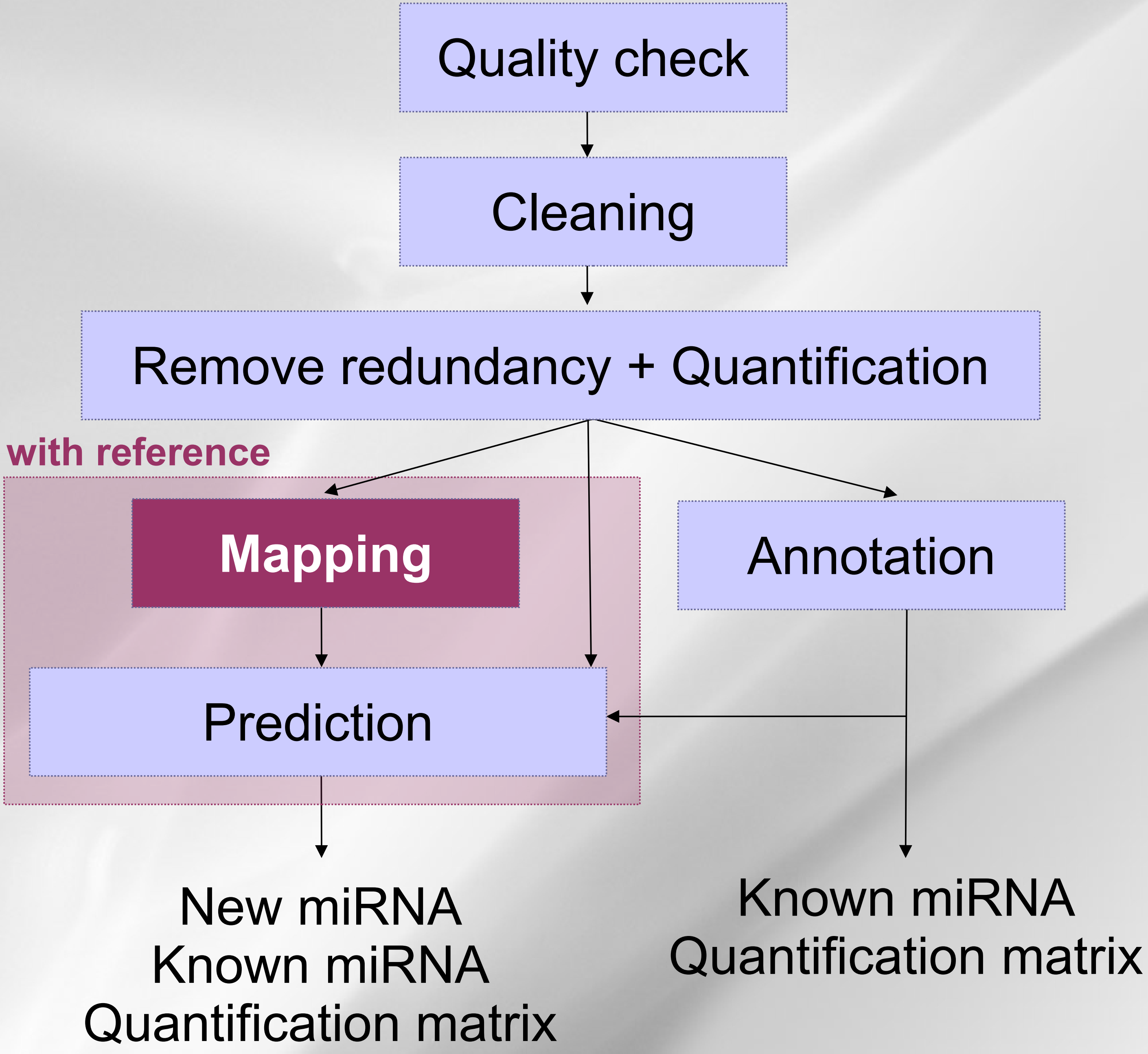
seq1328326#4#142	0	ata-MIR172	FJ966040.1.2409	RF00306;snoZ178;AAZX01013617.1/1306-1470	CABZ01109011.107.1605	4	142	52	8	0
seq487403#4#645	0	ata-MIR172	FJ966040.1.2409	RF00306;snoZ178;AAZX01015218.1/4829-4668	U94741.1.2950	4	645	226	4	0
seq487443#4#169	0	sbi-MIR396c	FJ966040.1.2409	RF00100;7SK;AAKN02002849.1/102766-102498	CABZ01109011.107.1605	4	169	69	2	0
seq1328328#5#144	0	smo-MIR1082a	FJ966040.1.2409	RF00306;snoZ178;AC114644.10/51094-51230	CABZ01109011.107.1605	5	144	52	11	5
seq653494#4#168	0	mmu-mir-5102	FJ605292.1.3569	RF01960;SSU_rRNA_eukarya;CABB01000342.1/31007-29320	0	4	168	53	0	34
seq686909#5#164	0	rlcv-mir-rL1-8	FJ424422.1.2497	RF01960;SSU_rRNA_eukarya;Z83748.1/1-1822	GQ352554.1.1846	5	164	6	4	140
seq1328311#5#316	0	ata-MIR172	FJ360703.1.2869	RF00009;RNaseP_nuc;AC102108.12/162476-162168	CABZ01109011.107.1605	5	316	80	24	6
seq667010#4#118	0	mmu-mir-5102	FJ040535.1.4142	RF00028;Intron_gpI;EU352794.1/2419-2809	0	4	118	42	0	8
seq1328321#4#323	0	osa-MIR408	EU921138.1.2387	RF00306;snoZ178;AAZX01015218.1/4829-4668	CABZ01109011.107.1605	4	323	91	23	0
seq487405#4#315	0	smo-MIR1082a	EU921138.1.2387	RF00306;snoZ178;AASC02015737.1/1625-1475	CABZ01109011.107.1605	4	315	124	3	0
seq1461535#5#1418	0	hsa-mir-4700	EU875589.109747.113671	RF00002;5_8S_rRNA;AJ270036.1/1-105	DM486508.4754.6504	5	1418	412	45	476
seq1861043#4#142	0	hsa-mir-4700	EU875589.109747.113671	RF00002;5_8S_rRNA;AF342795.1/144-297	AC211391.79568.81654	4	142	61	0	8

Plateforme Bioinformatique Midi-Pyrénées

small RNAseq pipeline



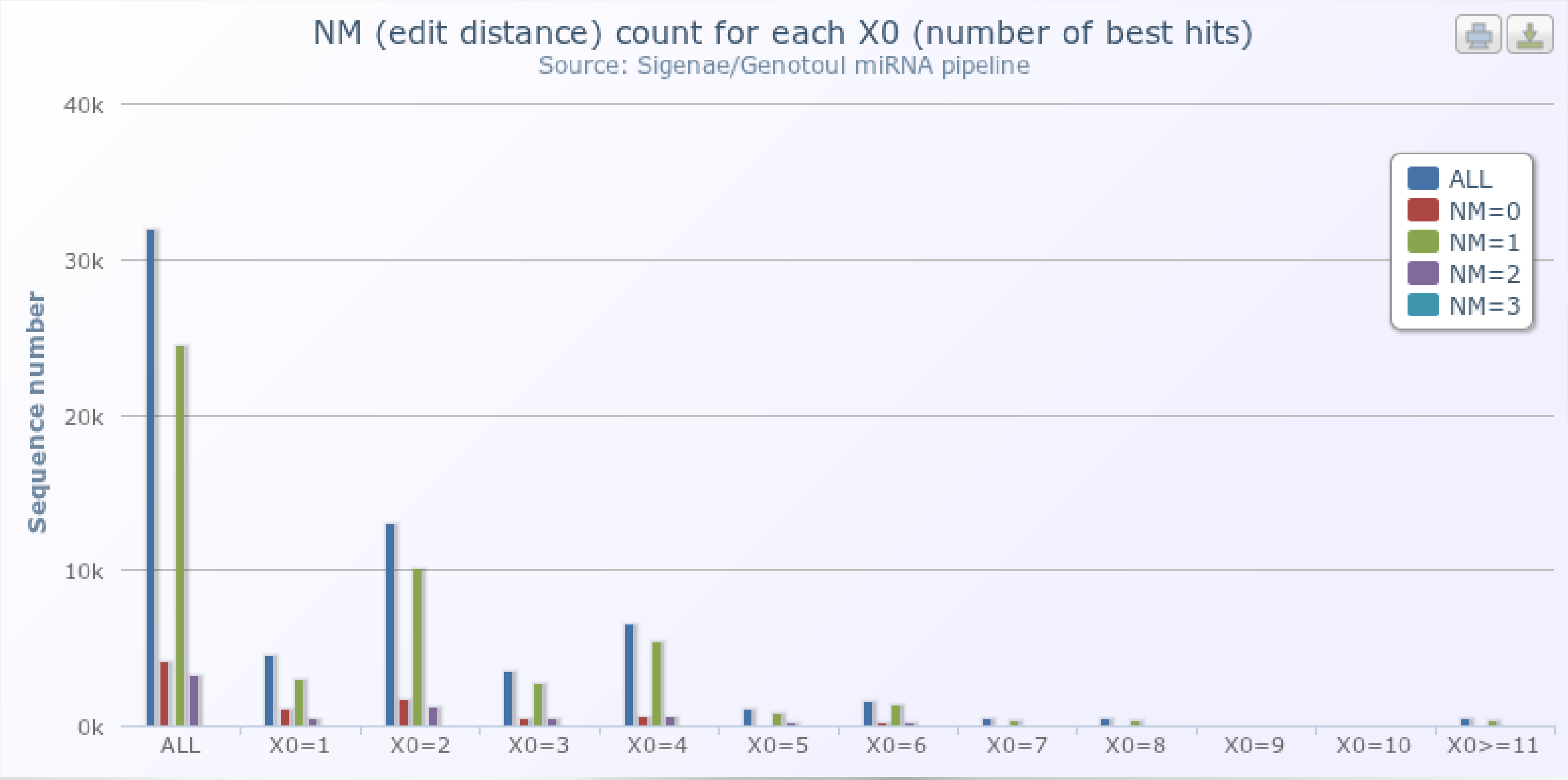
5. Mapping the reads

- Blat http://genome.ucsc.edu/cgi-bin/hgBlat
- Blast http://blast.ncbi.nlm.nih.gov/Blast.cgi
- Gmap http://www.gene.com/share/gmap/
- Bowtie http://bowtie-bio.sourceforge.net/index.shtml
- **BWA http://bio-bwa.sourceforge.net**
- ...

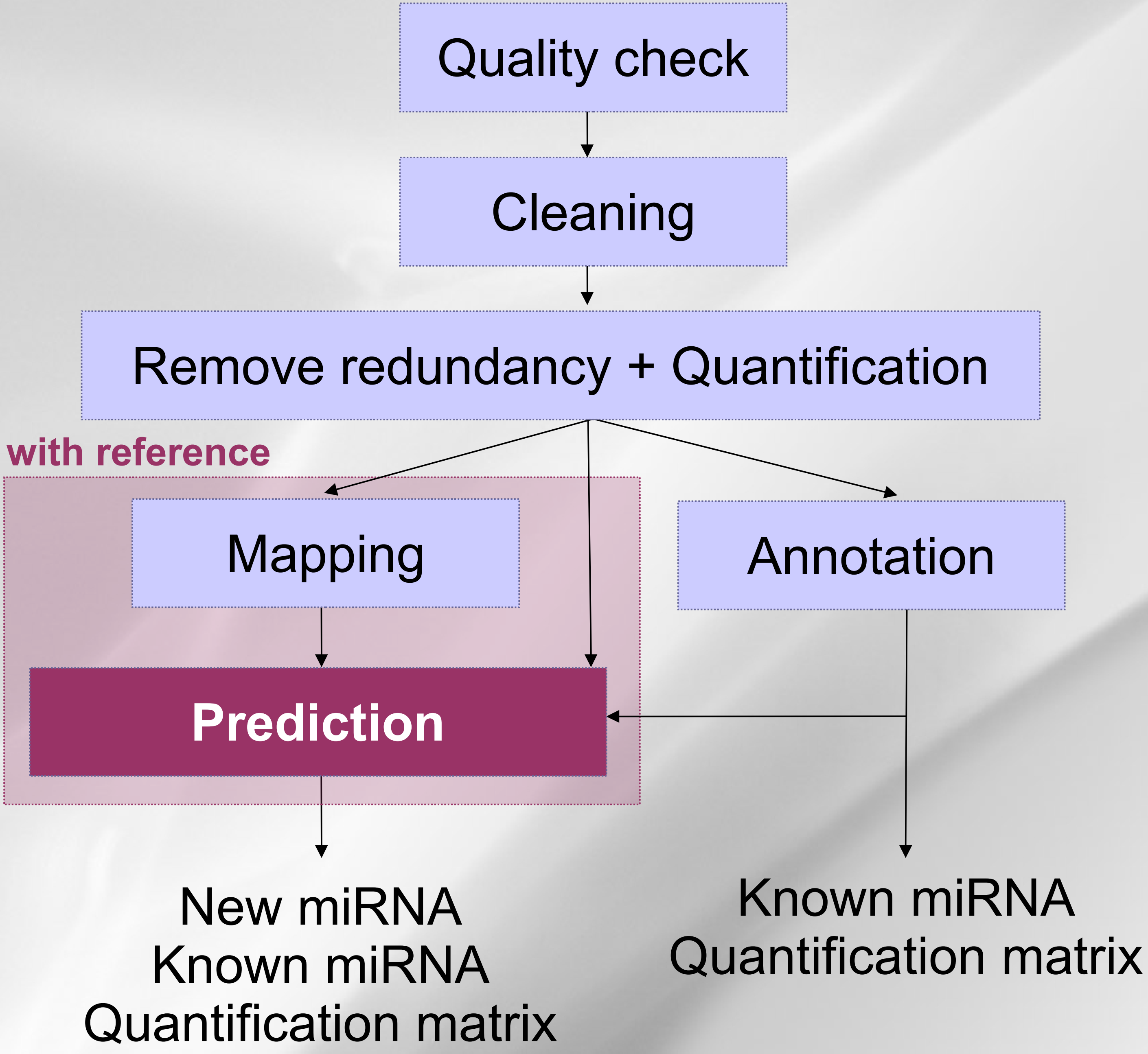
Plateforme Bioinformatique Midi-Pyrénées

5. Mapping the reads with bwa

- **Alignement of annotated reads**

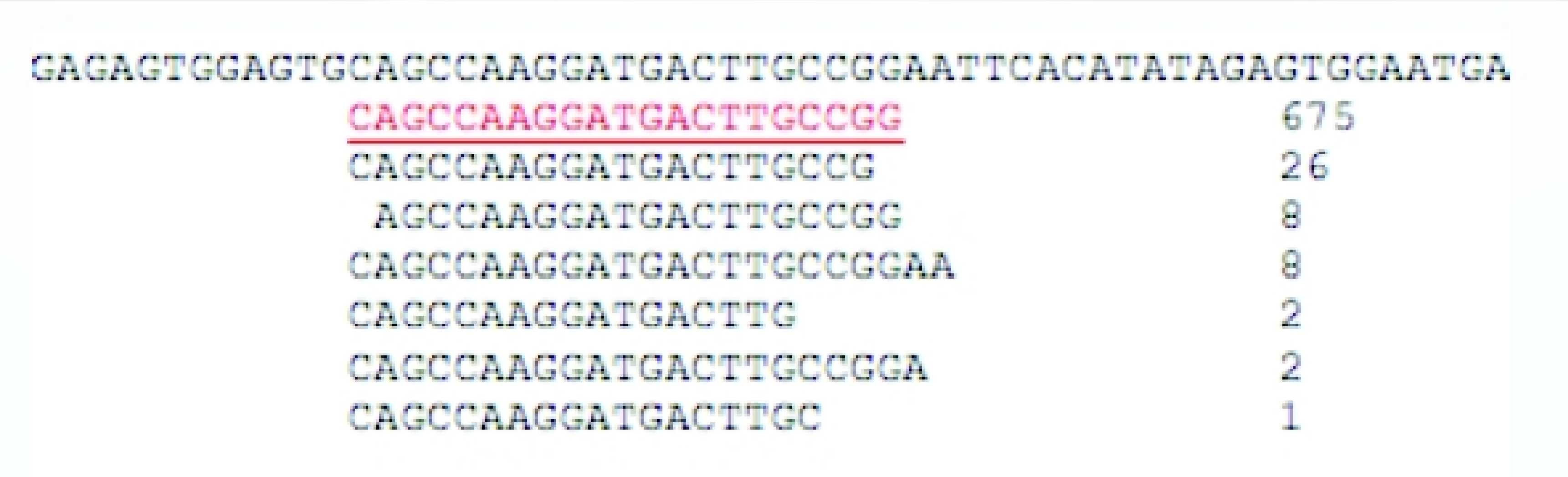


small RNAseq pipeline



6. Prediction

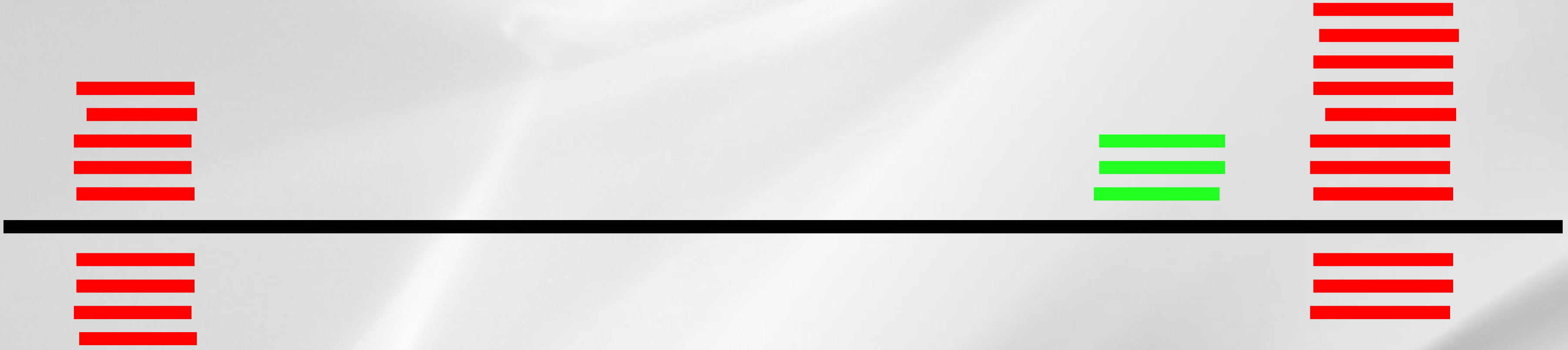
- Precise excision of a 21-22mer is typical of microRNA
  - less represented reads are products of Dicer errors and sequencing/sample preparation artifacts



```
GAGAGTGGAGTGCAGCCAAGGATGACTTGCCGGAATTCACATATAGAGTGGAATGA
            CAGCCAAGGATGACTTGCCGG               675
            CAGCCAAGGATGACTTGCCG                26
             AGCCAAGGATGACTTGCCGG               8
            CAGCCAAGGATGACTTGCCGGAA             8
            CAGCCAAGGATGACTTG                   2
            CAGCCAAGGATGACTTGCCGGA              2
            CAGCCAAGGATGACTTGC                  1
```

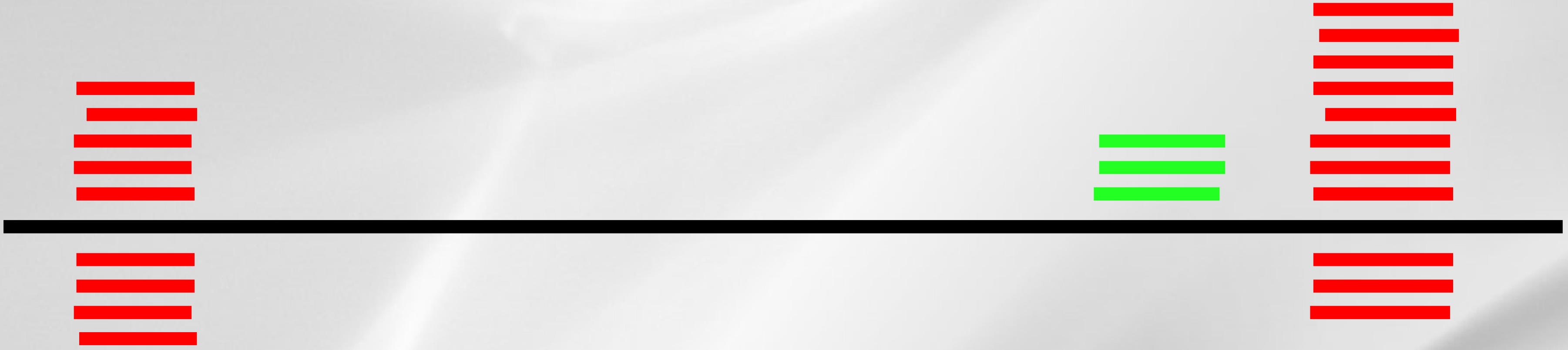
6. Prediction

- Once the reads mapped



6. Prediction

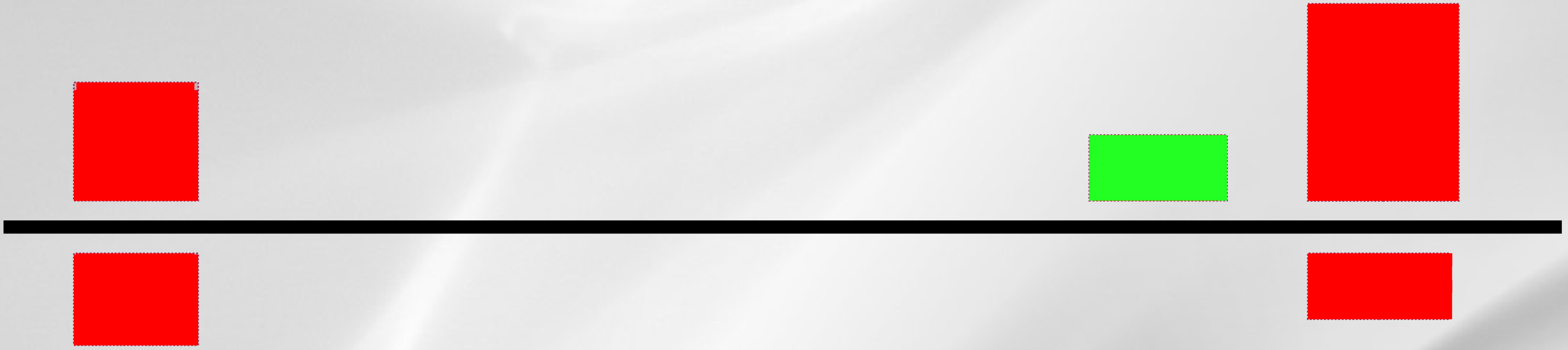
- Identify all contiguous read regions



Plateforme Bioinformatique Midi-Pyrénées

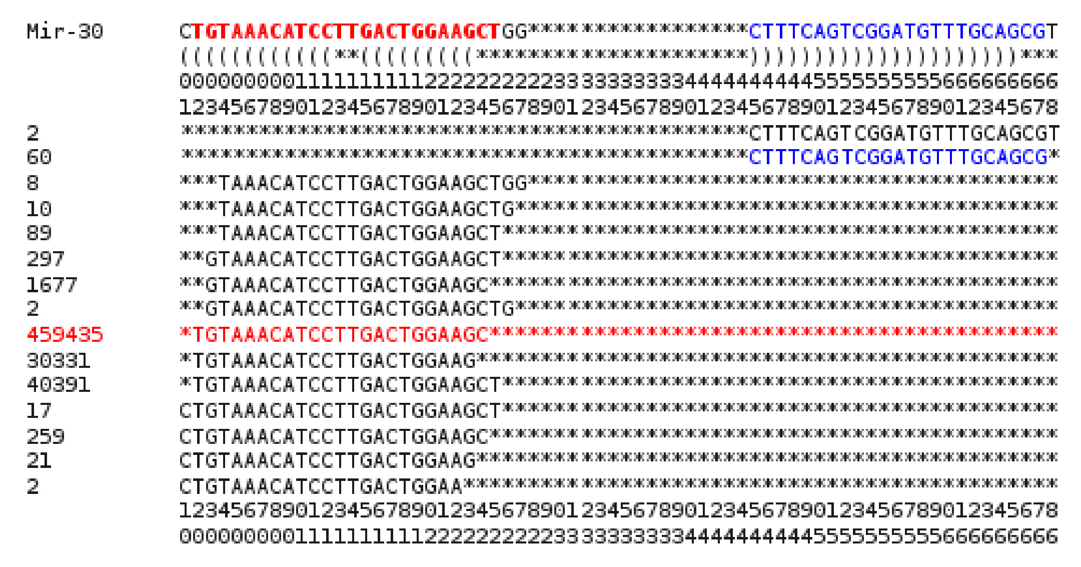
6. Prediction

- Identify all contiguous read regions

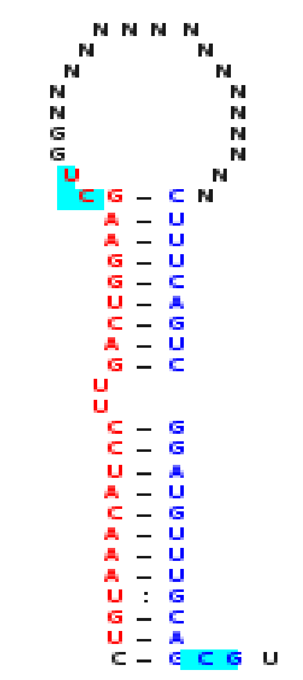


6. Prediction

- miRNA precursors have a characteristic secondary structure
  - The detection of a microRNA* sequence, opposing the most frequent read in a stable hairpin (but shifted by 2 bases), is sufficient to diagnose a microRNA.



```
Mir-30        CTGTAAACATCCTTGACTGGAAGCTGG*****************CTTTCAGTCGGATGTTTGCAGCGT
              ((((((((((((**(((((((((*********************)))))))))))))))))))))***
              00000000011111111112222222222333333333344444444445555555555666666666
              12345678901234567890123456789012345678901234567890123456789012345678
2             ********************************************CTTTCAGTCGGATGTTTGCAGCGT
60            ********************************************CTTTCAGTCGGATGTTTGCAGCG*
8             ***TAAACATCCTTGACTGGAAGCTGG*****************************************
10            ***TAAACATCCTTGACTGGAAGCTG******************************************
89            ***TAAACATCCTTGACTGGAAGCT*******************************************
297           **GTAAACATCCTTGACTGGAAGCT*******************************************
1677          **GTAAACATCCTTGACTGGAAGC********************************************
2             **GTAAACATCCTTGACTGGAAGCTG******************************************
459435        *TGTAAACATCCTTGACTGGAAGC********************************************
30331         *TGTAAACATCCTTGACTGGAAG*********************************************
40391         *TGTAAACATCCTTGACTGGAAGCT*******************************************
17            CTGTAAACATCCTTGACTGGAAGCT*******************************************
259           CTGTAAACATCCTTGACTGGAAGC********************************************
21            CTGTAAACATCCTTGACTGGAAG*********************************************
2             CTGTAAACATCCTTGACTGGAA**********************************************
              12345678901234567890123456789012345678901234567890123456789012345678
              00000000011111111112222222222333333333344444444445555555555666666666
```



6. Prediction

- Extend and fold read regions

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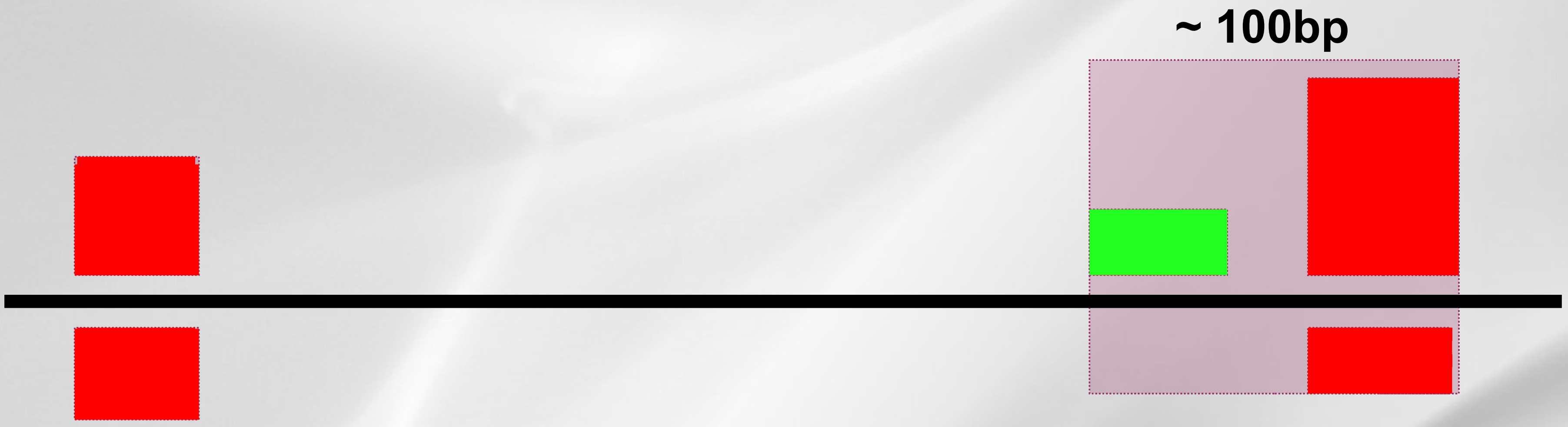
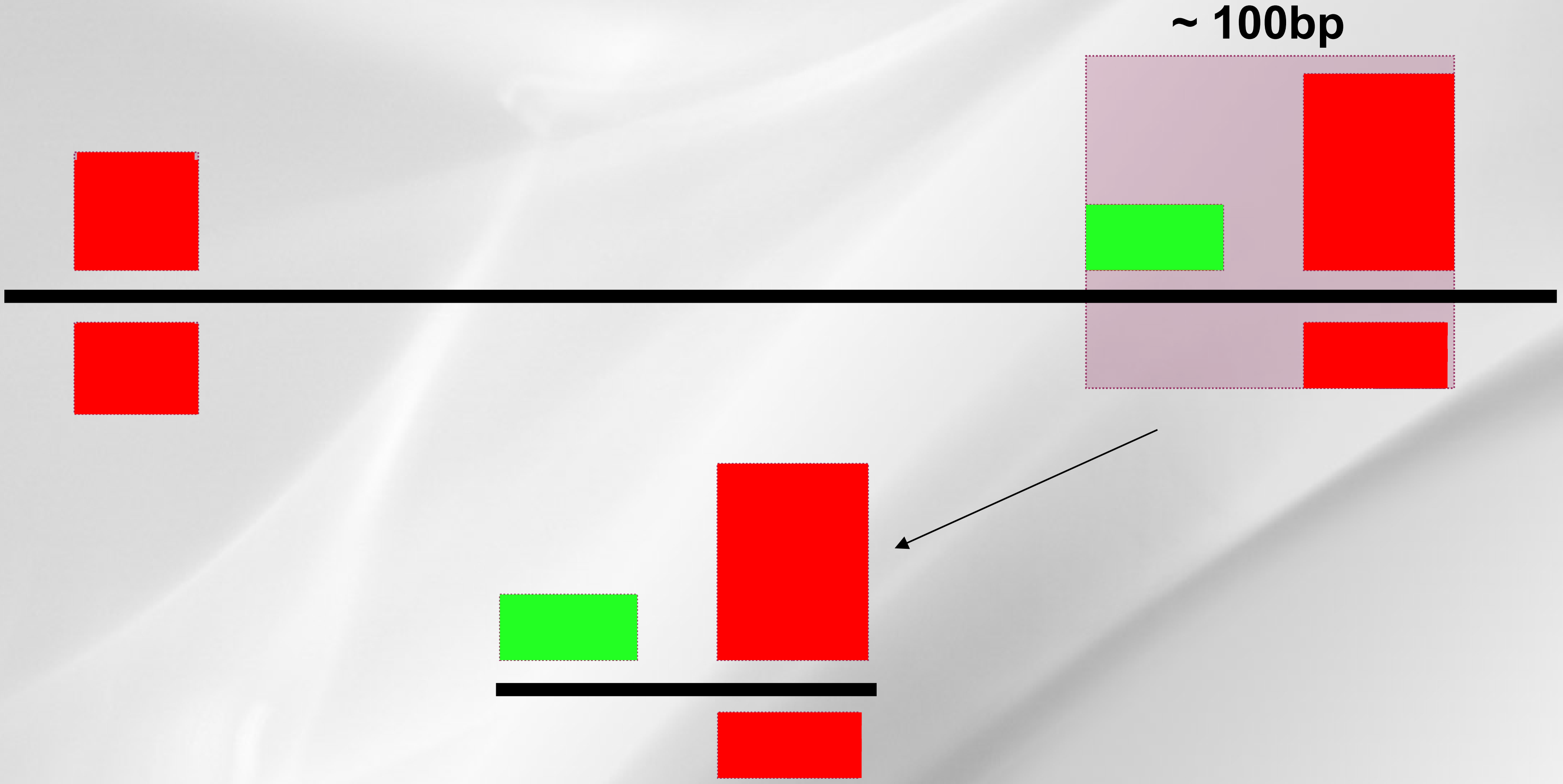


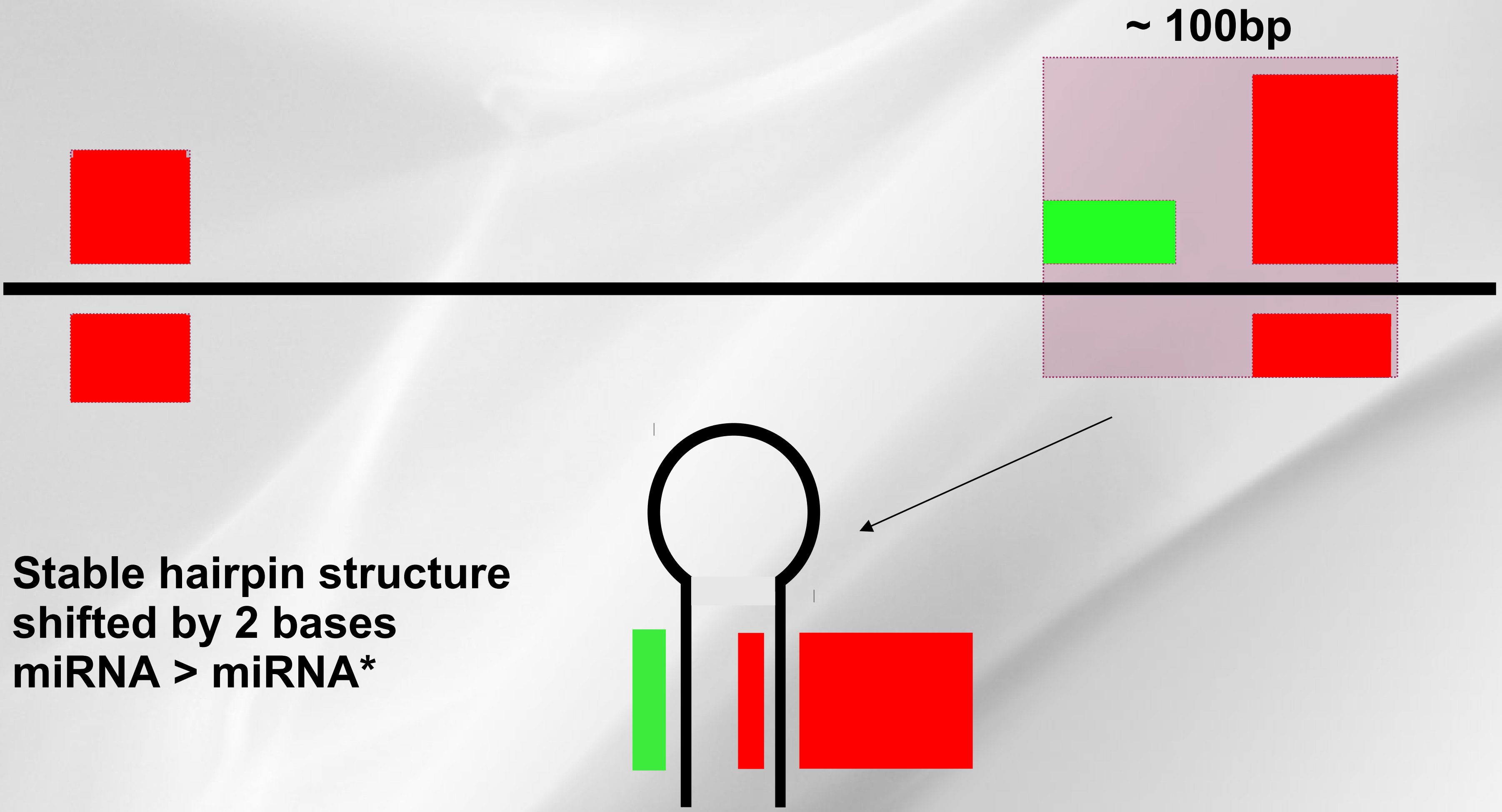
6. Prediction

- Extend and fold read regions



6. Prediction

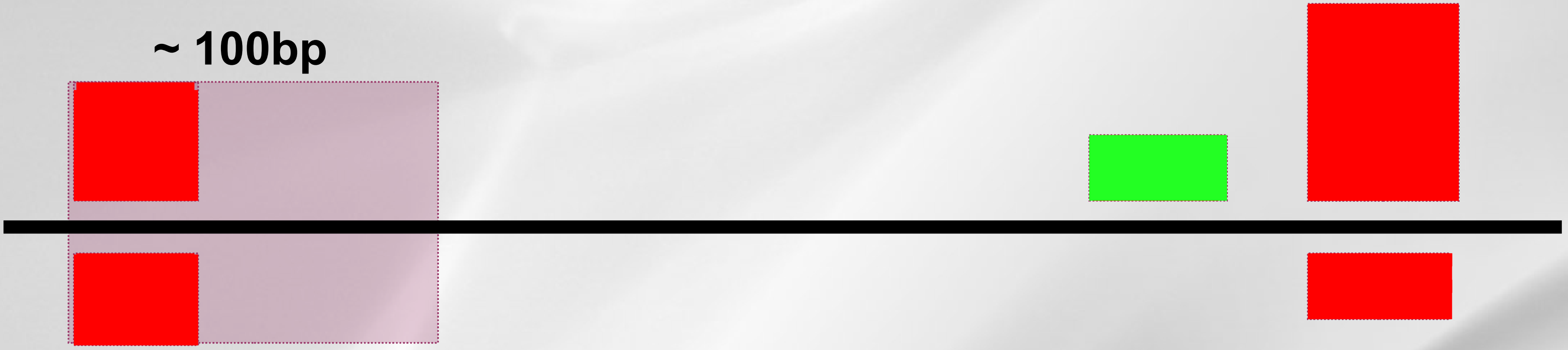
- Extend and fold read regions



- Stable hairpin structure shifted by 2 bases
- miRNA > miRNA*

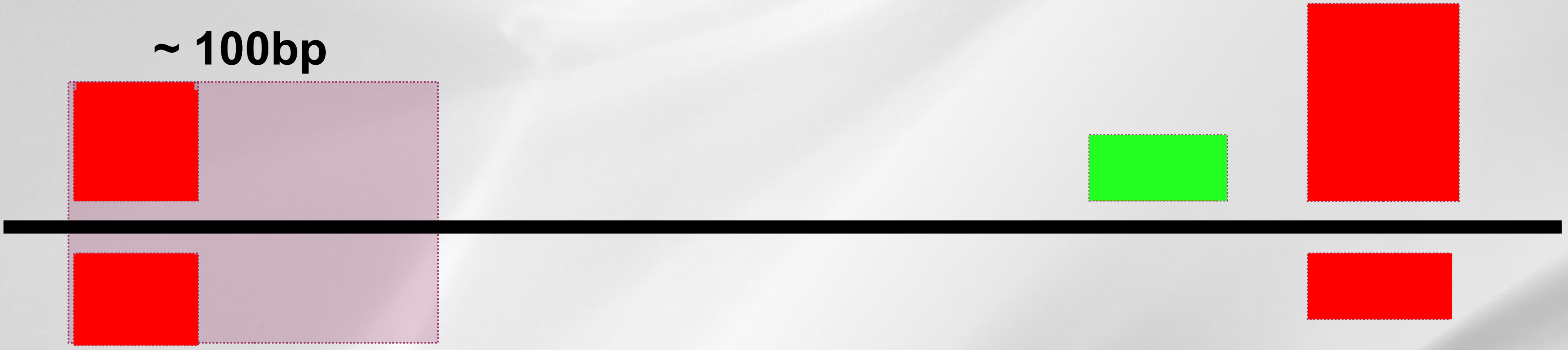
6. Prediction

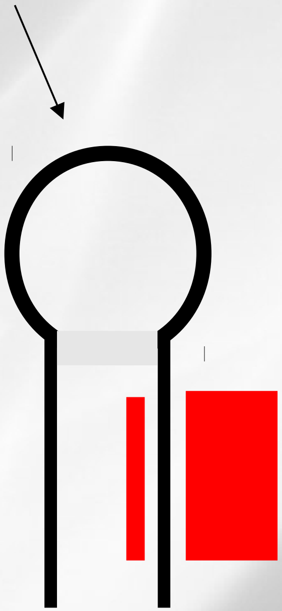
- Extend and fold read regions



6. Prediction

- Extend and fold read regions

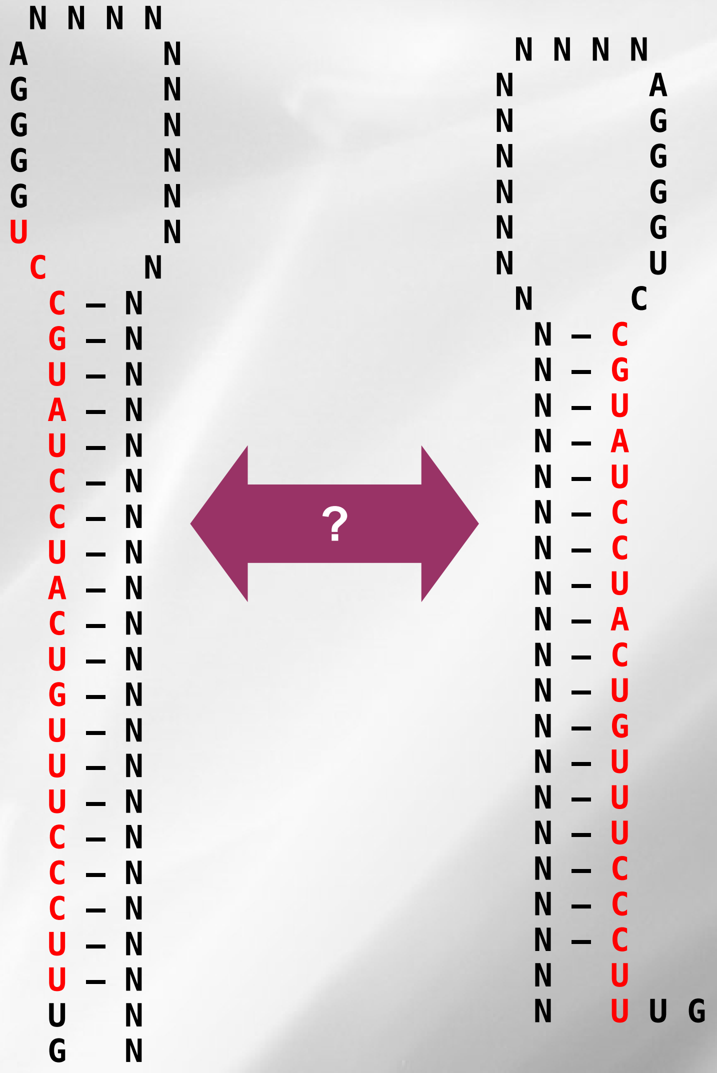




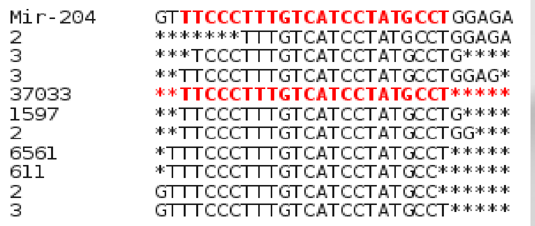
- **In the absence of reads corresponding to an expected miRNA*, additional checks on the structure are:**
  - Degree of pairing in the miRNA region
  - Hairpin: around 70nt in length
  - The secondary structure is significantly more stable than randomly shuffled versions of the same sequence
  - miRNA cluster

6. Prediction

- Which one should be used ?



```
  N N N N                      N N N N
 A       N                    N       A
 G       N                    N       G
 G       N                    N       G
 G       N                    N       G
 G       N                    N       G
 U       N                    N       U
  C     N                      N     C
   C – N                        N – C
   G – N                        N – G
   U – N                        N – U
   A – N                        N – A
   U – N                        N – U
   C – N                        N – C
   C – N                        N – C
   U – N                        N – U
   A – N                        N – A
   C – N                        N – C
   U – N                        N – U
   G – N                        N – G
   U – N                        N – U
   U – N                        N – U
   U – N                        N – U
   C – N                        N – C
   C – N                        N – C
   C – N                        N – C
   U – N                        N   U
   U – N                        N   U U G
   U   N
   G   N
```



```
Mir-204    GTTTCCCTTTGTCATCCTATGCCTGGAGA
2          *******TTTGTCATCCTATGCCTGGAGA
3          ***TCCCTTTGTCATCCTATGCCTG****
3          **TTCCCTTTGTCATCCTATGCCTGGAG*
37033      **TTCCCTTTGTCATCCTATGCCT*****
1597       **TTCCCTTTGTCATCCTATGCCTG****
2          **TTCCCTTTGTCATCCTATGCCTGG***
6561       *TTTCCCTTTGTCATCCTATGCCT*****
611        *TTTCCCTTTGTCATCCTATGCC******
2          GTTTCCCTTTGTCATCCTATGCC******
3          GTTTCCCTTTGTCATCCTATGCCT*****
```

Exercices

- Exercice 2: Ma séquence peut-elle représenter un miRNA ?
    - Chacune des séquences contenues dans le fichier /work/gaspin/L3/TP1 correspond au précurseur possible d'un miRNA. Dites quelles sont les informations qui, à votre connaissance, vous permettraient d'attribuer l'annotation miRNA.
    - Sachant que le programme RNAfold permet de calculer la structure secondaire la plus stable pour une séquence donnée, utilisez le pour replier les séquences de TP1:
        - » Quelles sont les séquences qui contiennent potentiellement des miRNA?
        - » Dites comment vous pourriez exploiter ce programme dans le cadre de l'analyse de séquences issues de sRNAseq lorsque vous disposez d'un génome de référence.