Characterization of the small RNA transcriptome

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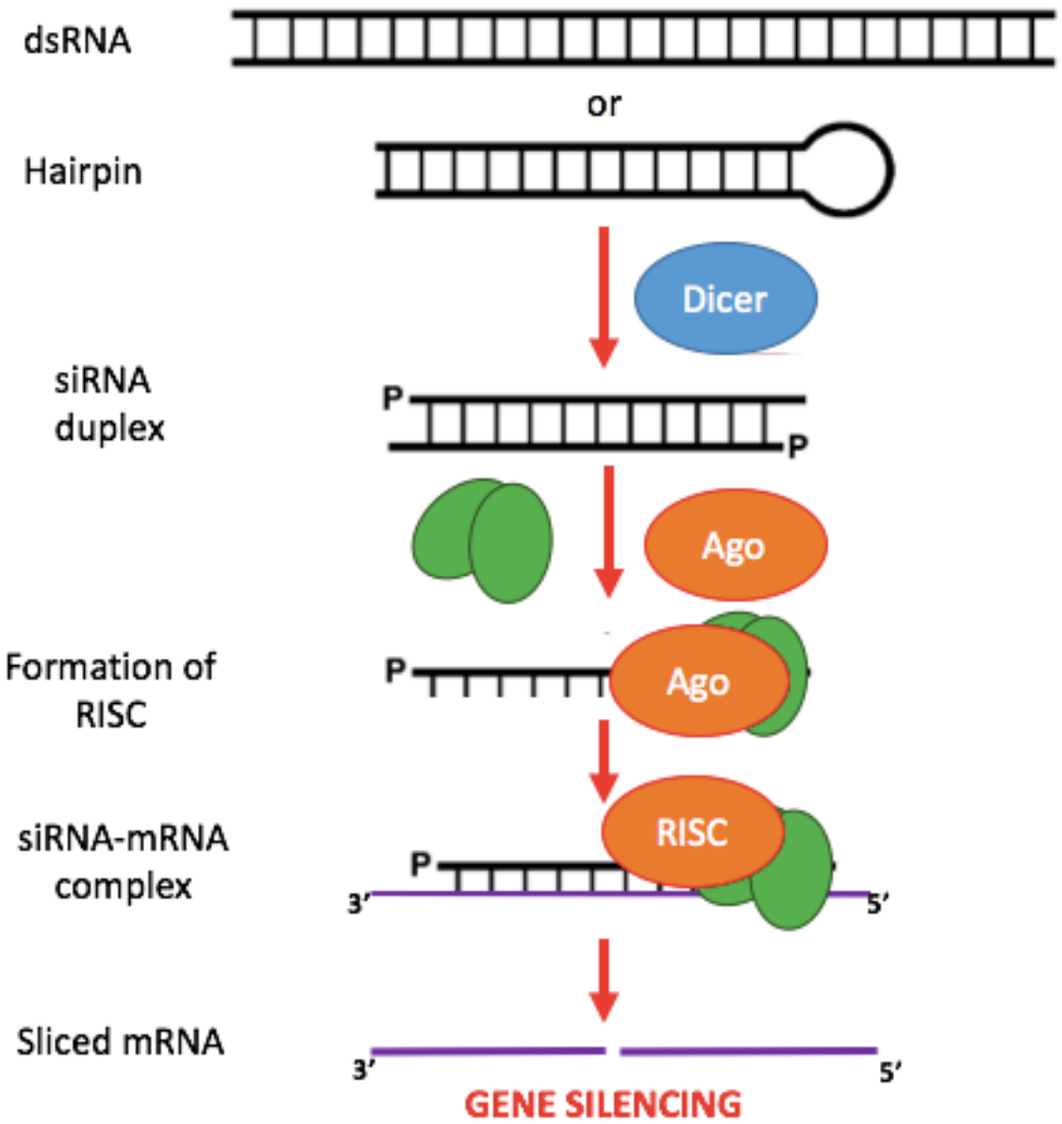
Harvard TH Chan School of Public Health

https://goo.gl/B9iDgm

2017-06-22

small interference RNA

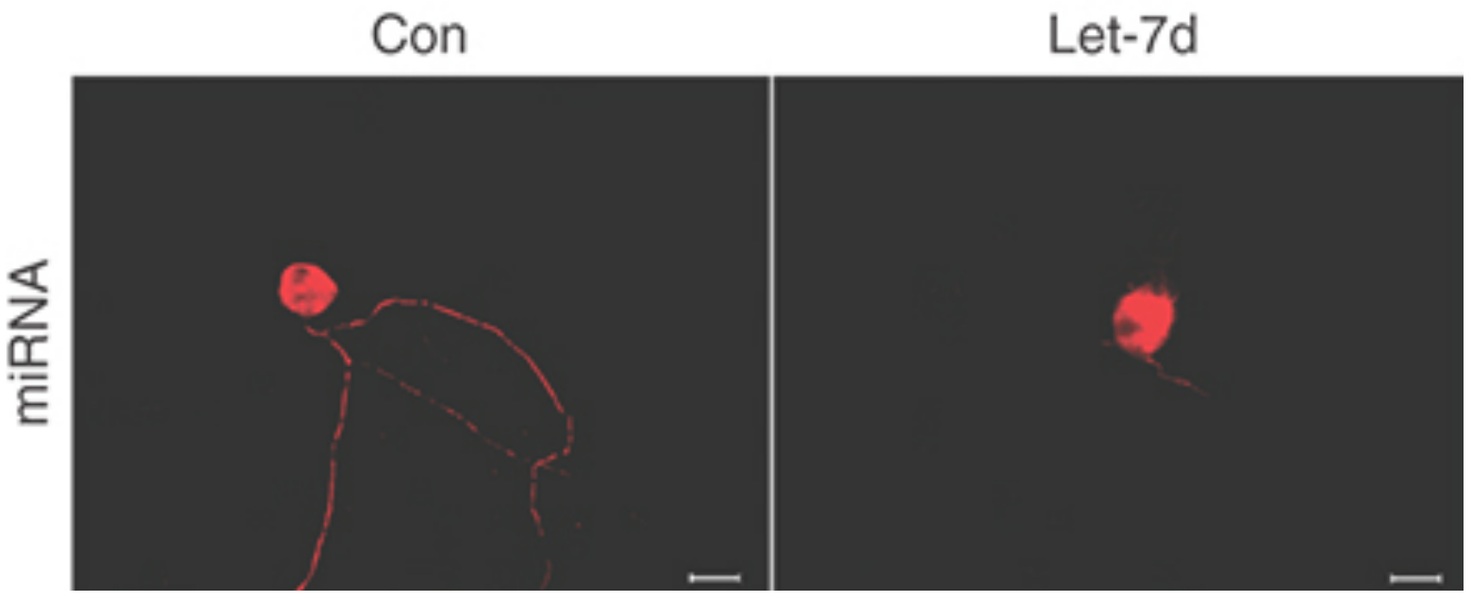


https://en.wikipedia.org/wiki/Small_interfering_RNA

- miRNA (18-25nt)
- endo-siRNA (20-25nt)
- piRNA (25-33nt)

miRNA

axon outgrowth



Let-7 microRNAs Regenerate Peripheral Nerve Regeneration by Targeting Nerve Growth Factor
Shiying Li, Xinghui Wang, Yun Gu, Chu Chen, Yaxian Wang, Jie Liu, Wen Hu, Bin Yu, Yongjun Wang, Fei Ding, Yan Liu and Xiaosong Gu

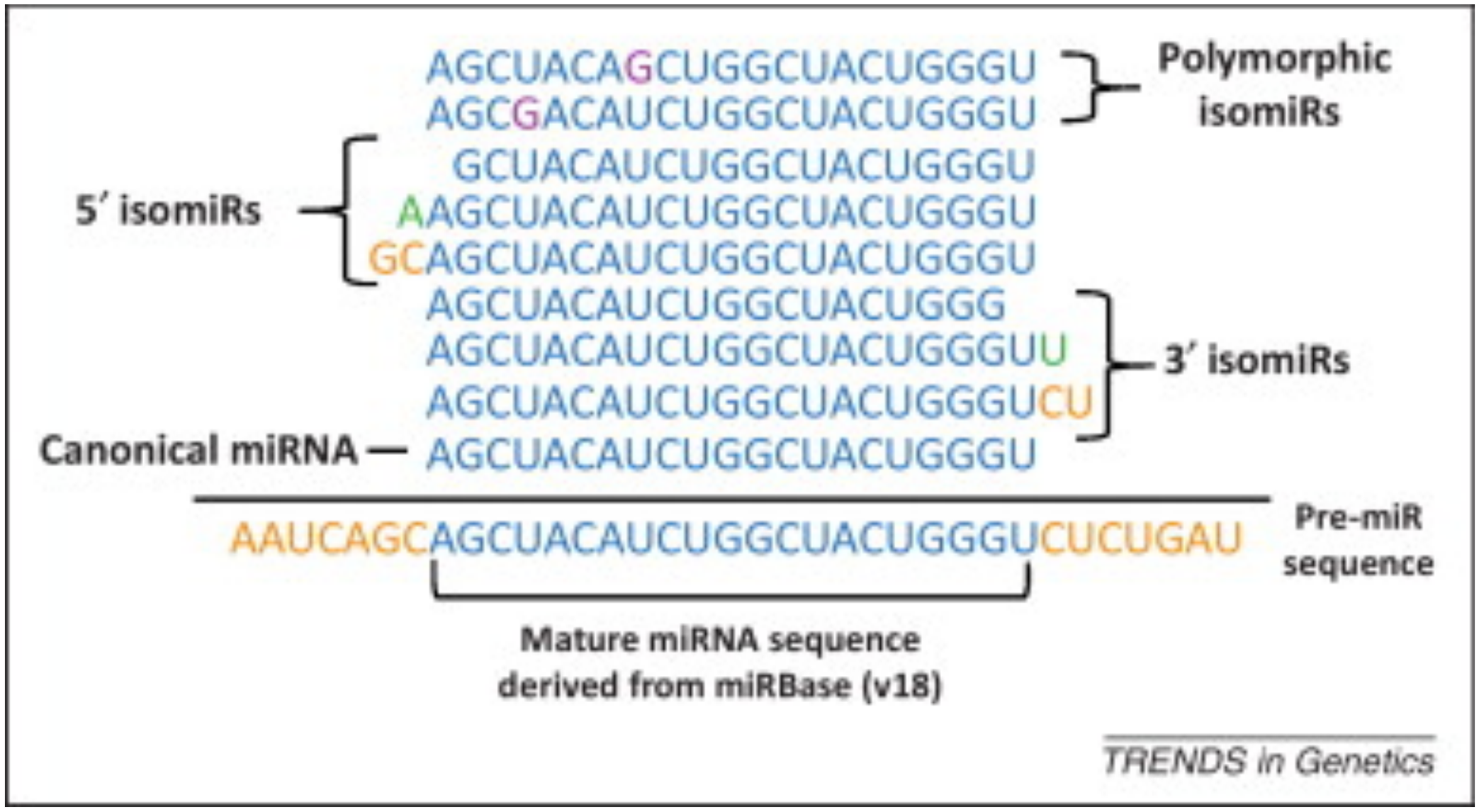
isomiRs

```
     hsa-miR-24-1-5p                                hsa-miR-24-3p
....GGUGCCUACUGAGCUGAUAUC.....................................
.....GUGCCUACUGAGCUGAUAUCAGU..................................
.....GUGCCUACUGAGCUGAUAUCAG...................................
.....GUGCCUACUGAGCUGAUA.......................................
......UGCCUACUGAGCUGAUAUCA....................................
......UGCCUACUGAGCUGAUAUCAGU..................................
......UGCCUACUGAGCUGAUAUC.....................................
......UGCCUACUGAGCUGAUA.......................................
........CCUACUGAGCUGAUAUCA....................................
........CCUACUGAGCUGAUAUCAGU..................................
.........CUACUGAGCUGAUAUCA....................................
.........CUACUGAGCUGAUAUC.....................................
```

```
CUCCGGUGCCUACUGAGCUGAUAUCAGUUCUCAUUUUACACACUGGCUCAGUUCAGCAGGAACAGGAG
((((.((.(((.(((((((((..(((((.............))))).)))))))))).))).)).)))) (-26.32)
```

precursor

types of isomiRs



isomiRs

Search results

Items: 1 to 20 of 146

<< First < Prev Page 1 of 8 Next > Last >>

1. Chronic low-dose exposure to a mixture of environmental endocrine disruptors induces microRNAs/**isomiRs** deregulation in mouse concomitant with intratesticular estradiol reduction.
   Buñay J, Larriba E, Moreno RD, Del Mazo J.
   Sci Rep. 2017 Jun 13;7(1):3373. doi: 10.1038/s41598-017-02752-7.
   PMID: 28611354
   Similar articles

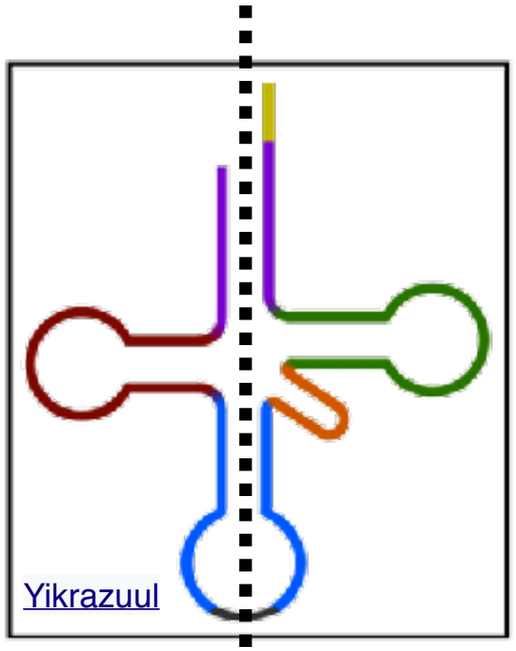
2. Expression profile of Epstein-Barr virus and human adenovirus small RNAs in tonsillar B and T lymphocytes.
   Assadian F, Kamel W, Laurell G, Svensson C, Punga T, Akusjärvi G.
   PLoS One. 2017 May 25;12(5):e0177275. doi: 10.1371/journal.pone.0177275. eCollection 2017.
   PMID: 28542273 **Free PMC Article**
   Similar articles

3. isomiR2Function: An Integrated Workflow for Identifying MicroRNA Variants in Plants.
   Yang K, Sablok G, Qiao G, Nie Q, Wen X.
   Front Plant Sci. 2017 Mar 21;8:322. doi: 10.3389/fpls.2017.00322. eCollection 2017.
   PMID: 28377776 **Free PMC Article**
   Similar articles

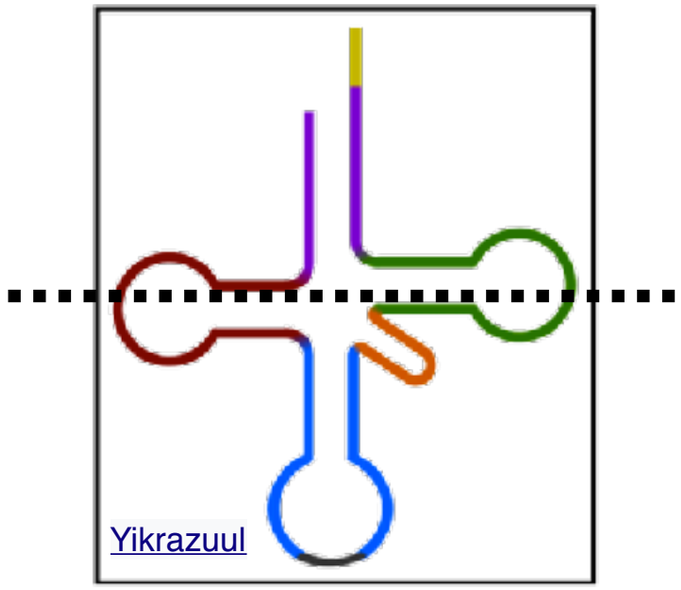
4. 3' Uridylation controls mature microRNA turnover during CD4 T-cell activation.
   Gutiérrez-Vázquez C, Enright AJ, Rodríguez-Galán A, Pérez-García A, Collier P, Jones MR, Benes V, Mizgerd JP, Mittelbrunn M, Ramiro AR, Sánchez-Madrid F.
   RNA. 2017 Jun;23(6):882-891. doi: 10.1261/rna.060095.116. Epub 2017 Mar 28.
   PMID: 28351886
   Similar articles

tRNA derived fragments

tRNAs function as carriers that transport amino acids to the growing polypeptide chain during the translation of mRNA.



tRNA-halves (30-33nt)



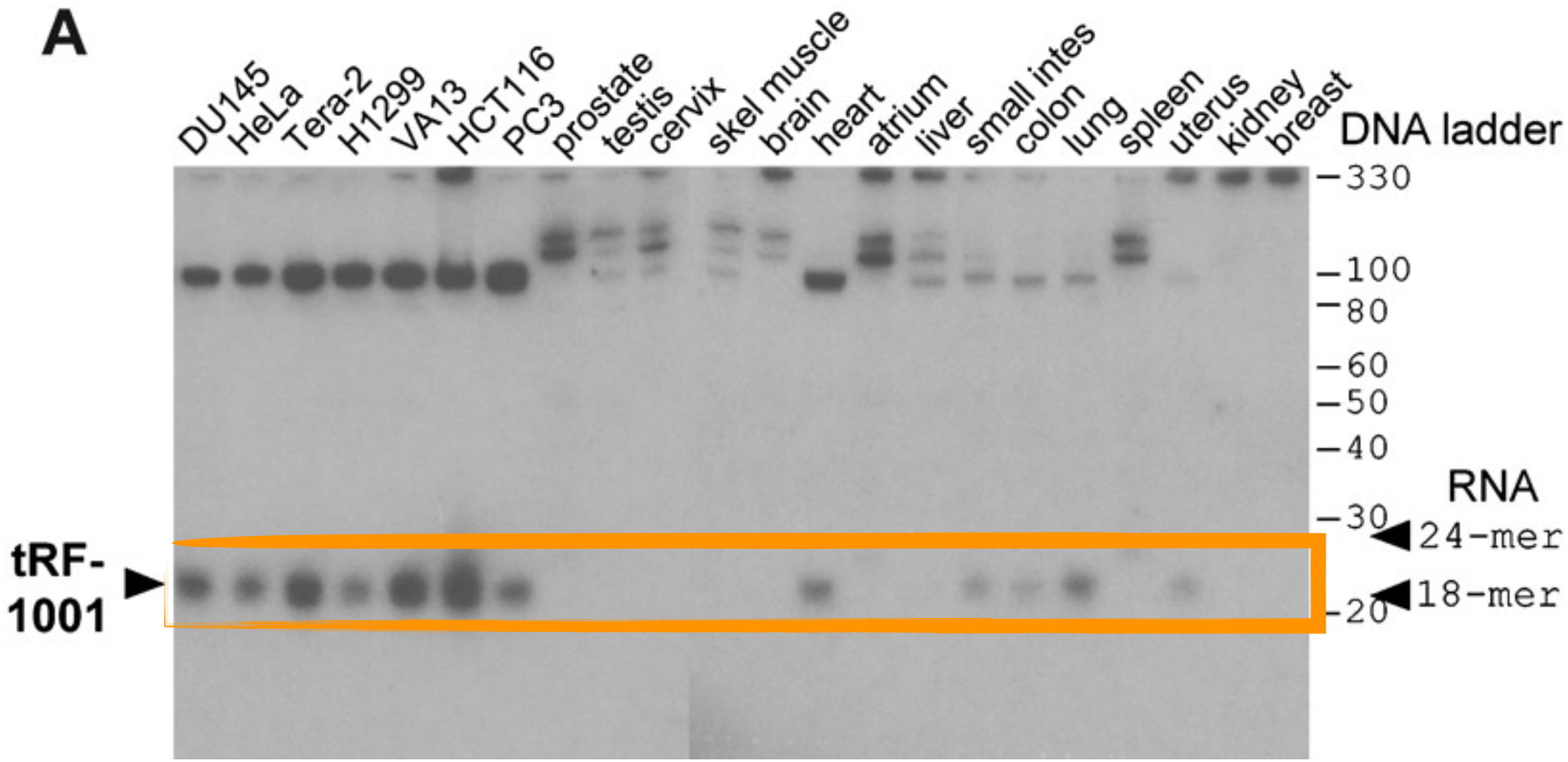
5’-tRNAs (18-22) 3’-tRNAs (18-22)

associated with **genetic disorders** and malignancies such as **prostate, liver, lung (tRF-Leu-CAG) or breast cancer,** and related processes like **aging, oxidative stress ,** and embryonic development

In Arabidopsis, they are miRNA-like sequences, targeting transposable elements.

They have been found in extracellular samples like: plasma, saliva and urine.

small tRNAs



Yong Sun Lee et al. Genes Dev. 2009;23:2639-2649

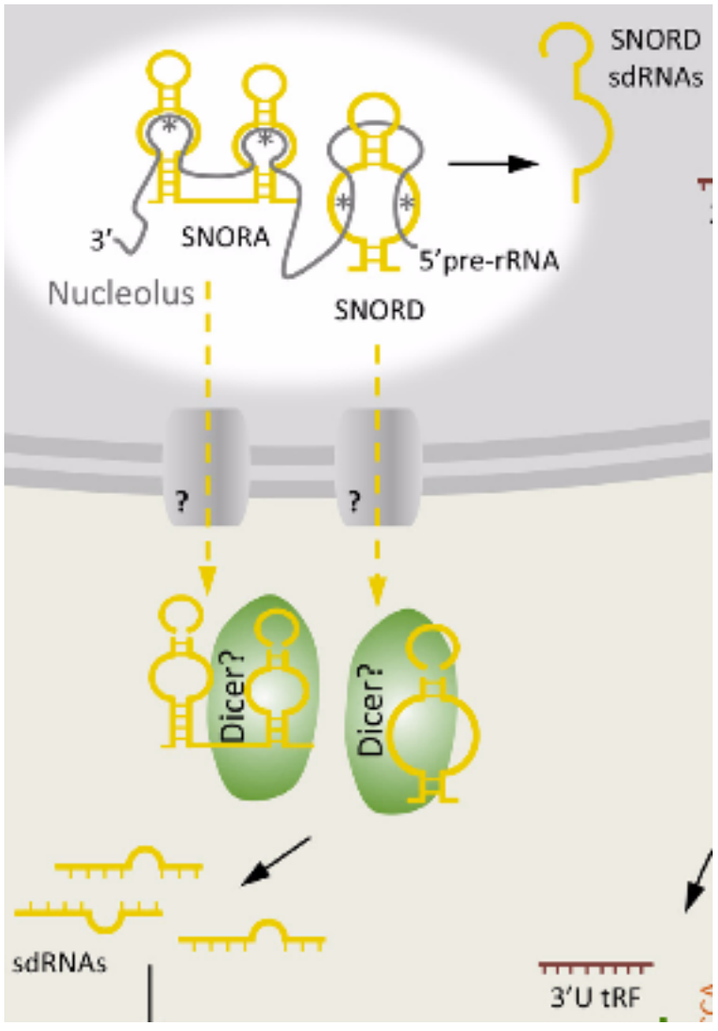
sncRNA fragments

Small nucleolar RNAs (snoRNA) are well-conserved, abundant, short non-coding RNA molecules, 60–300 nucleotides (nt) in length, which localize to a specific compartment of the cell nucleus – the nucleolus

In HEK293, SCARNA15 **miRNA-like** sequence targeting CDK11B (22nt)

SNORD88C-sdRNAs can regulates **alternative splicing** of fibroblast growth factor receptor 3. (FGFR3)

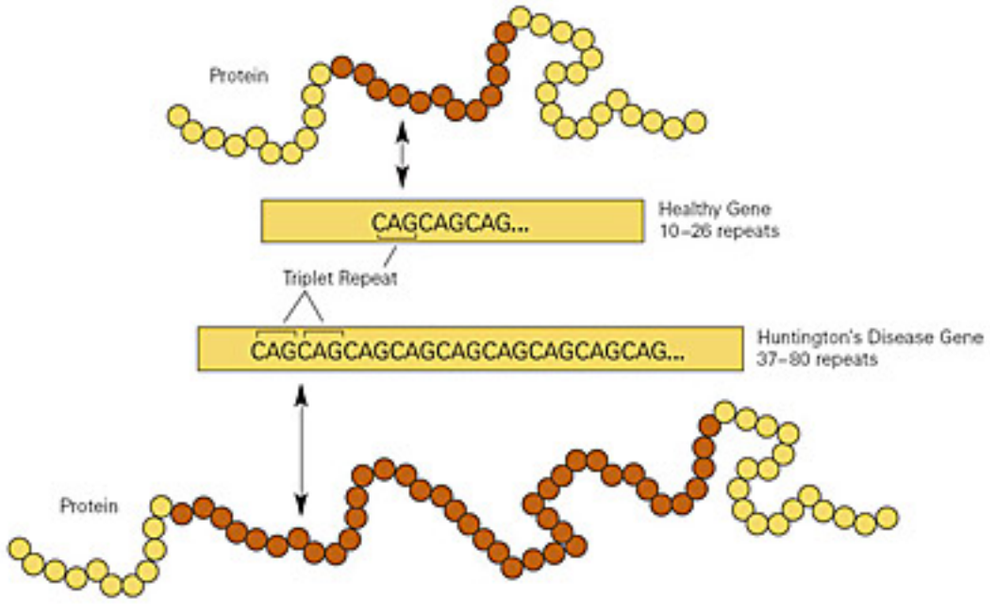
SNORD44/78 up-regulated in **prostate caner**.

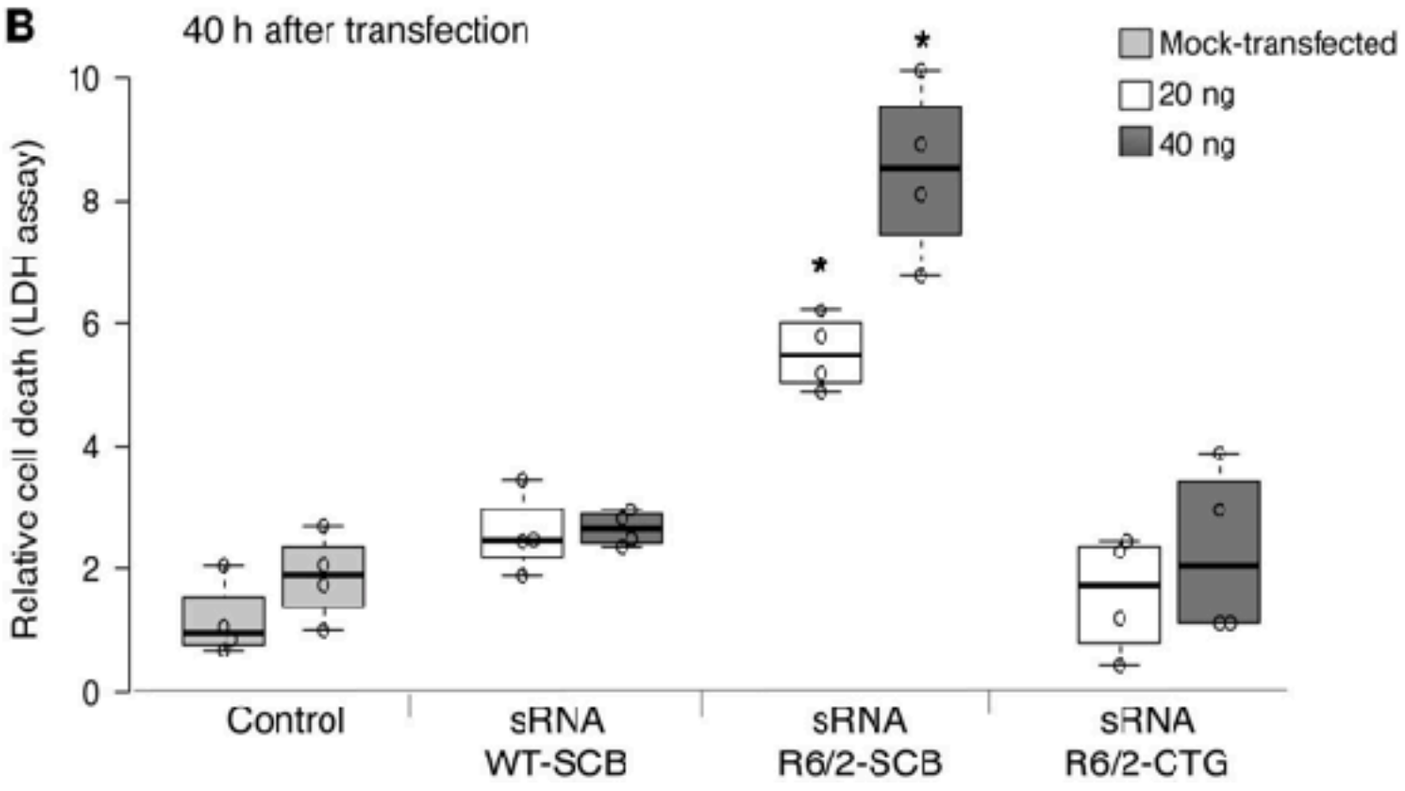


snoRNA-derived RNAs (sdRNAs)

small RNA

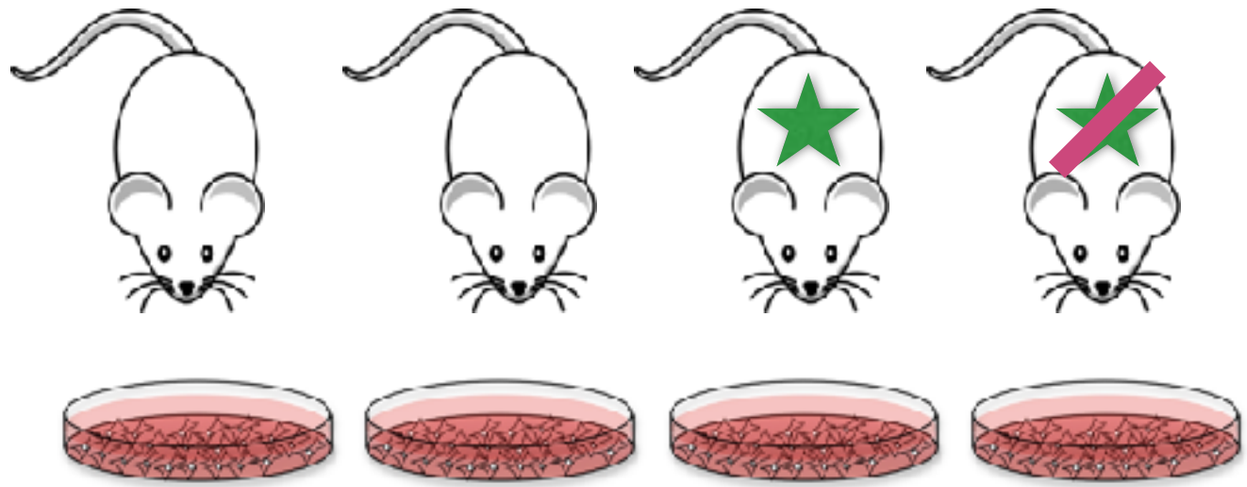
Huntington disease therapy



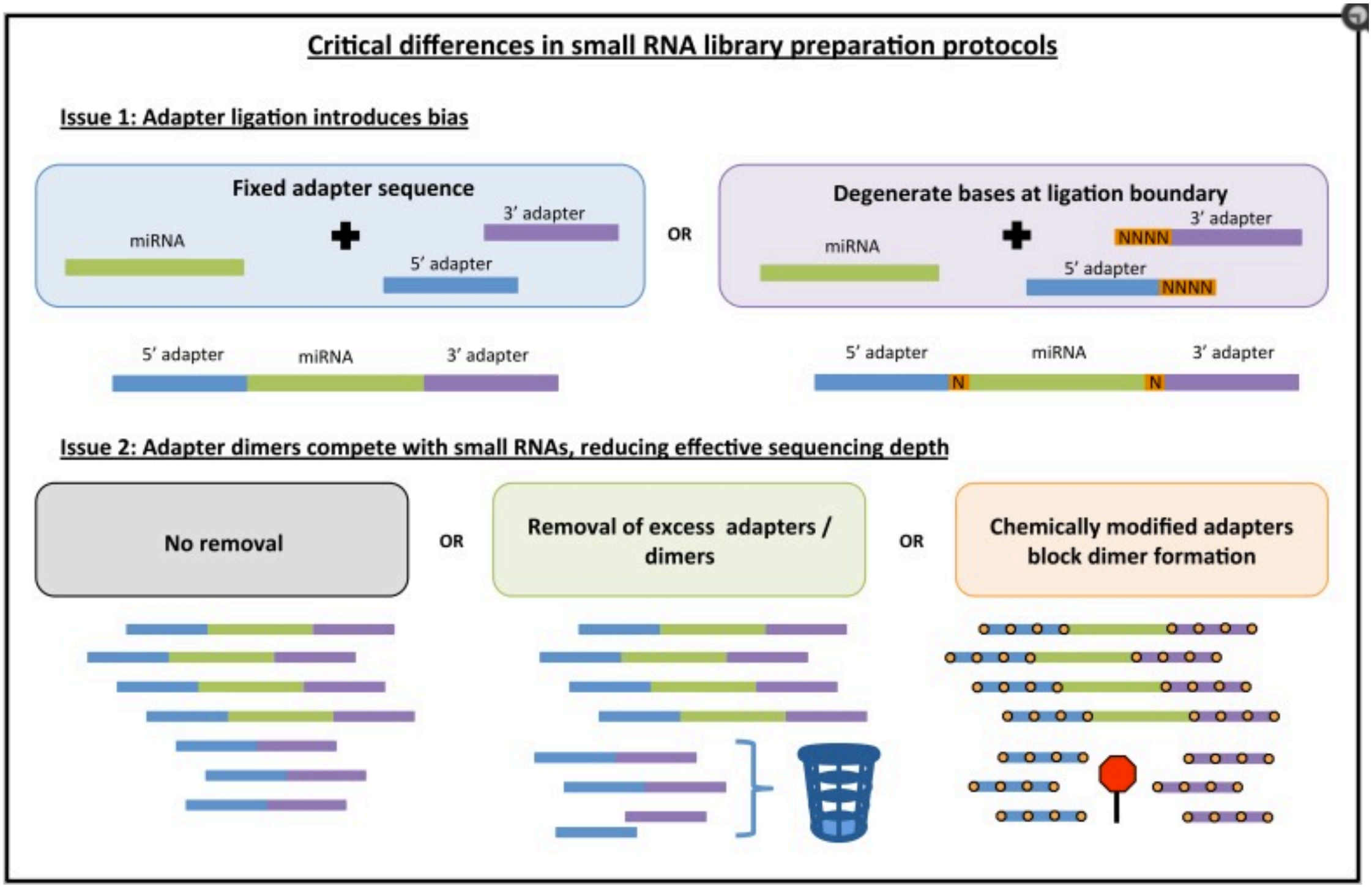


synthetic small RNA

CTGCTGCTGCTGCTGCTGCT

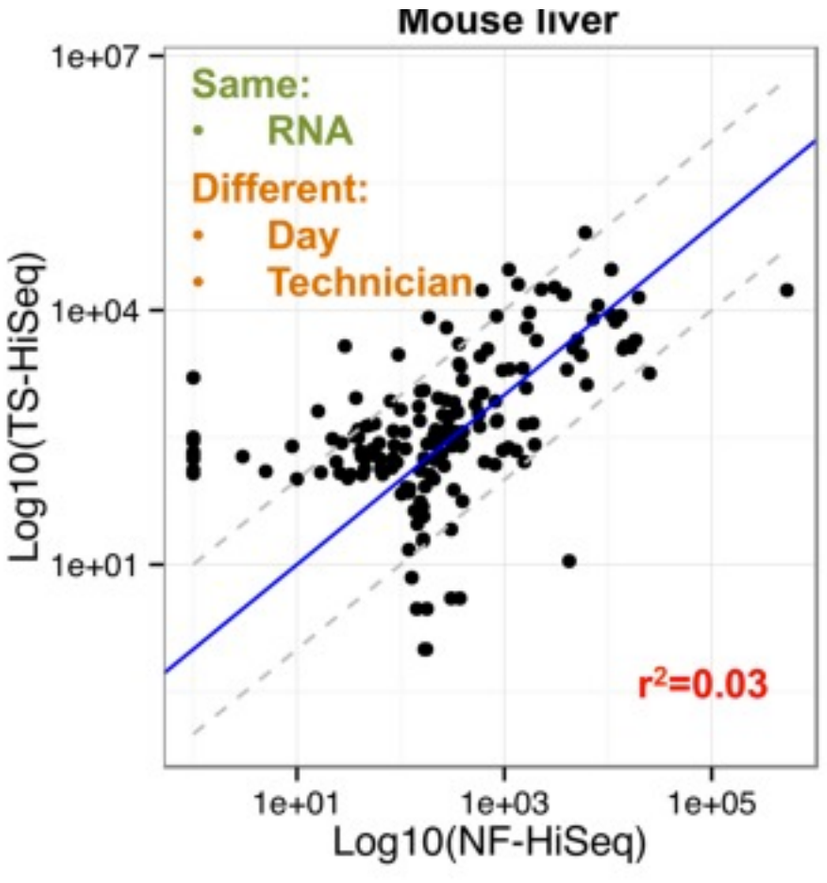


Protocols

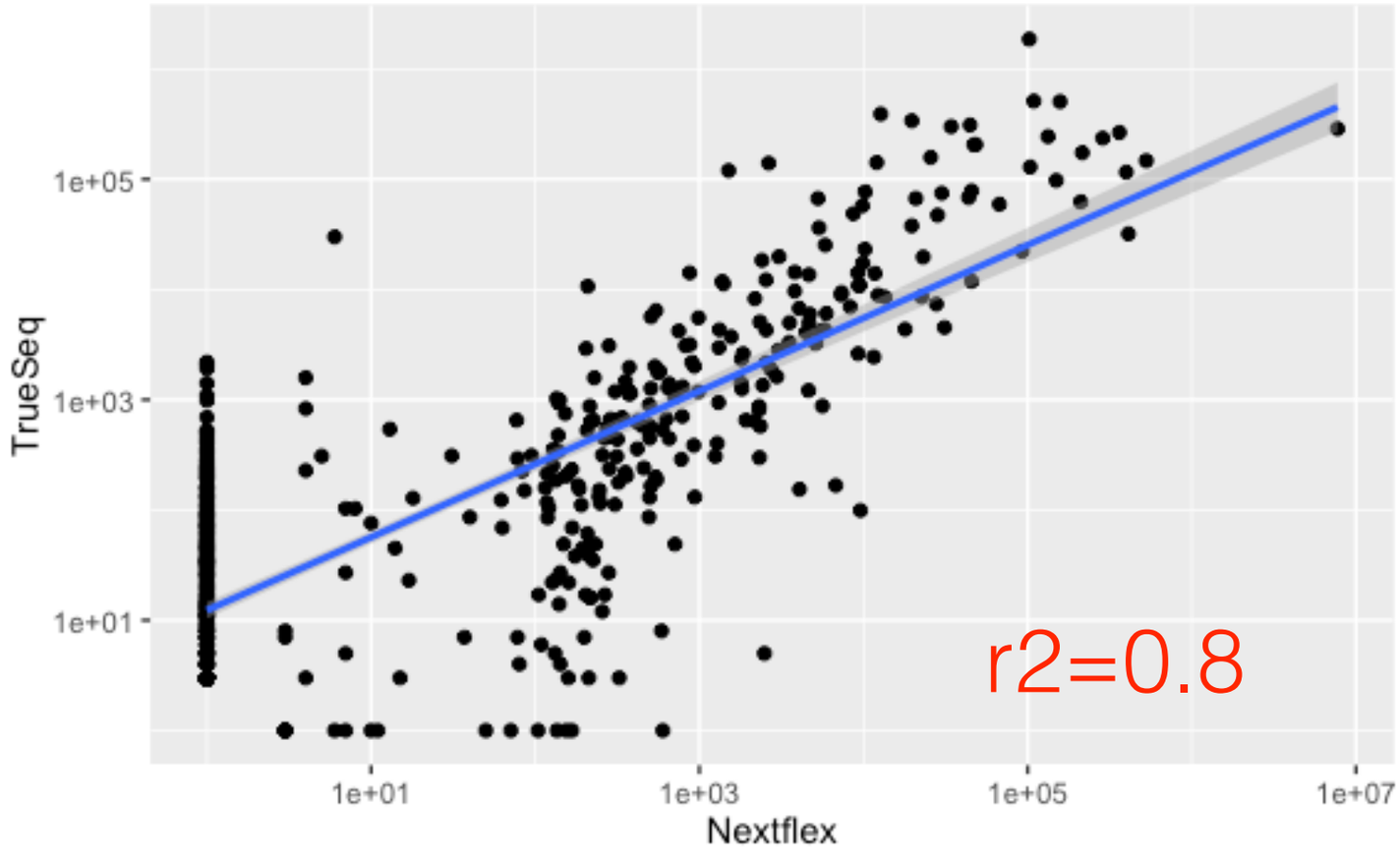

Critical differences in small RNA library preparation protocols
Issue 1: Adapter ligation introduces bias
Fixed adapter sequence
miRNA
3' adapter
5' adapter
OR
Degenerate bases at ligation boundary
miRNA
3' adapter
NNNN
5' adapter
NNNN
5' adapter
miRNA
3' adapter
5' adapter
N
miRNA
N
3' adapter
Issue 2: Adapter dimers compete with small RNAs, reducing effective sequencing depth
No removal
OR
Removal of excess adapters / dimers
OR
Chemically modified adapters block dimer formation

Protocol correlation

Paper figure

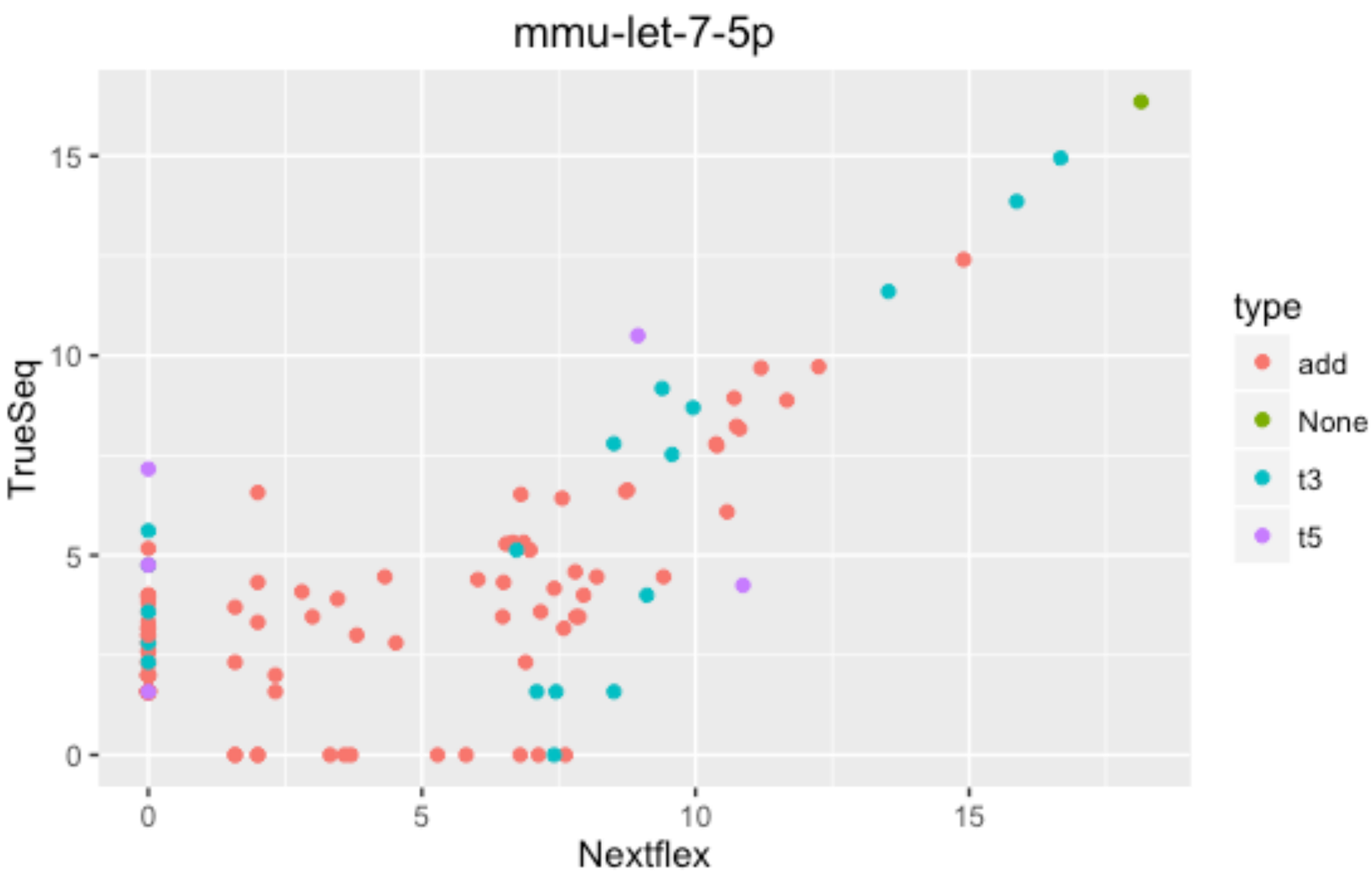


bcbio pipeline



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4686641/

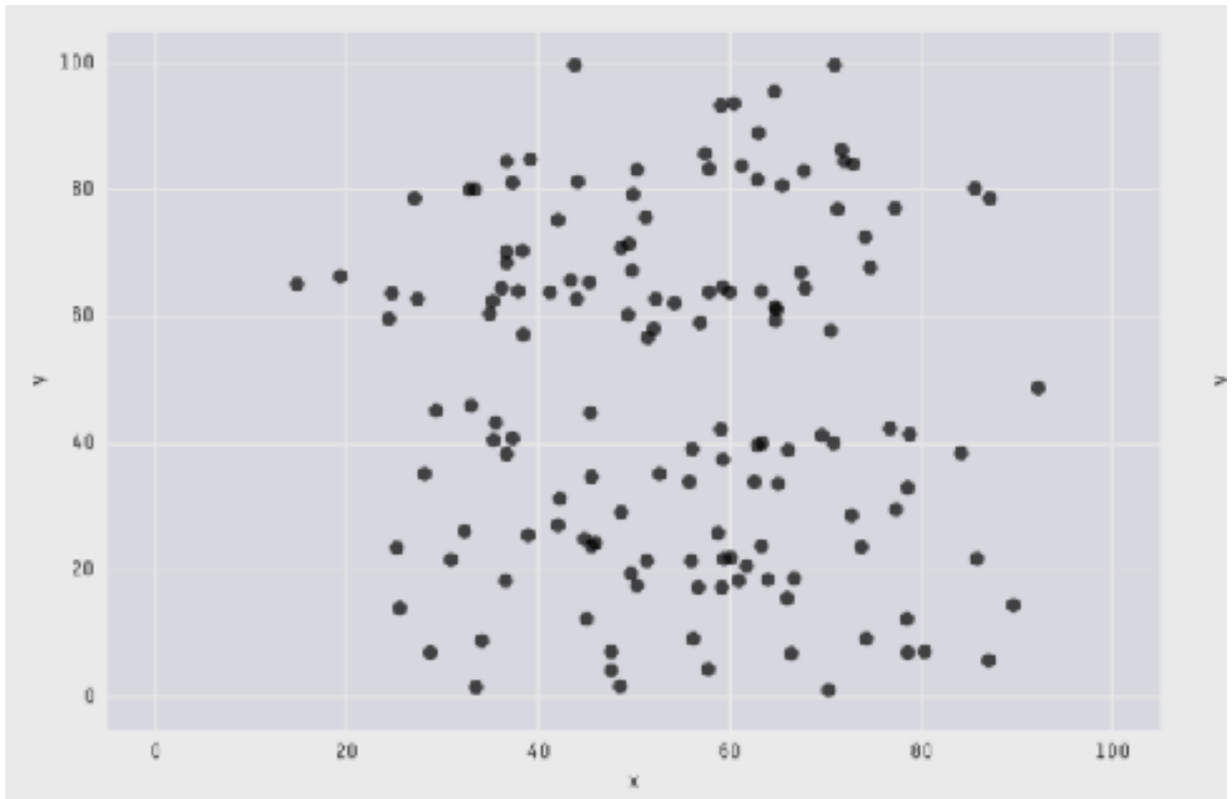
let7-a-5p miRNA



Caveats

- TrueSeq Illumina: ligation bias
- NextFlex Bioo Scientific: generation of random sequences?. We lose the accuracy to detect isomiRs

Same Stats, Different graphs



X Mean: 54.26

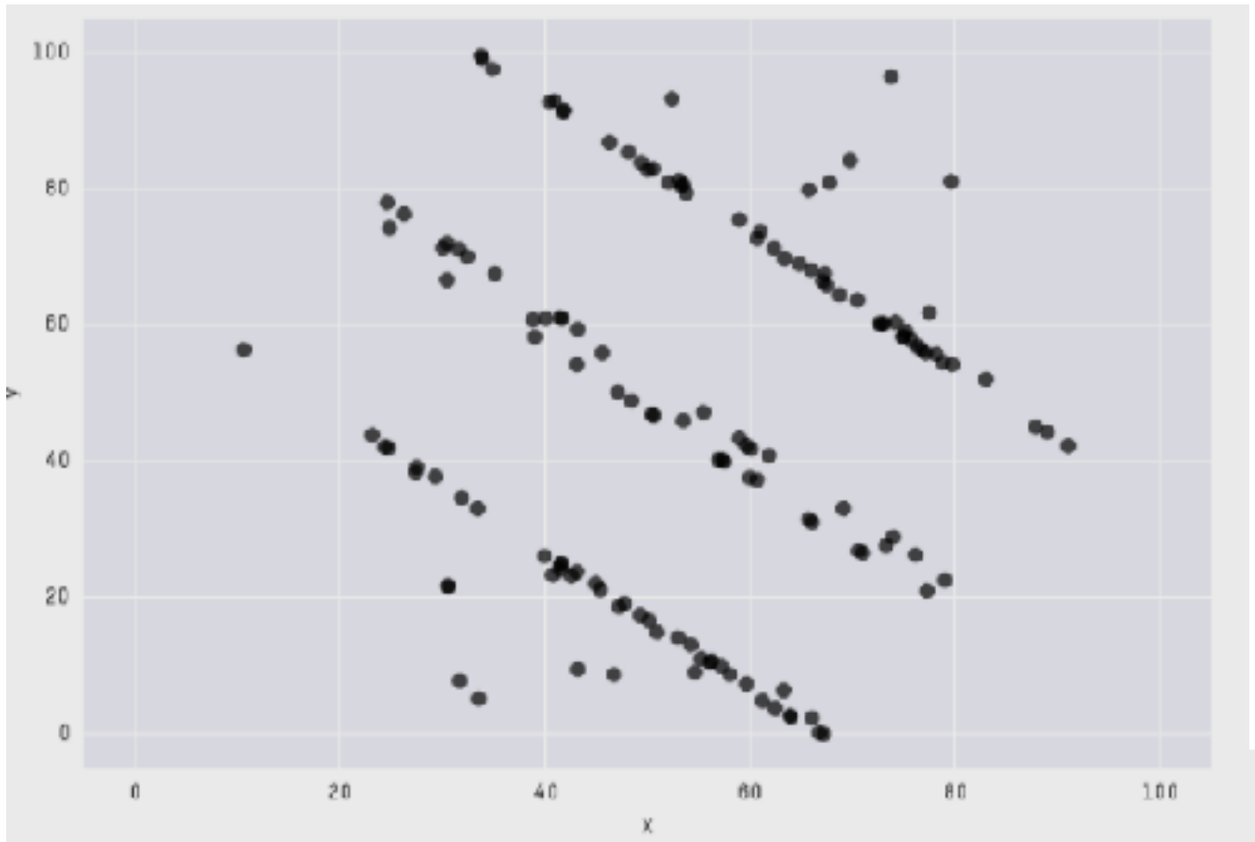
Y Mean: 47.83

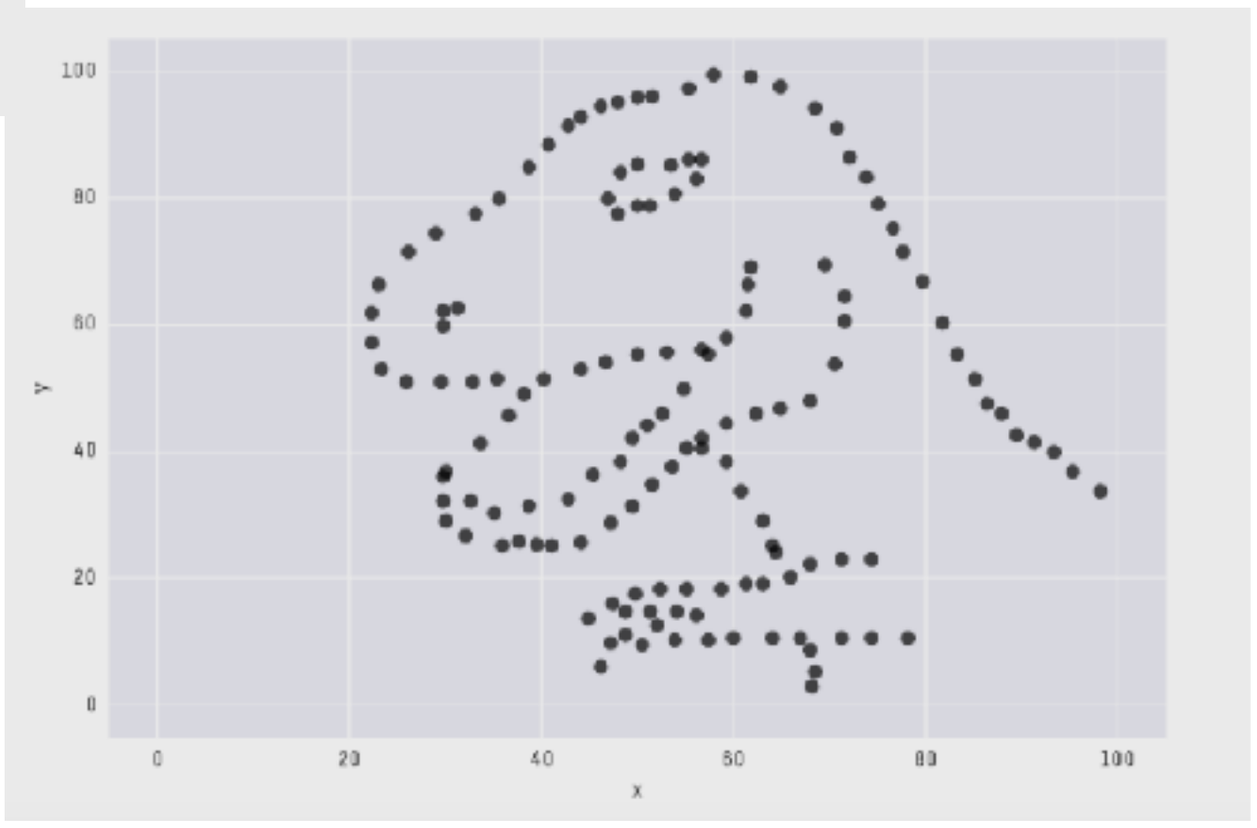
X SD : 16.76

Y SD : 26.93

Corr. : -0.06

https://www.autodeskresearch.com/publications/samestats

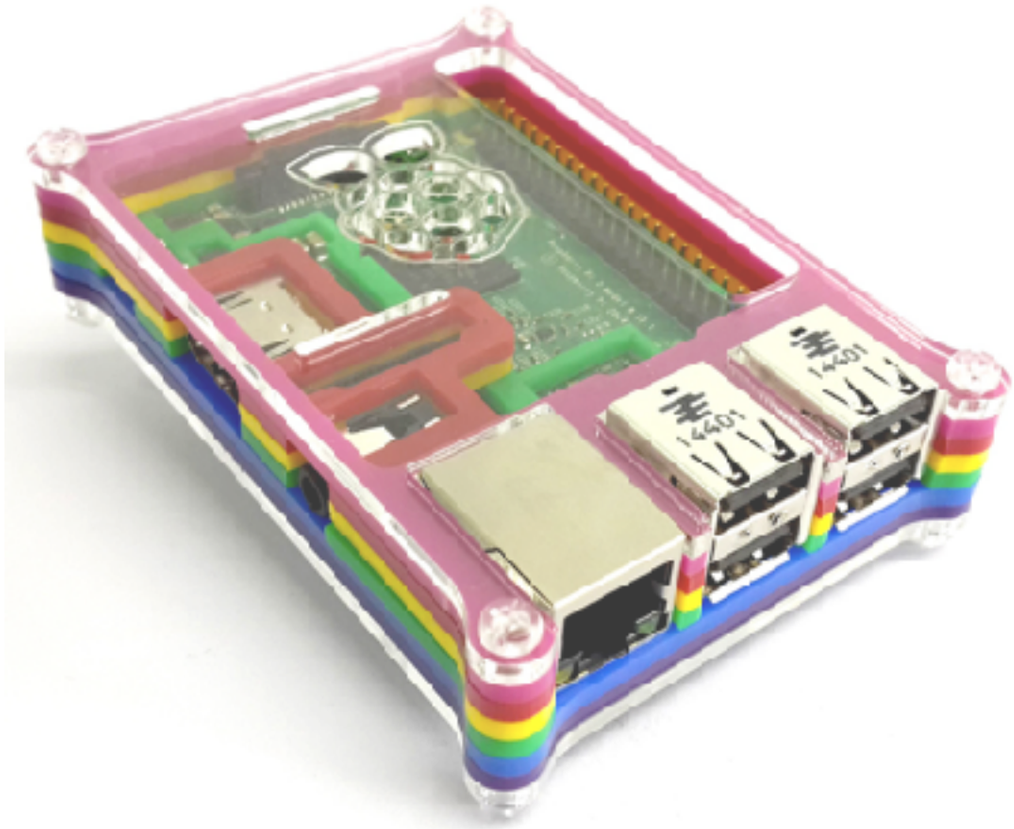

100
80
60
40
20
0
y
0
20
40
60
80
100
x


100
80
60
40
20
0
y
0
20
40
60
80
100
x

challenges

- isomiRs detection
- small RNAs coming from multiple precursors over the genome (multi-mapped reads can be 40% of the data.)
- differentiate degradation and functional molecules
- non-model organism

bcbio-nextgen



Variant calling, RNA-seq, small RNA-seq

over 200 peer reviewed tools BIOCONDA®

May 20, 2017 – June 20, 2017

Period: **1 month**

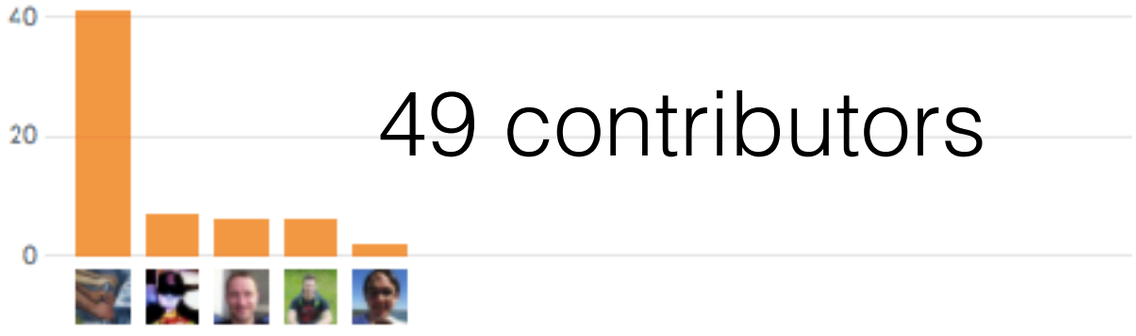
Overview

6 Active Pull Requests

39 Active Issues

6 Merged Pull Requests	**0** Proposed Pull Requests	**29** Closed Issues	**10** New Issues

Excluding merges, **5 authors** have pushed **62 commits** to master and **62 commits** to all branches. On master, **91 files** have changed and there have been **3,836 additions** and **536 deletions**.



small RNA-seq analysis

processing & QC

cutadapt
fastqc
qualimap
multiqc

de-novo

seqcluster
mirdeep2 for miRNA
protac for piRNA

detection & annotation

miraligner
miRAnalyzer
miRGe
tdrmapper
MINTmapper

Detection & Annotation

trimmed and collapsed reads

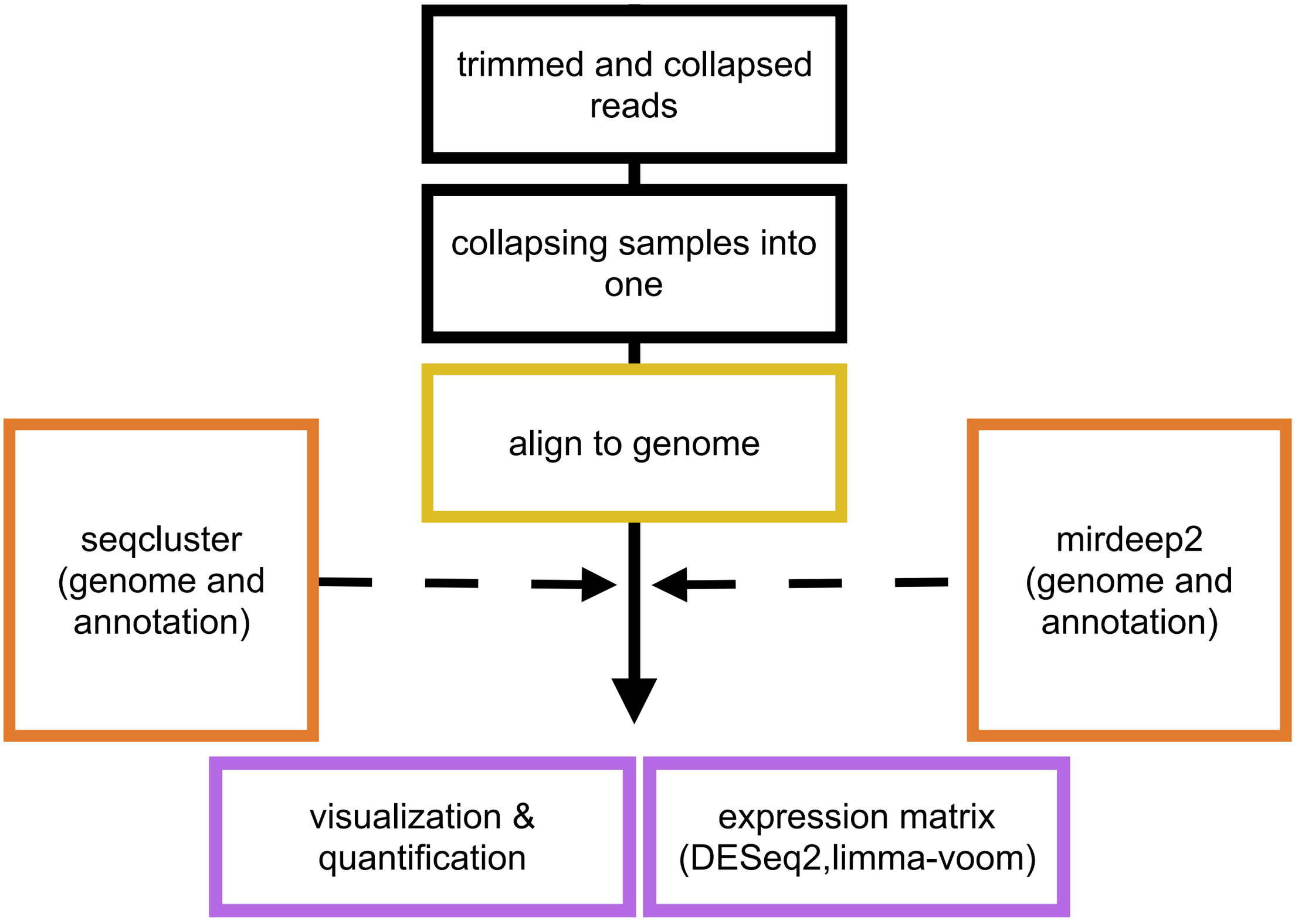
miraligner
miRAnalyzer
miRGe
(miRBase)

tdrMapper
MINTmapper
(tRNA reference)

visualization & quantification

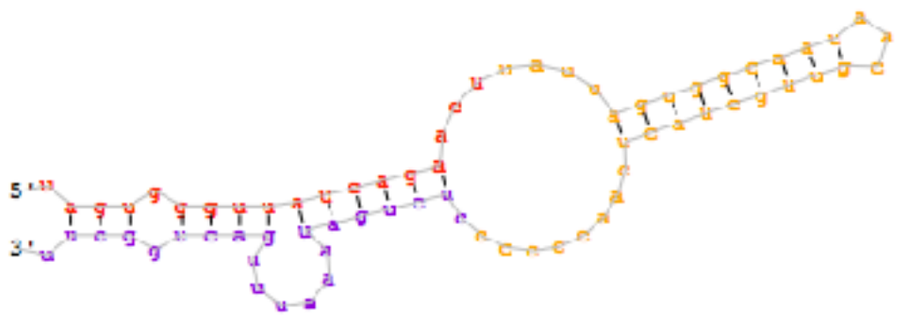
expression matrix (DESeq2,limma-voom)

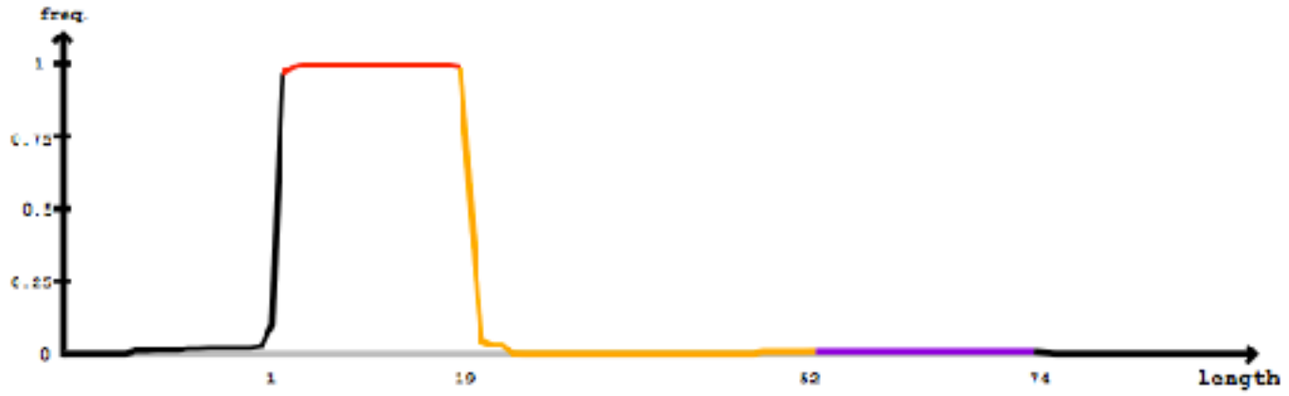
De-novo detection



miRDeep2 output

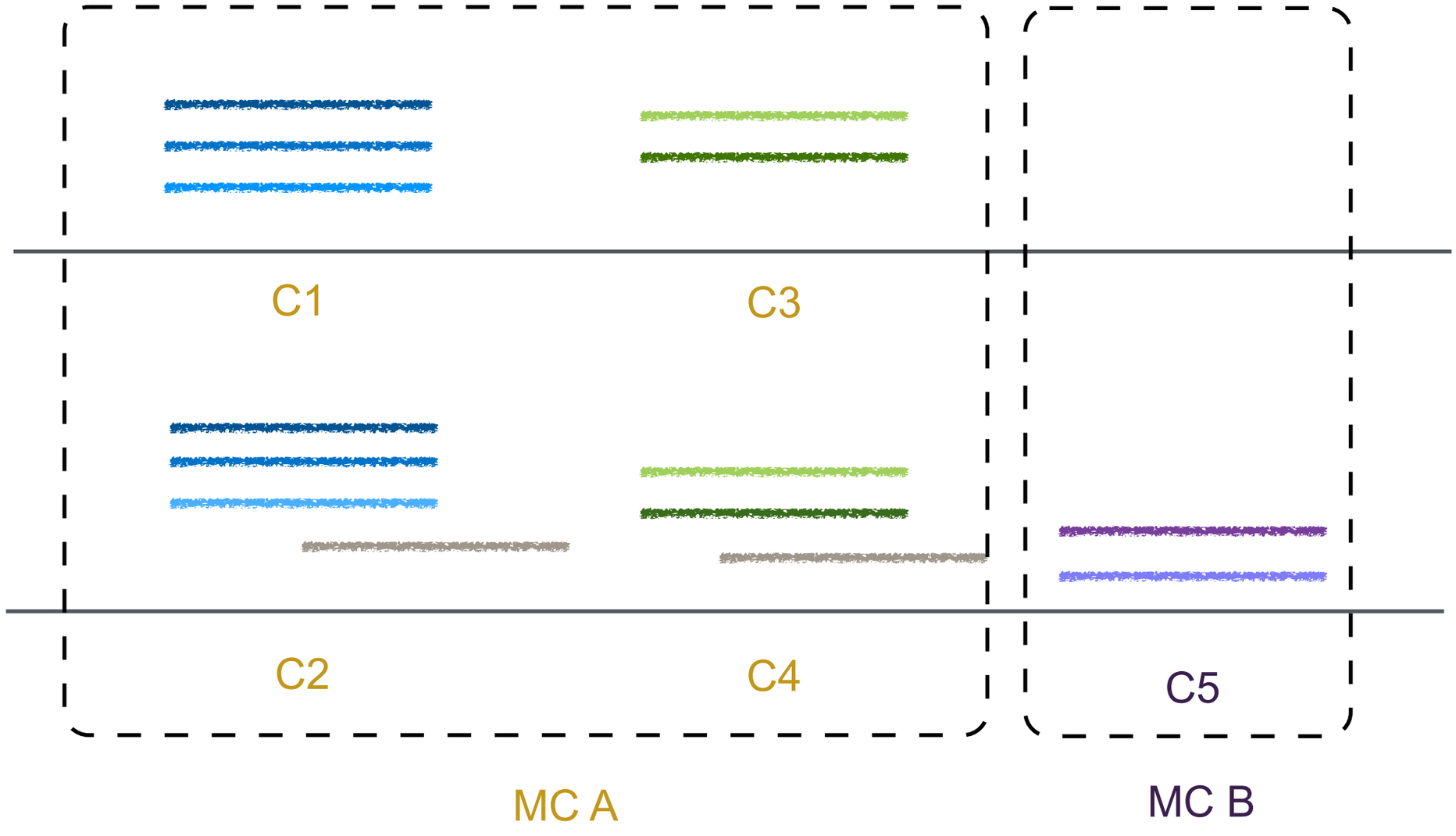
Provisional ID	:	chr12_16160
Score total	:	1869.4
Score for star read(s)	:	3.9
Score for read counts	:	1866.6
Score for mfe	:	-1
Score for randfold	:	
Score for cons. seed	:	
Total read count	:	3673
Mature read count	:	3670
Loop read count	:	0
Star read count	:	3



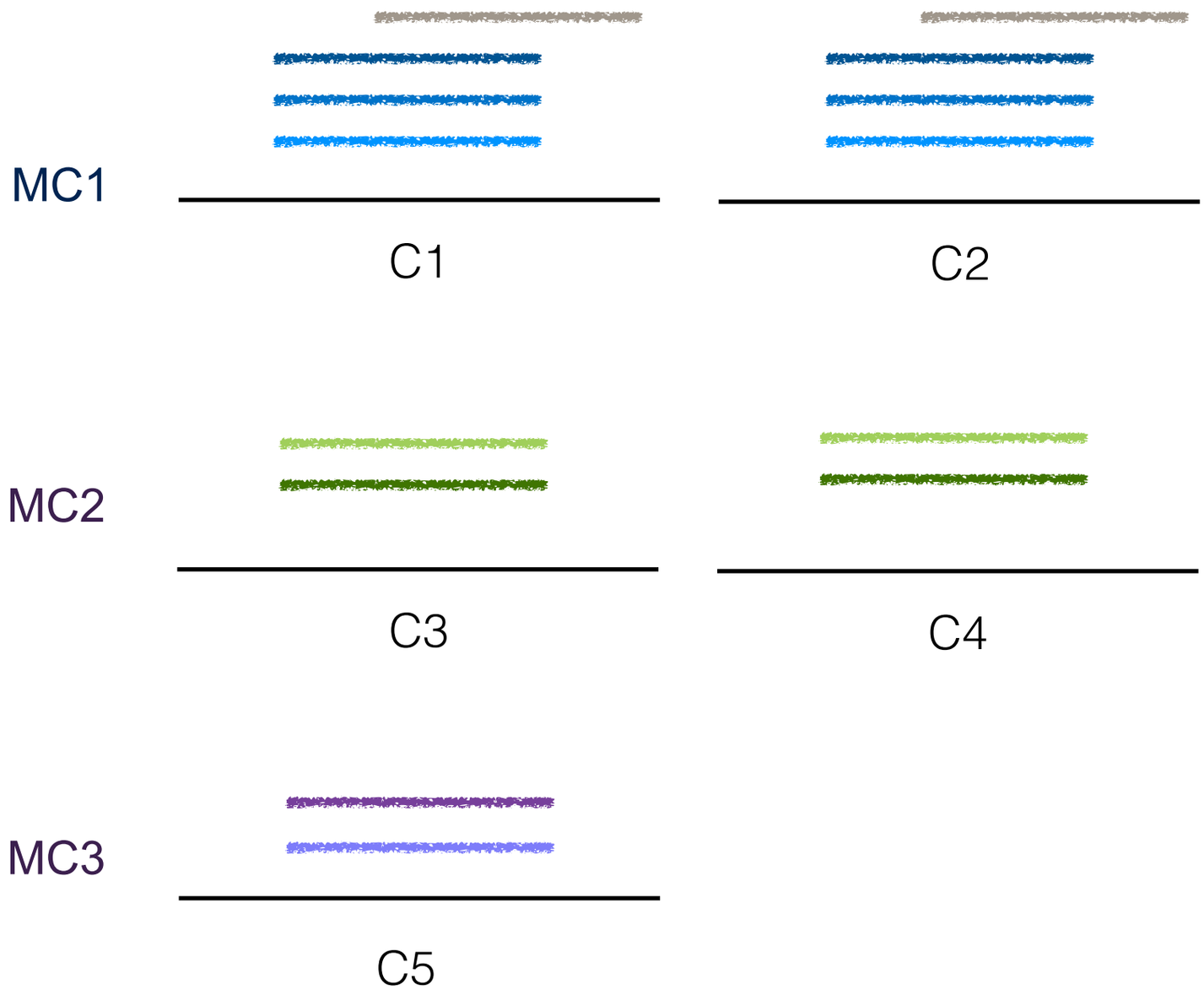


seqcluster deals with multi-mapped reads

Step 1: clustering

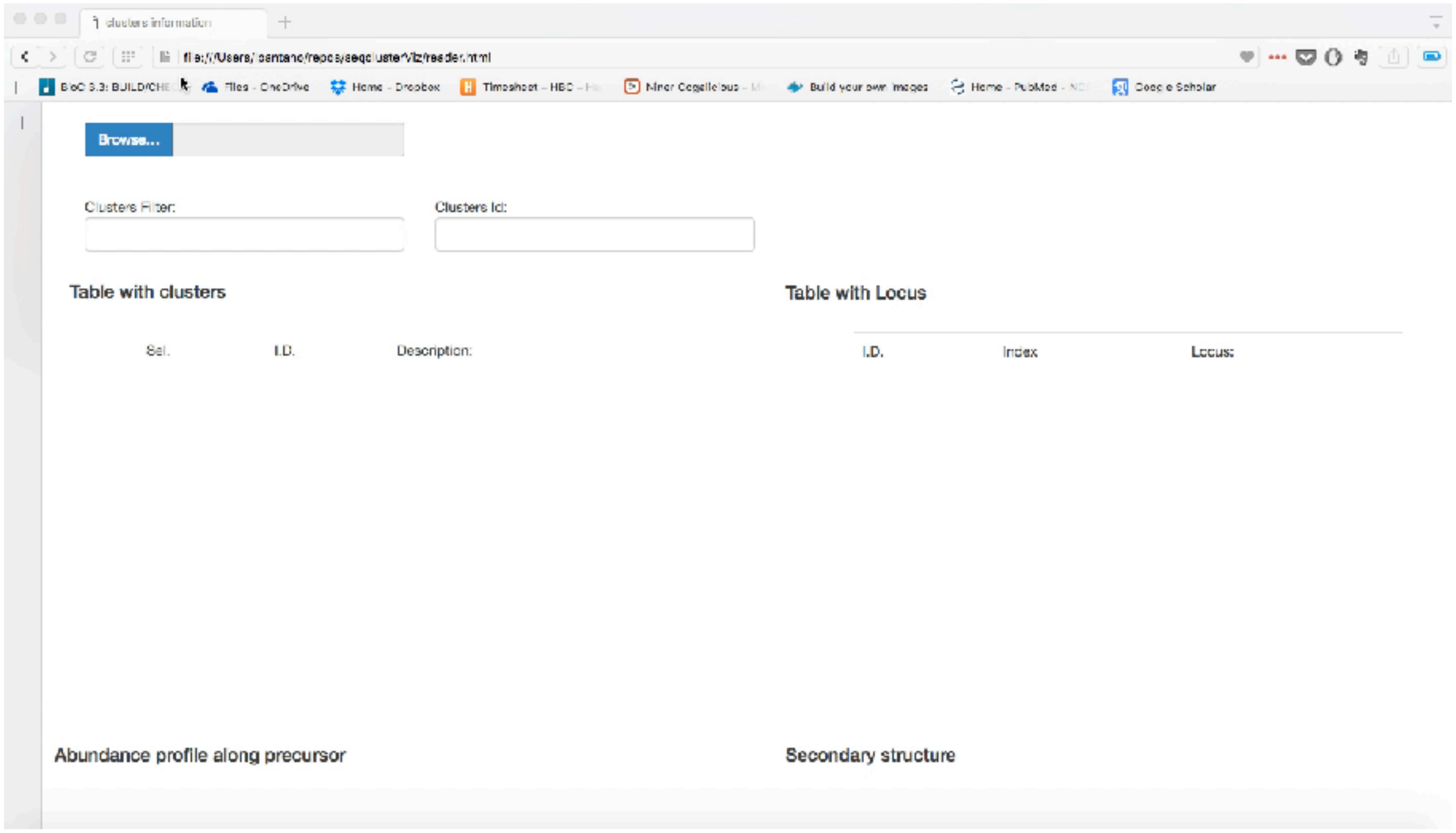


Step 2: cleaning



seqcluster.readthedocs.io

seqcluster visualization



https://github.com/lpantano/seqclusterViz

isomiRs: R package

- General Characterization of isomiRs.
  `biocLite("isomiRs")`

- Collapsing isomiRs in different ways

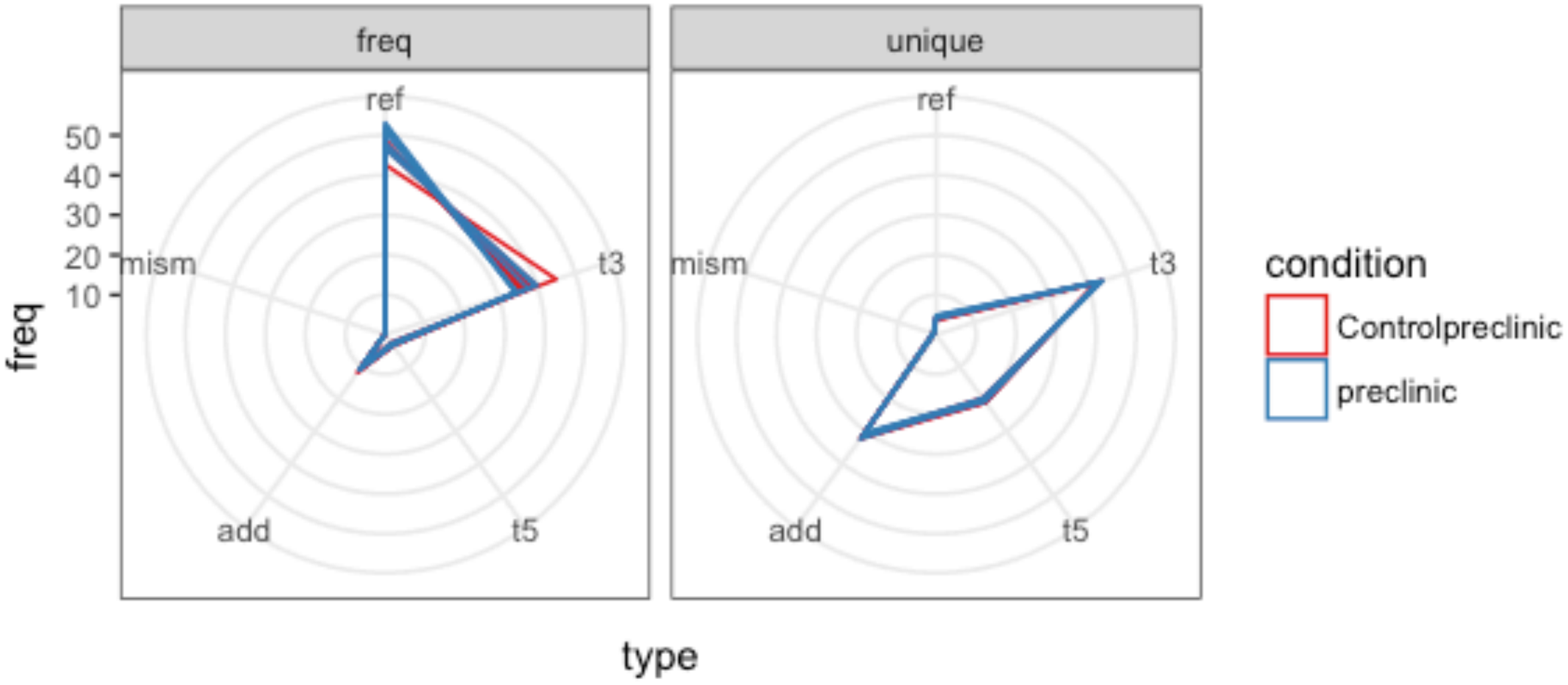
- Supervised clustering analysis to detect important miRNAs (PLS-DA)

- RNAseq and miRNA time serie data

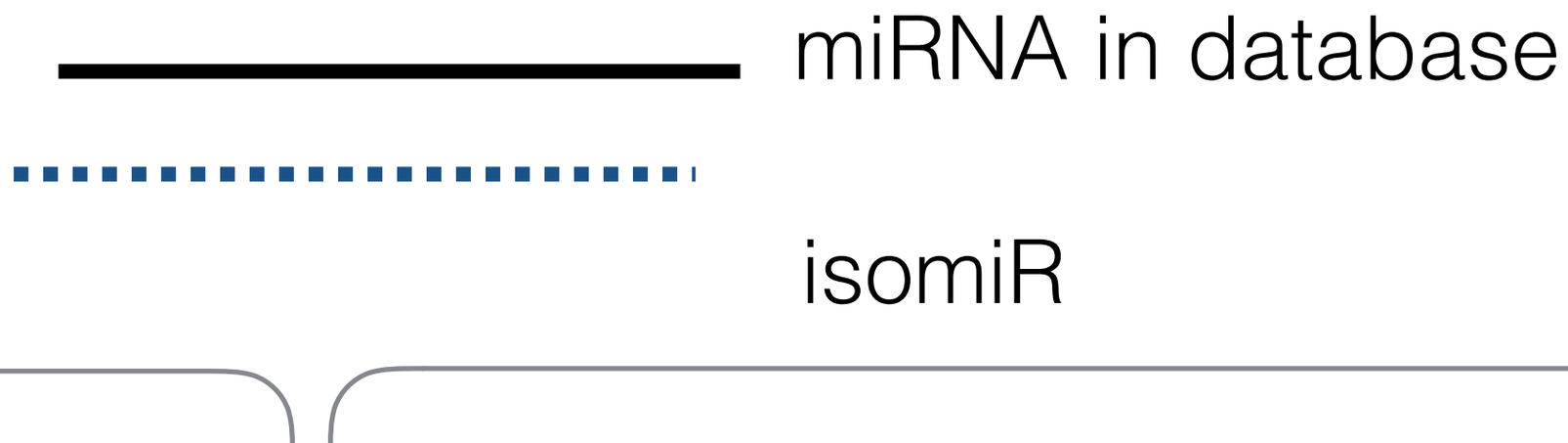
- Help with DE analysis

http://bioconductor.org/packages/release/bioc/html/isomiRs.html

isomiRs: R package



miRNA naming



UPPER CASE: addition

lower cases: deletion

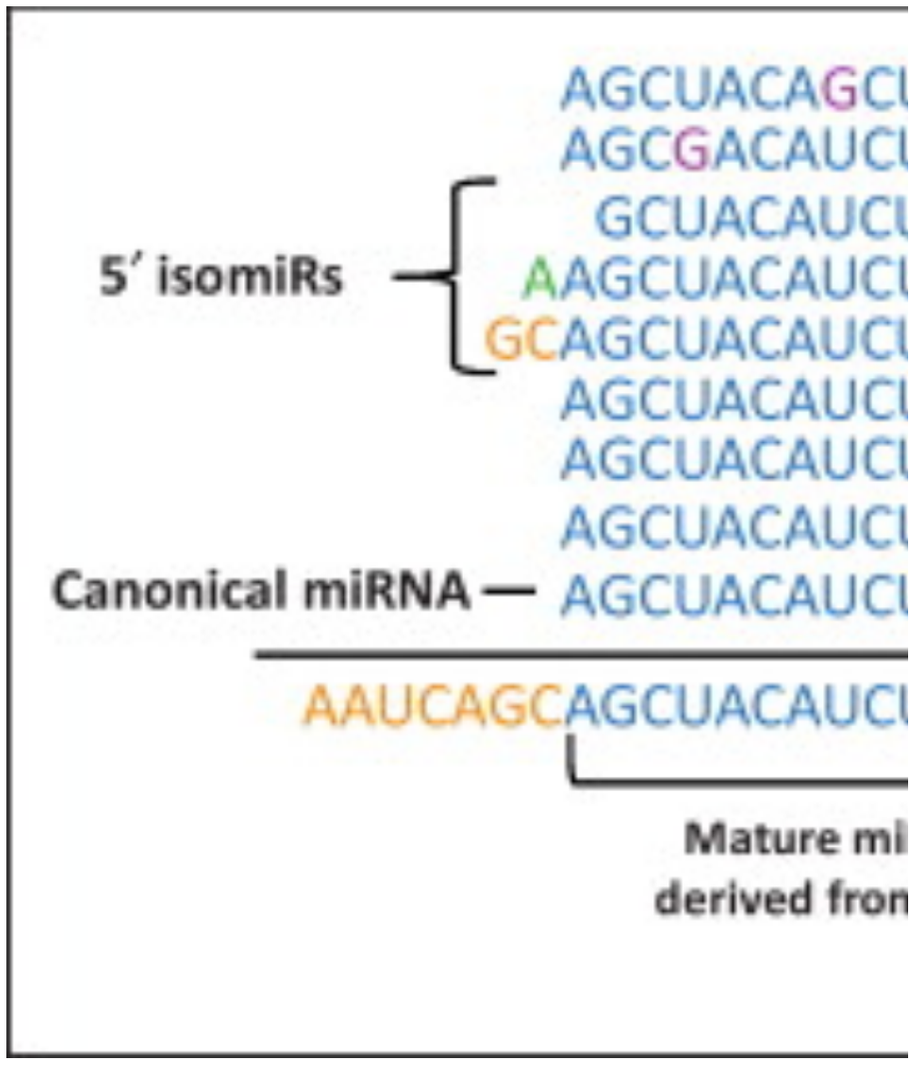
mismatch addition

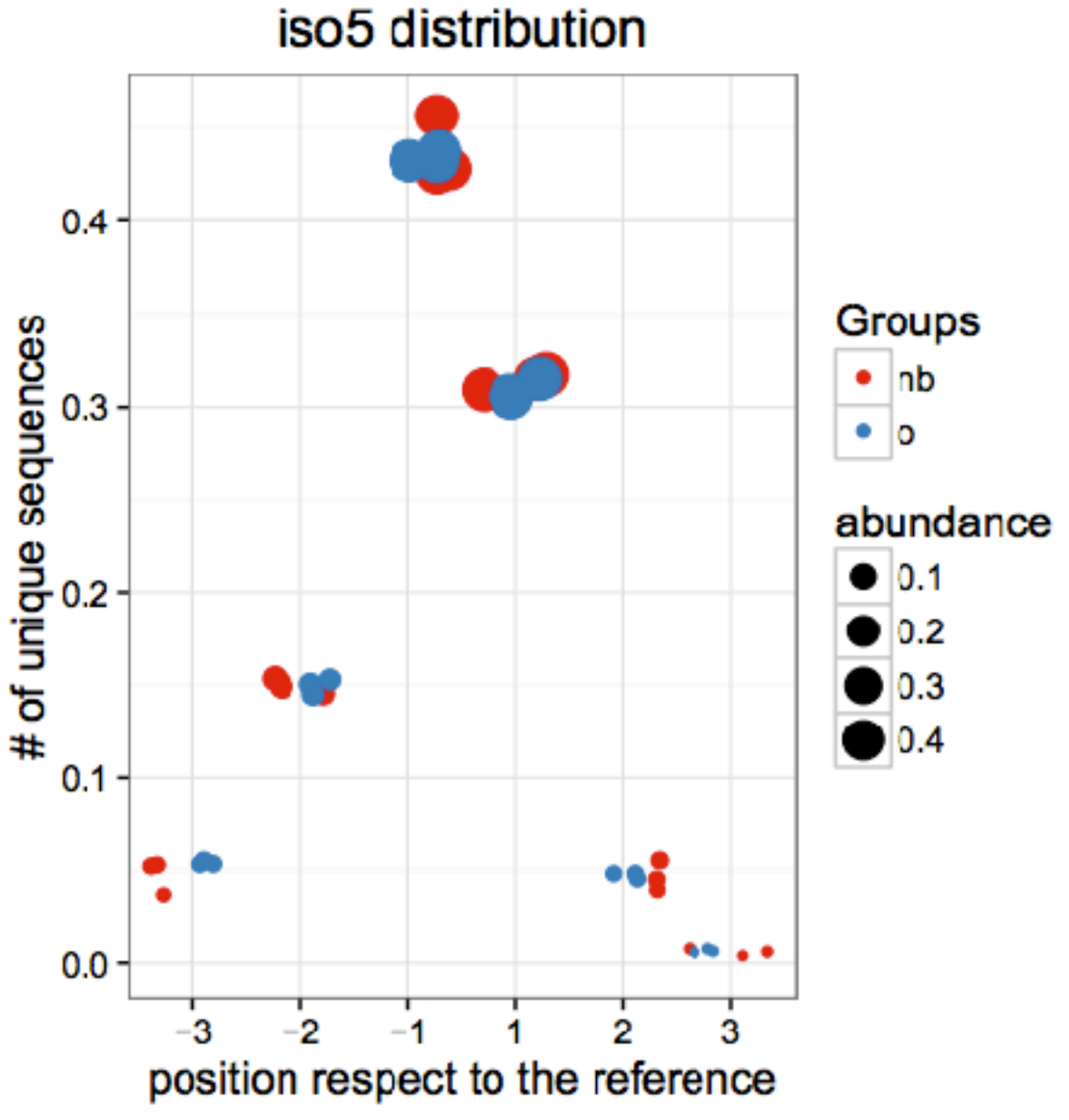
trimming 5’ trimming 3’

miRNA_name:mismatch:addition:t5:t3

hsa-let-7a-5p:0:0:GT:t

isomiRs: R package



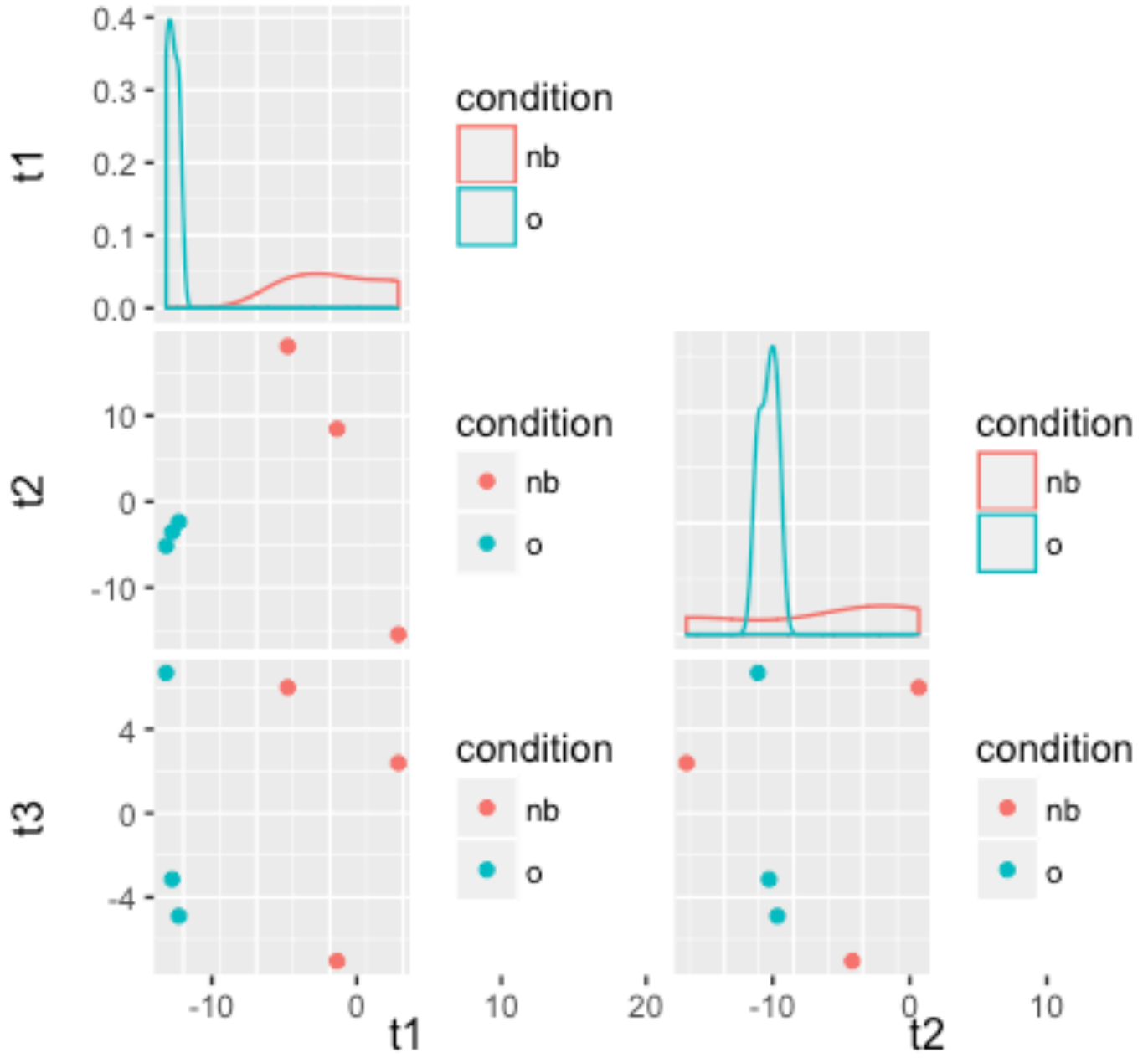


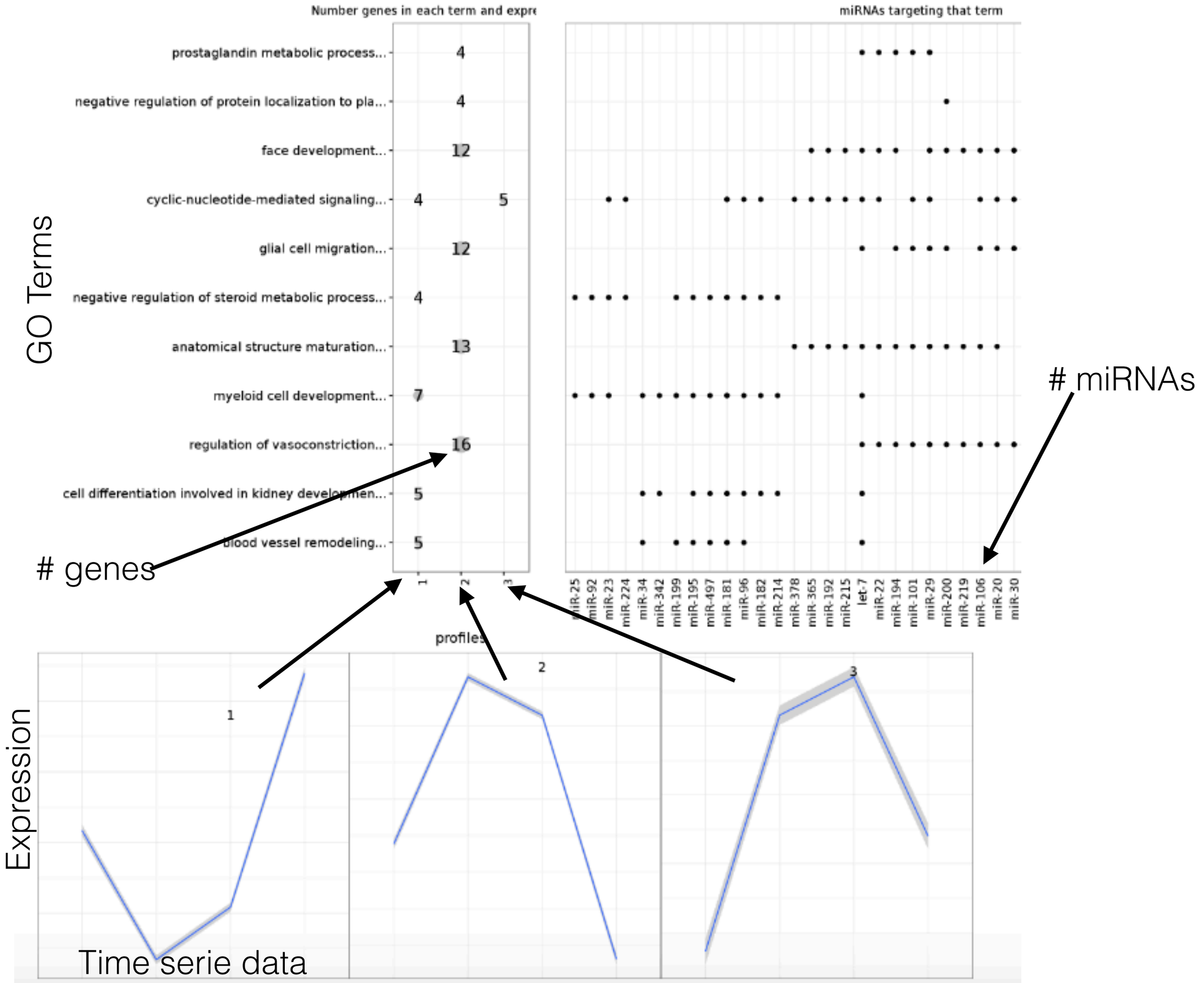
PLS-DA

Similar to PCA

It outputs the most important miRNAs

It is compatible with correlation of miRNAs




Number genes in each term and expr
miRNAs targeting that term
GO Terms
prostaglandin metabolic process...
negative regulation of protein localization to pla...
face development...
cyclic-nucleotide-mediated signaling...
glial cell migration...
negative regulation of steroid metabolic process...
anatomical structure maturation...
myeloid cell development...
regulation of vasoconstriction...
cell differentiation involved in kidney developmen...
blood vessel remodeling...
4
4
12
4
5
12
4
13
7
16
5
5
miRNAs
genes
profiles
miR-25
miR-92
miR-23
miR-224
miR-34
miR-342
miR-199
miR-195
miR-497
miR-181
miR-96
miR-182
miR-214
miR-378
miR-365
miR-192
miR-215
let-7
miR-22
miR-194
miR-101
miR-29
miR-200
miR-219
miR-106
miR-20
miR-30
Expression
Time serie data

MultiQC

Phil Ewels

ewels

Bioinformatician working with next generation sequencing data.

- Science for Life Laboratory
- Stockholm, Sweden
- phil.ewels@scilifelab.se
- http://phil.ewels.co.uk
- Joined on Nov 3, 2010

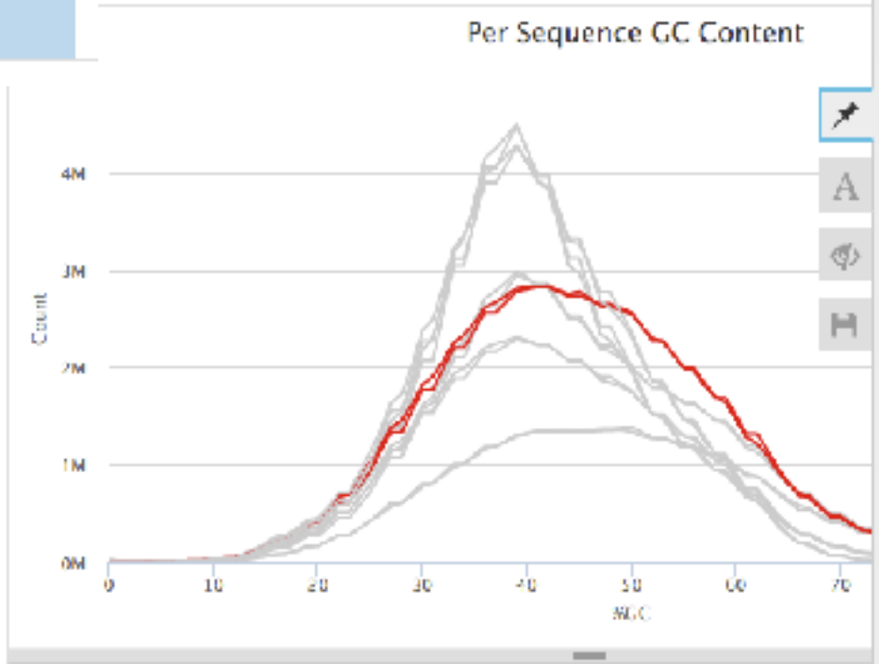
48	21	23
Followers	Starred	Following

STAR: % Uniquely mapped reads

signed	% Aligned	M Aligne
0.9	81.1%	
1.5	79.1%	
1.9	70.2%	
0.9	63.2%	
0.7	61.8%	
0.6	50.6%	

equence GC Content 6 5 1

ge GC content of reads. Normal random library typically have a roughly normal d
C help.



MultiQC Toolbox

Highlight Samples

5:0

Regex mode off

5:14

Evaluation of quantitative miRNA expression platforms in the microRNA quality control (miRQC) study

Pieter Mestdagh, Nicole Hartmann, Lukas Baeriswyl, Ditte Andreasen, Nathalie Bernard, Caifu Chen, David Cheo, Petula D'Andrade, Mike DeMayo, Lucas Dennis, Stefaan Derveaux, Yun Feng, Stephanie Fulmer-Smentek, Bernhard Gerstmayer, Julia Gouffon, Chris Grimley, Eric Lader, Kathy Y Lee, Shujun Luo, Peter Mouritzen, Aishwarya Narayanan, Sunali Patel, Sabine Peiffer, Silvia Rüberg, Gary Schroth + *et al.*

Affiliations | **Contributions** | **Corresponding author**

Nature Methods **11**, 809–815 (2014) | doi:10.1038/nmeth.3014
Received 27 February 2014 | Accepted 22 May 2014 | Published online 29 June 2014
| Corrected online **30 July 2014**

Analyze Public Dataset

Samples (20)
Less...

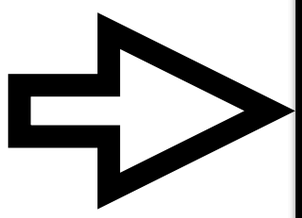
GSM1207643	miRQC A
GSM1207644	miRQC A repeat
GSM1207645	miRQC B
GSM1207646	miRQC B repeat
GSM1207647	miRQC C
GSM1207648	miRQC C repeat
GSM1207649	miRQC D
GSM1207650	miRQC D repeat

```
samplenames,description,group
GSM1207643,miRQCA,A
GSM1207644,miRQCArepeat,A
GSM1207645,miRQCB,B
GSM1207646,miRQCBrepeat,B
GSM1207647,miRQCC,B
GSM1207648,miRQCCrepeat,B
GSM1207649,miRQCD,B
GSM1207650,miRQCDrepeat,B
```

```
lp113@loge:~$ bcbio_prepare_samples.py --csv test.csv --out fastq
```

test-merged.csv

fastq/*fastq.gz



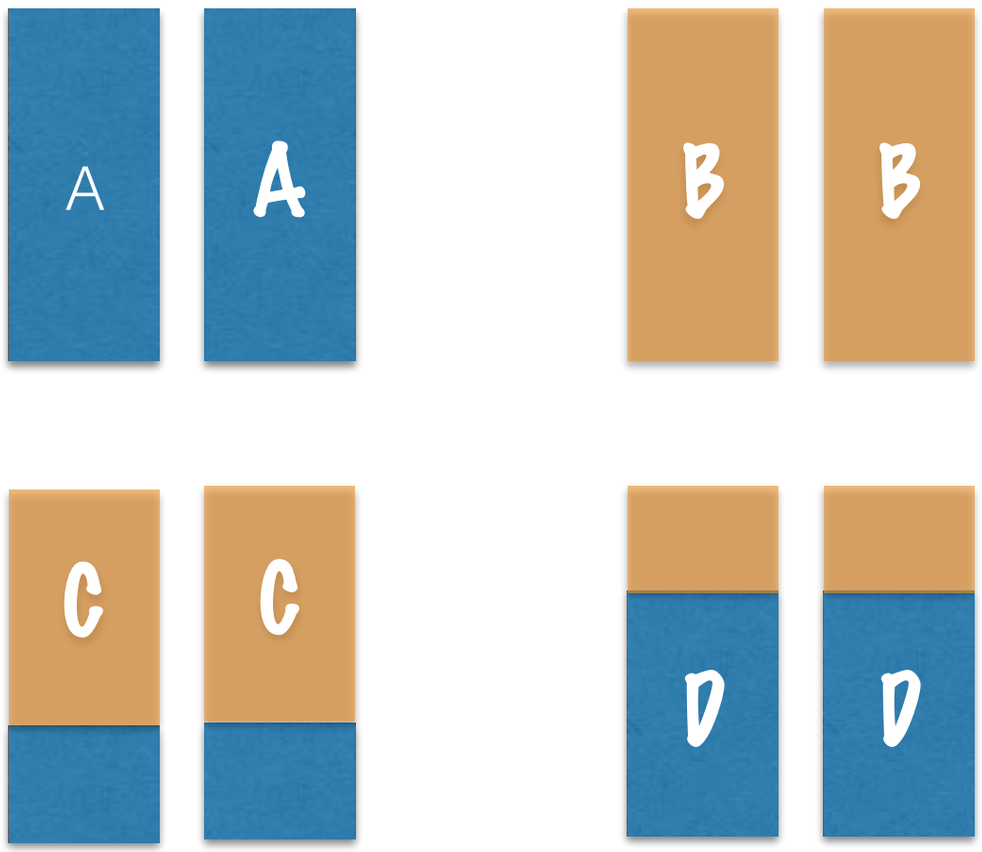
```
bcbio_nextgen.py -w template ….

bcbio_nextgen.py config.yaml ….
```

Support of remote files

```
details:
- algorithm:
    adapters:
    - AGATCGGAAGAG
    aligner: star
    expression_caller:
    - trna
    - seqcluster
    species: mmu
    spikein_fasta: /home/lp113/scratch/charest_egfr_srna/spikeins/all.fa
  analysis: smallRNA-seq
  description: sampleone
  files:
  - ftp://ftp.sra.ebi.ac.uk/vol1/ERA169/ERA169754/fastq/NA07000.1.MI_120104_3_1.fastq.gz
  genome_build: mm10
  metadata: {}
fc_date: '2017-06-21'
fc_name: sample
```

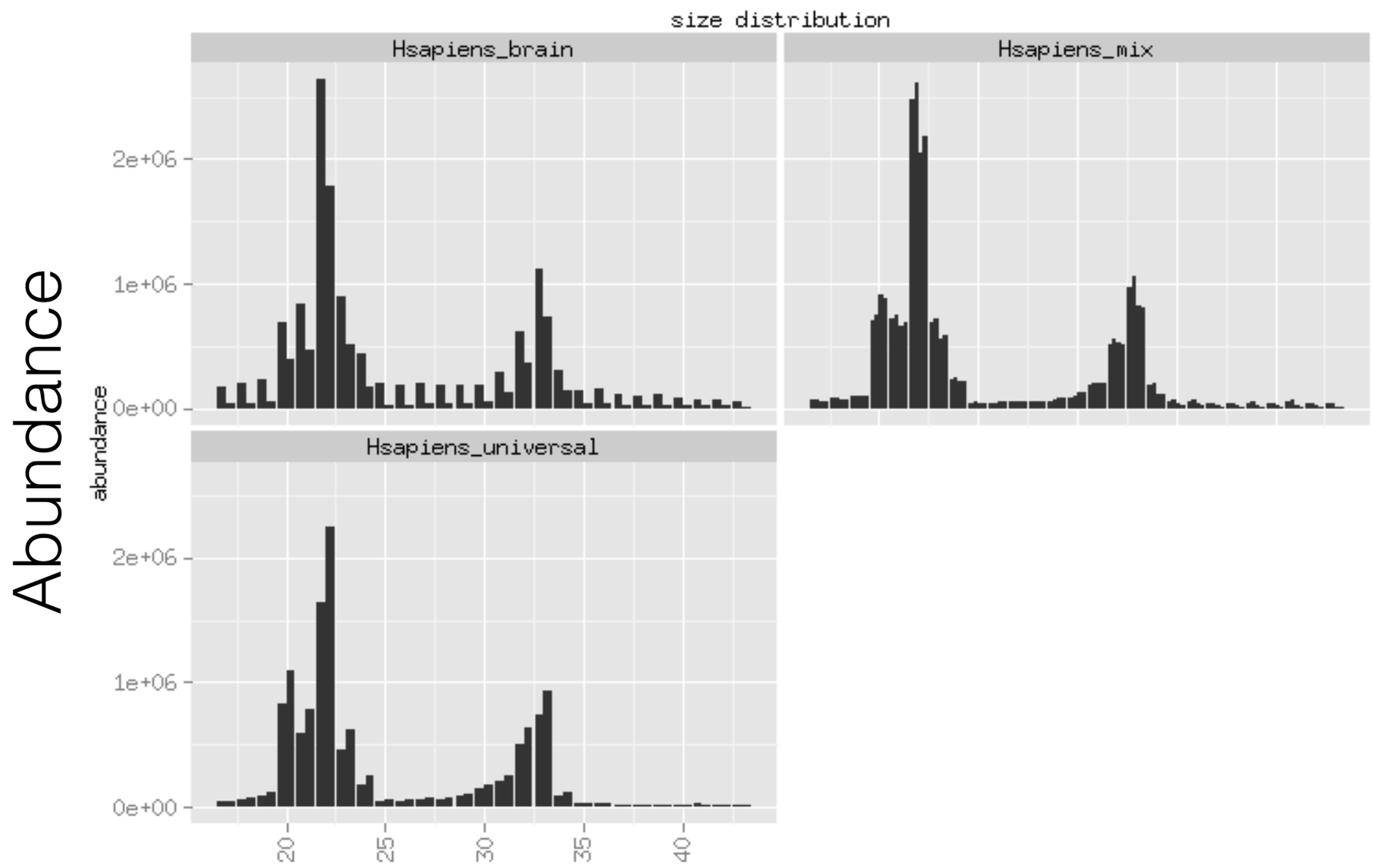
Quality Control samples



For each molecule:

* If A > B then A > D > C > B
* If B > A then A < D < C < B

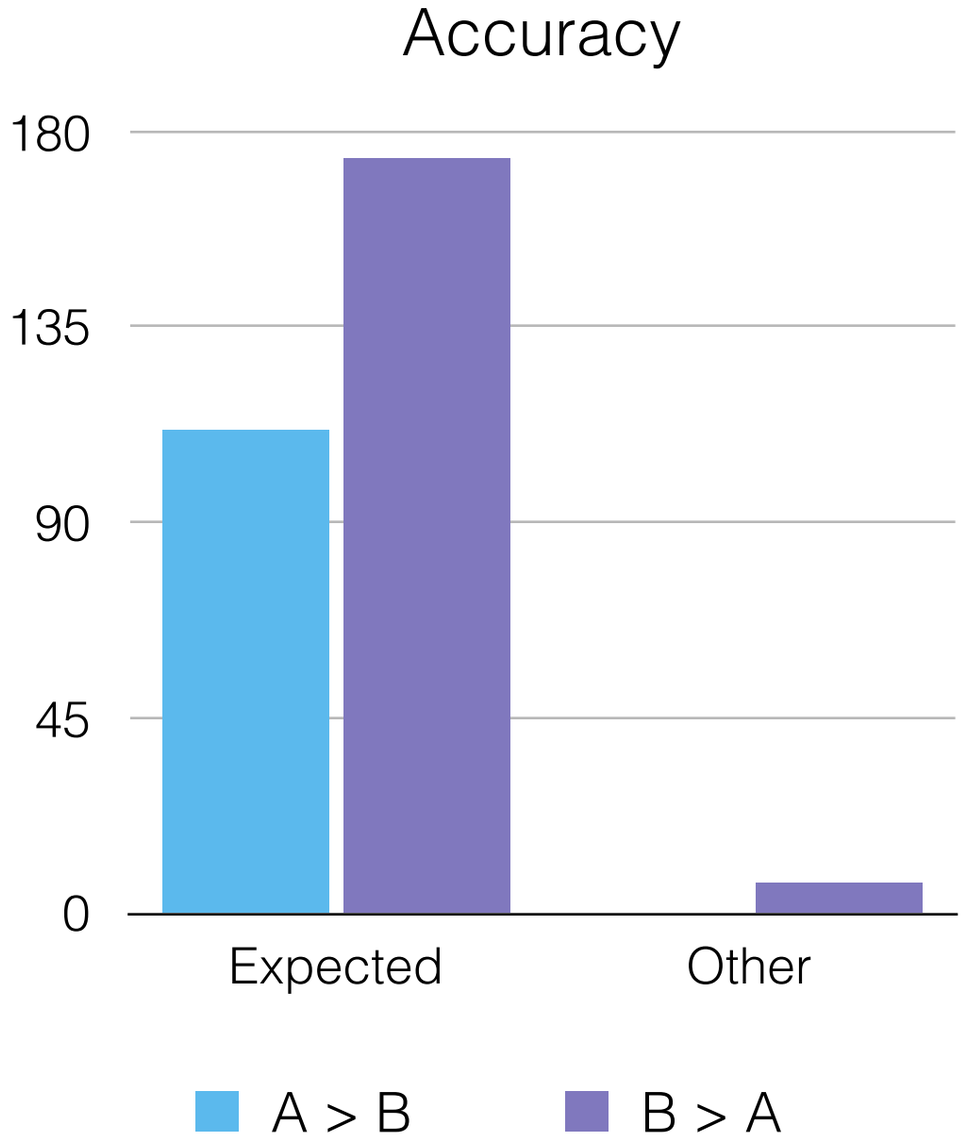
Good samples



miRNA quantification

miRNAs > 5 counts in average

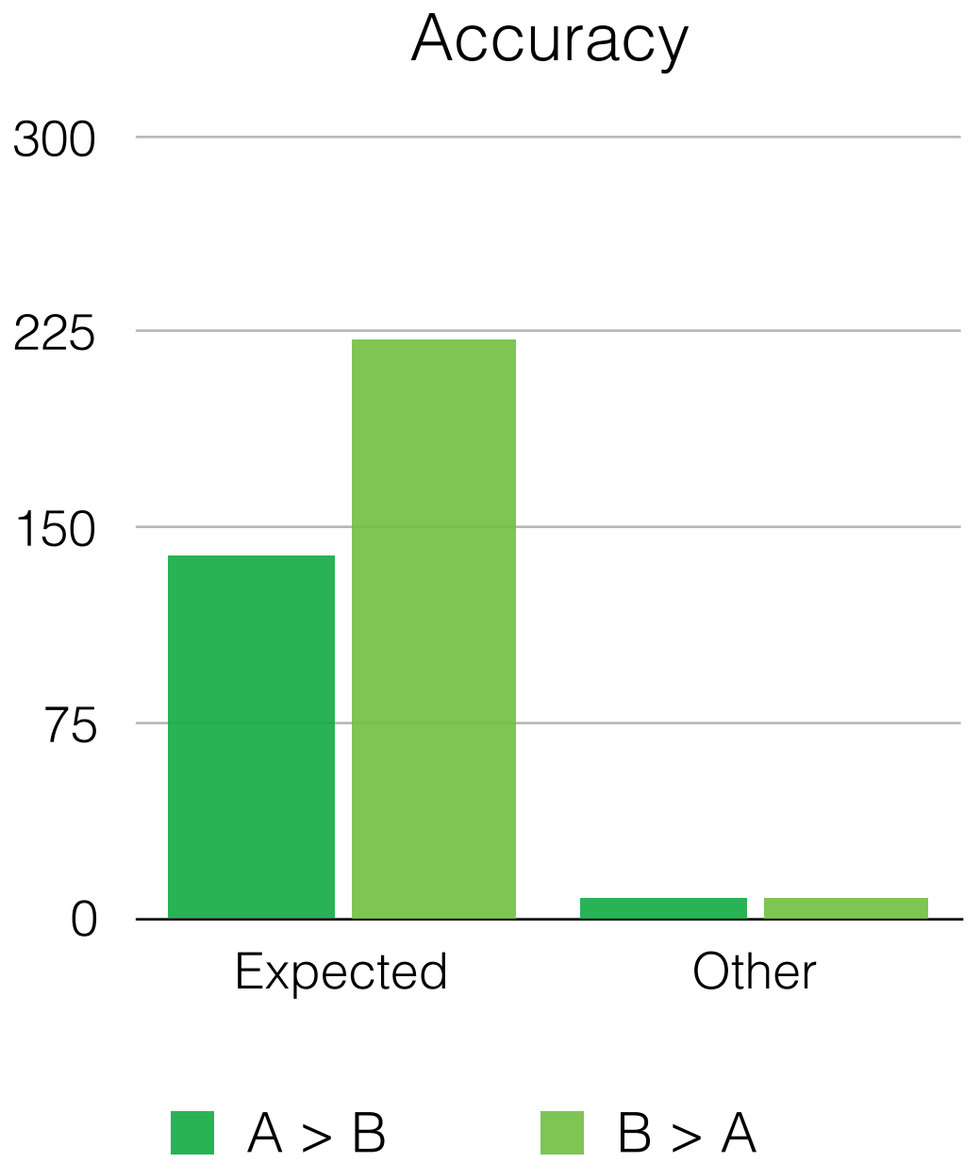
upper quantile normalization



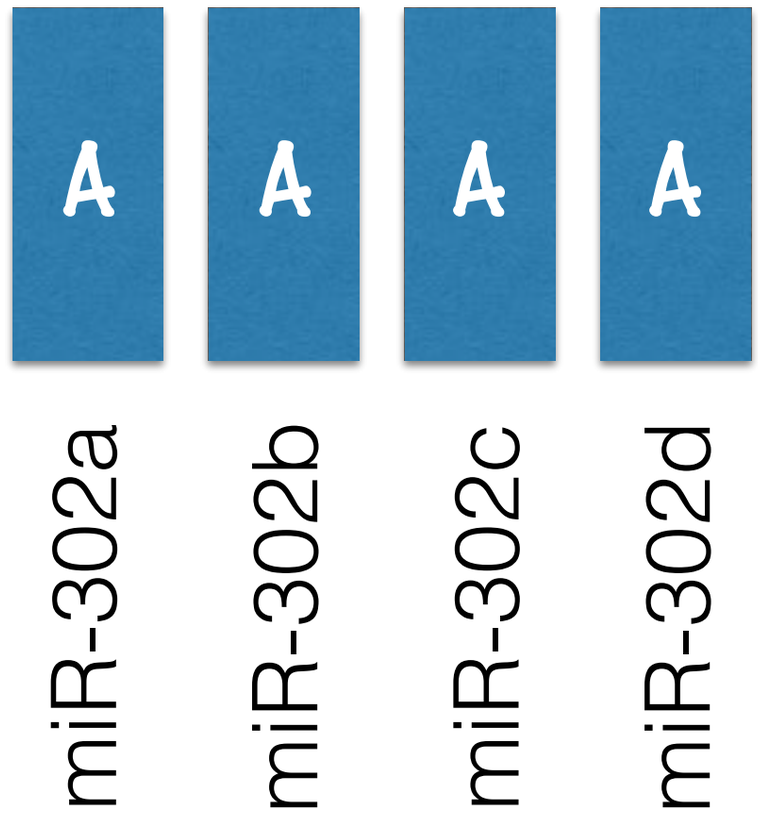
clusters quantification

expression > 5 counts in average

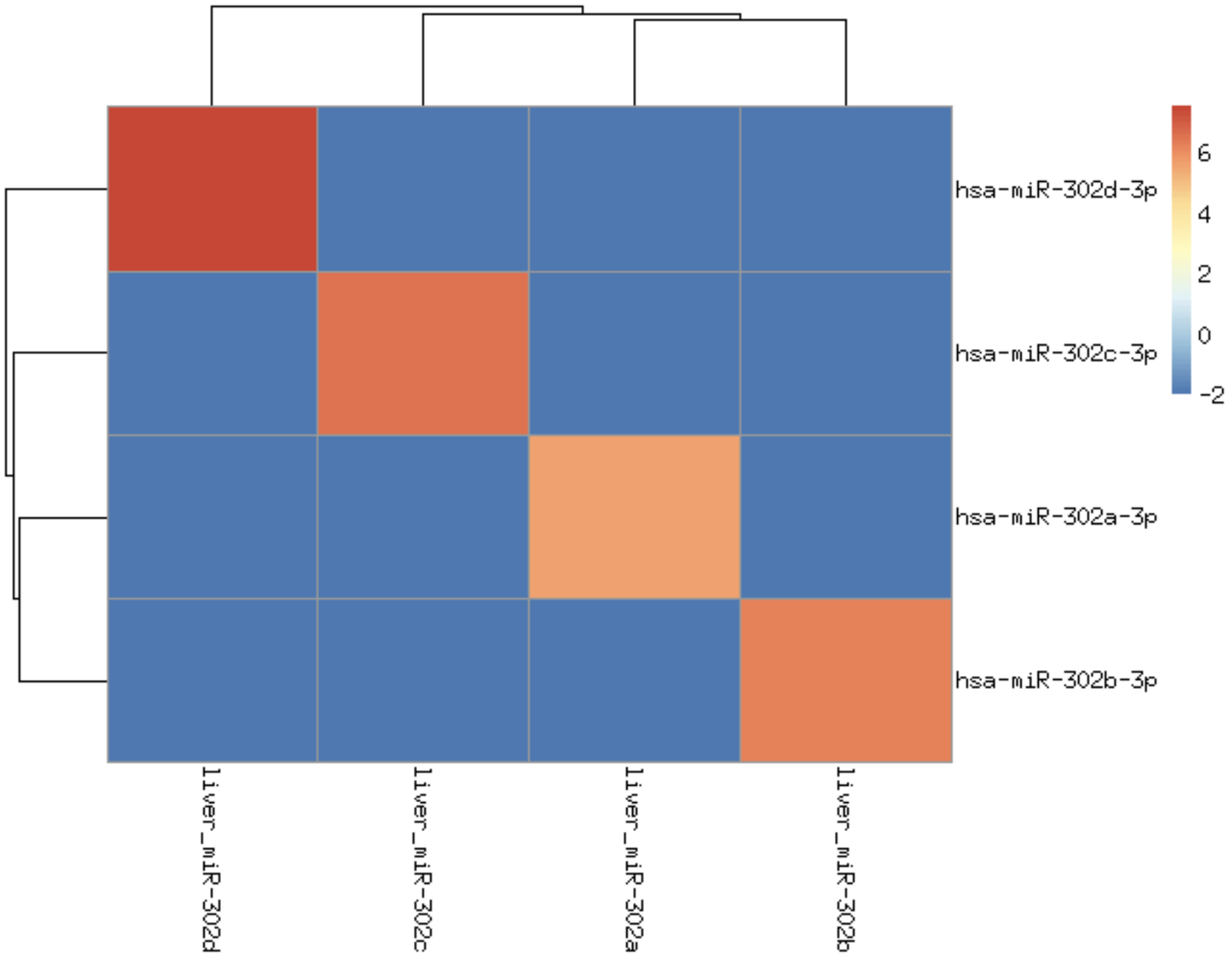
upper quantile normalization



Positive controls



Specificity



bcbio template

```
upload:
  dir: ../final
details:
  - analysis: smallRNA-seq
    algorithm:
      aligner: star
      # change adapter according project
      adapters: ["TGGAATTCTCGGGTGC"]
      expression_caller: [trna, seqcluster, mirdeep2]
      species: hsa
    genome_build: hg19
```

https://github.com/chapmanb/bcbio-nextgen/blob/master/config/templates/illumina-srnaseq.yaml

Resources

	Time (h)
organize	0:01
adapter	0:27
alignment	0:26
annotation	3:43
cluster + mirdeep2	4:15
qc	0:04

The time for 8 samples with 6 millions reads each was 8 hours and 57 minutes.

open project for small RNA annotation and analysis

http://mirtop.github.io

standard formats

naming rules

best-practices

miRNAs, tRNAs ...

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Upcoming (1) | Past | Draft (1) | Calendar

Rshiny app to browse RNAseq data

Harvard University: Countway Library
10 Shattuck St, Boston, Ma (map)

Hi, Join us in the last meeting of the year to create an easy app to browse RNAseq data. The goal is to have a small working code to visualize the expression of selected...

Learn more

Hosted by: Lorena Pantano (Organizer)

Tue Dec 13
5:45 PM

I'm going

16 going
4 spots left
0 comments

thanks

* Harvard T.H. Chan School of Public Health

* Research Computing at Harvard Medical School: Chris Botka, Director of Research Computing and all the people in the team.

* Special thanks to the authors of those papers to make data available.