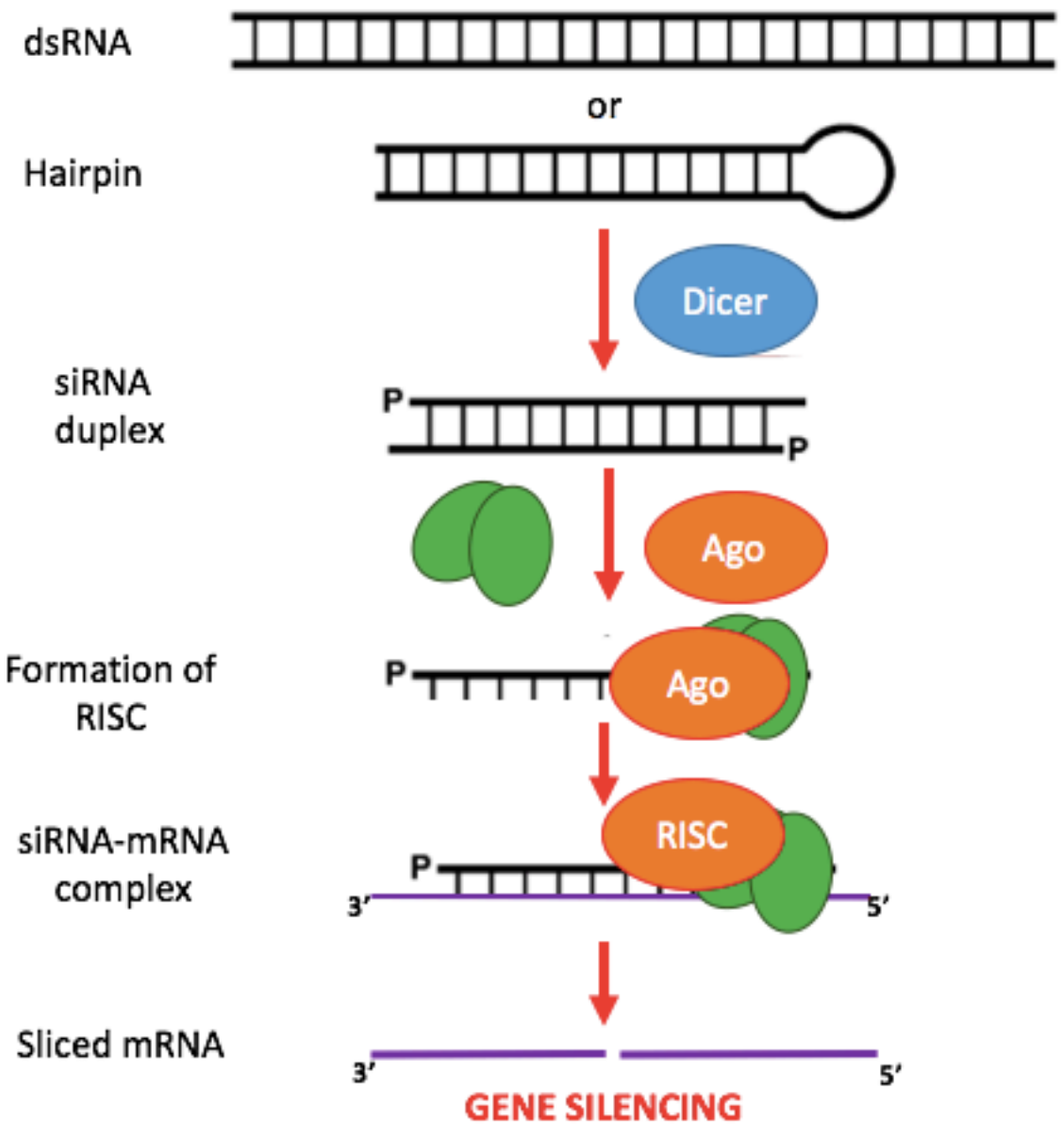
Characterization of the small RNA transcriptome

Lorena Pantano
@lopantano lpantano@hsph.harvard.edu
Harvard TH Chan School of Public Health

2018-9-26

small interference RNA

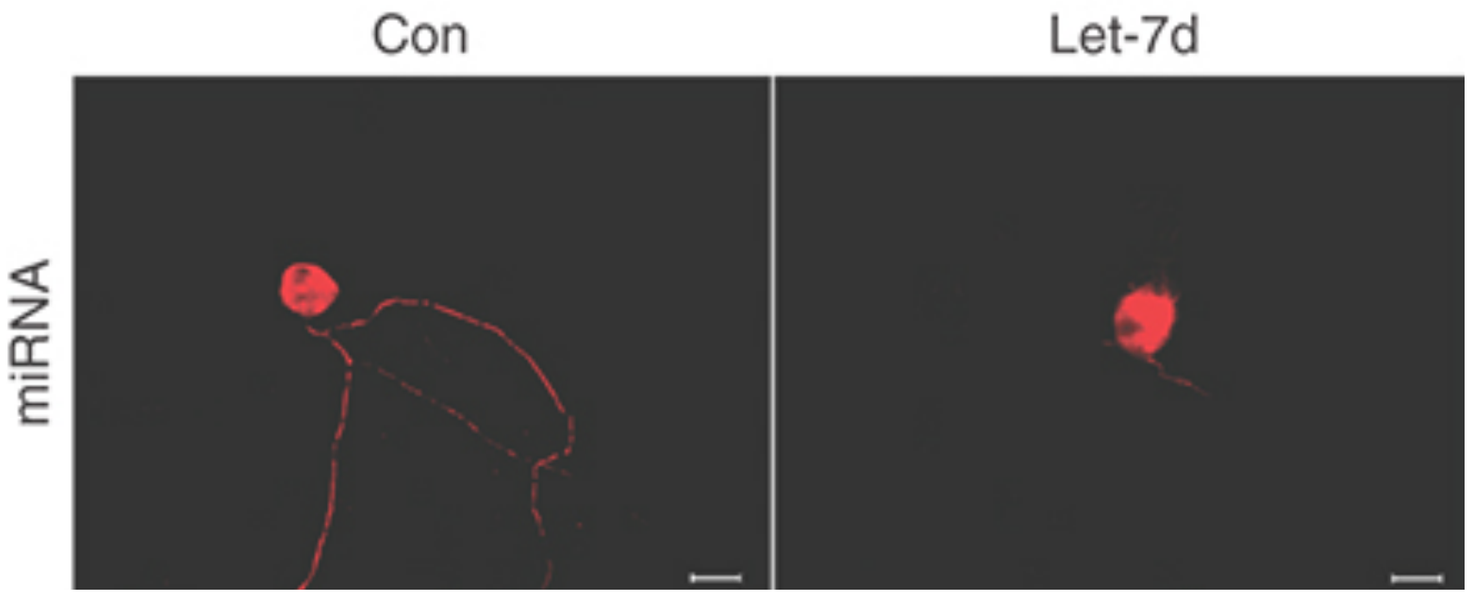


https://en.wikipedia.org/wiki/Small_interfering_RNA

- 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

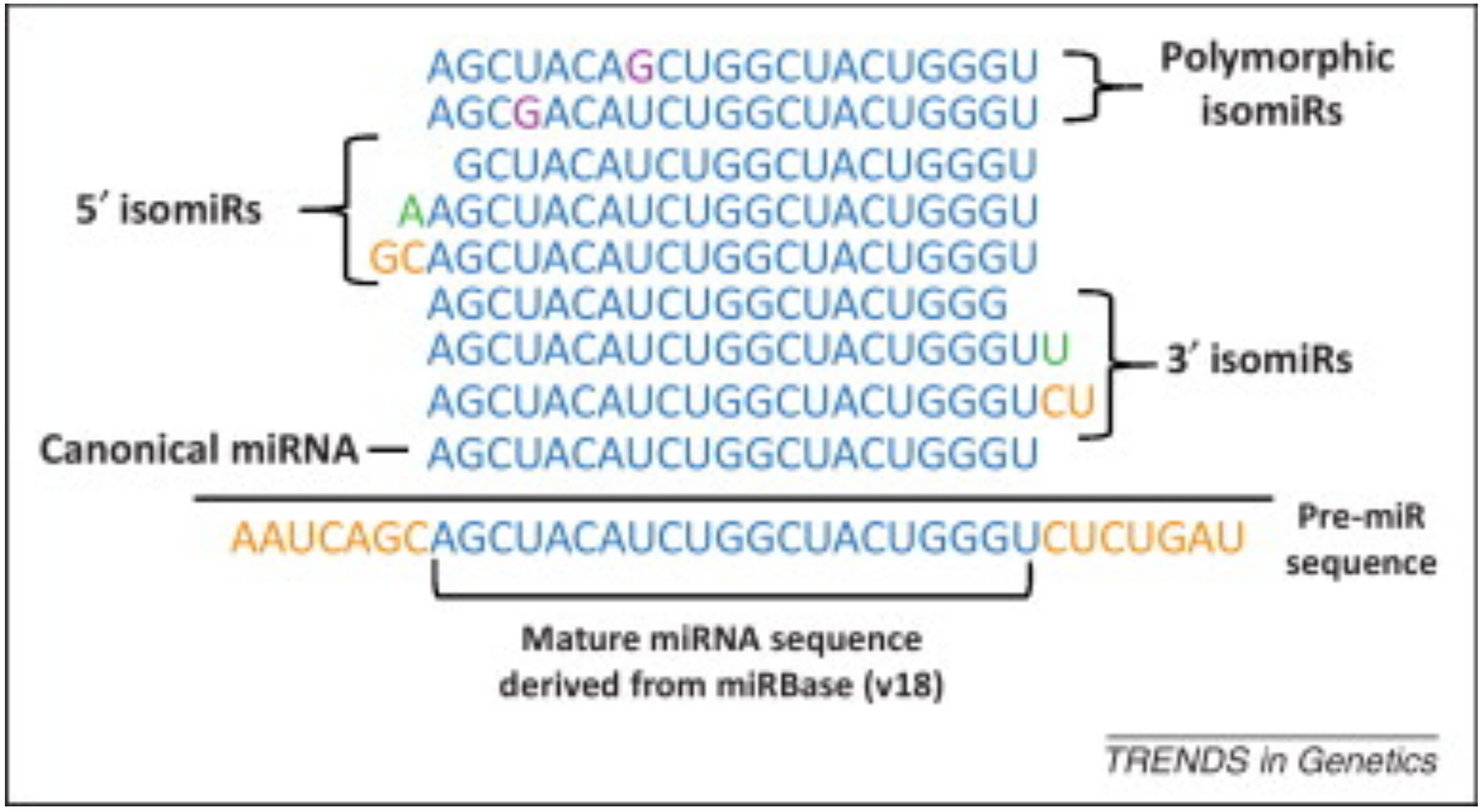
isomiRs

```
     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 186

<< First < Prev Page 1 of 10 Next > Last >>

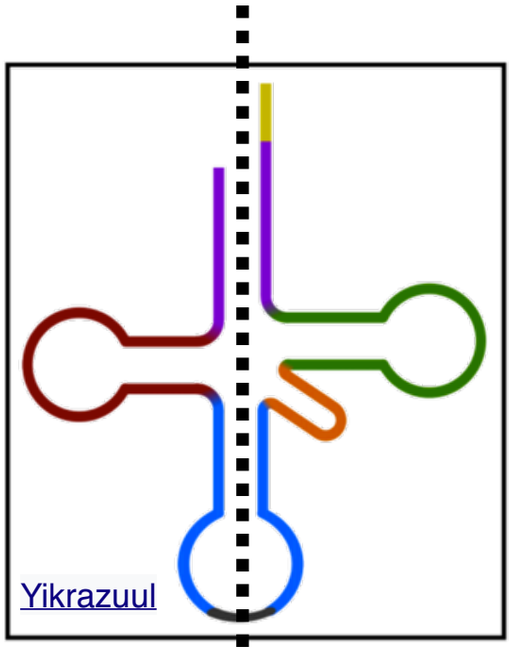
1. NGS analysis of total small non coding RNAs from low input RNA from dried blood sampling.
Pirritano M, Fehlmann T, Laufer T, Ludwig N, Gasparoni G, Li Y, Meese E, Keller A, Simon M.
Anal Chem. 2018 Sep 10. doi: 10.1021/acs.analchem.8b03557. [Epub ahead of print]
PMID: 30198258
Similar articles

2. miRge 2.0 for comprehensive analysis of microRNA sequencing data.
Lu Y, Baras AS, Halushka MK.
BMC Bioinformatics. 2018 Jul 23;19(1):275. doi: 10.1186/s12859-018-2287-y.
PMID: 30153801 **Free PMC Article**
Similar articles

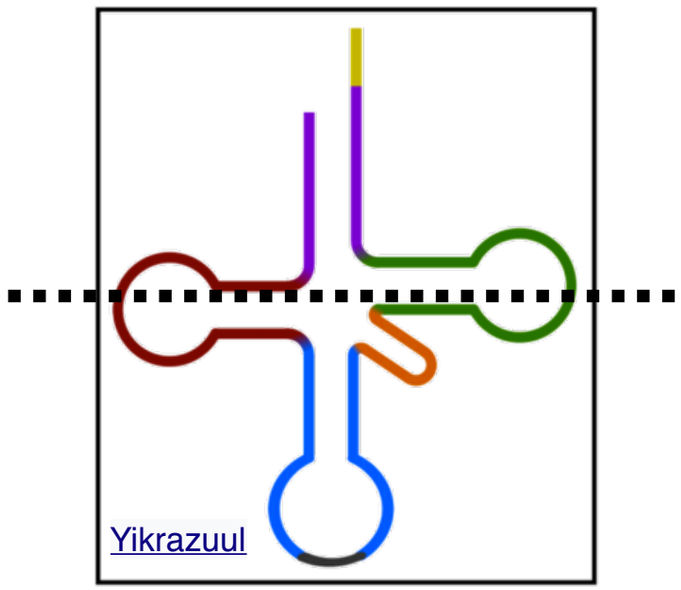
3. Analysis of the expression, function, and evolution of miR-27 isoforms and their responses in metabolic processes.
Ma M, Yin Z, Zhong H, Liang T, Guo L.
Genomics. 2018 Aug 23. pii: S0888-7543(18)30297-0. doi: 10.1016/j.ygeno.2018.08.004. [Epub ahead of print]
PMID: 30145283

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)



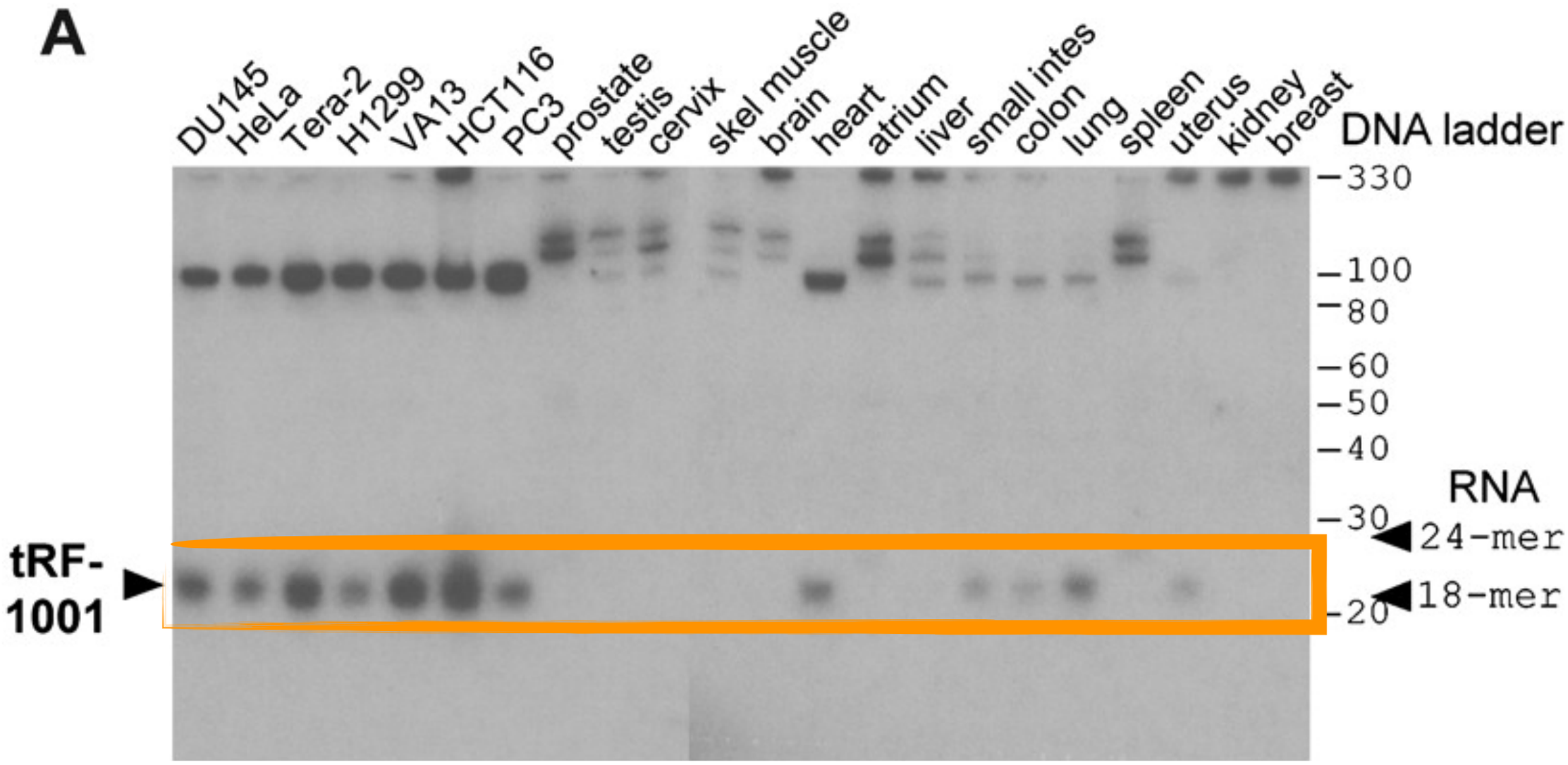
5’-tRNAs (18-22) 3’-tRNAs (18-22)

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

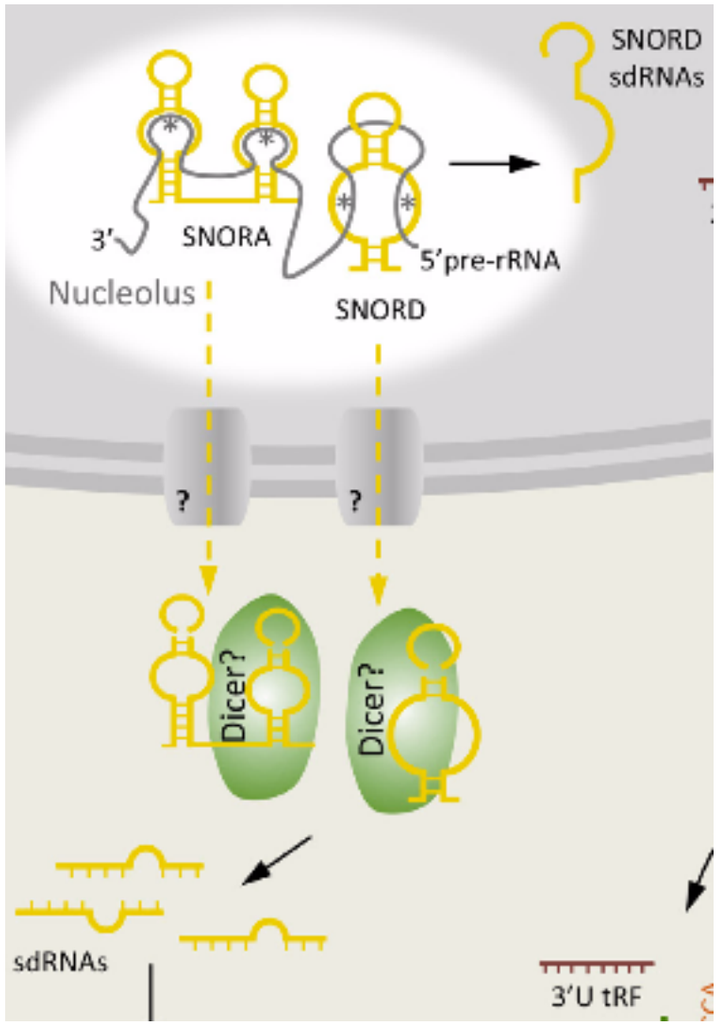
sncRNA fragments

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)

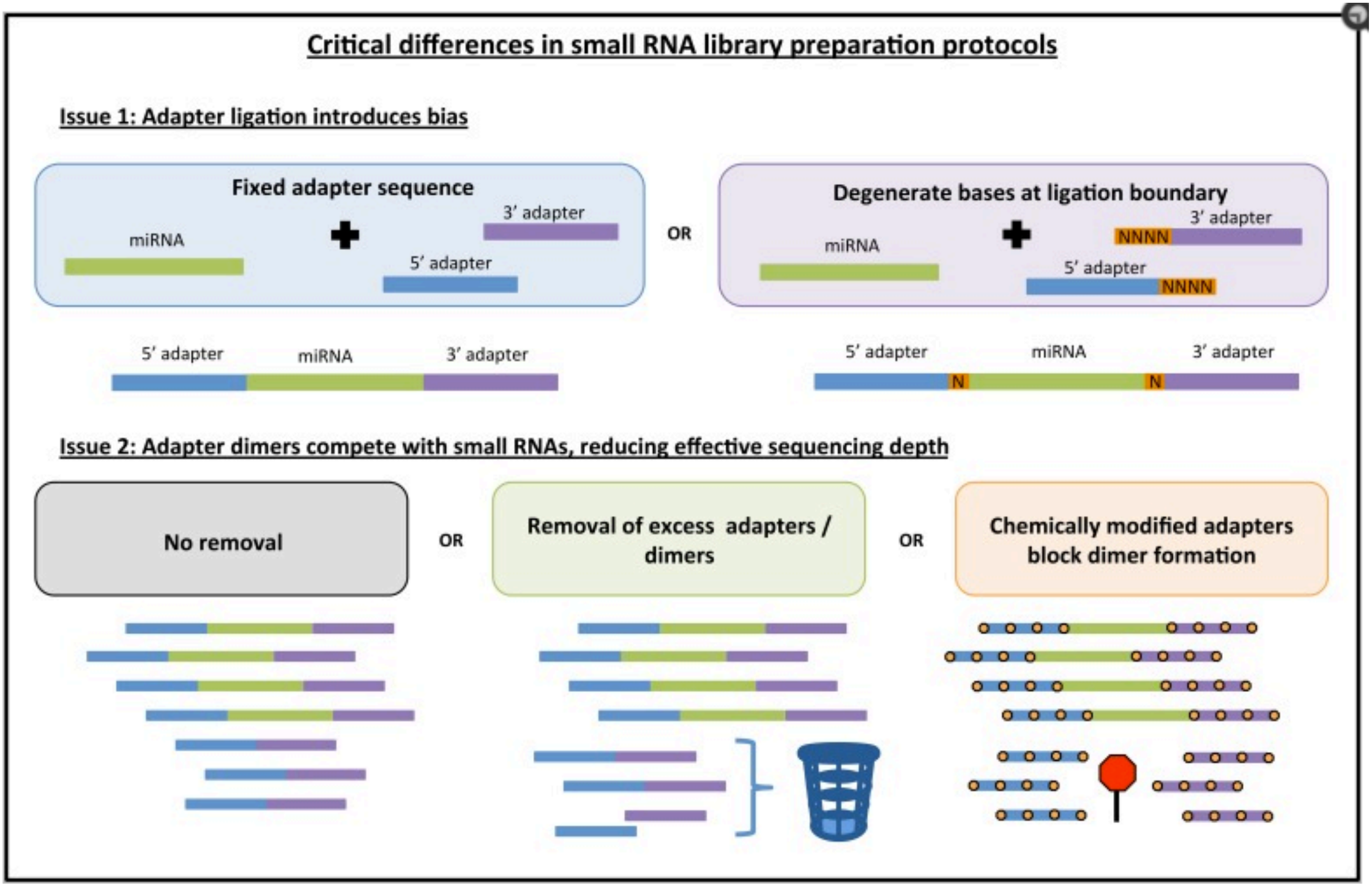
SNORD88C-sdRNAs can regulates **alternative splicing** of fibroblast growth factor receptor 3. (FGFR3)

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



snoRNA-derived RNAs (sdRNAs)

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

TRILINK: Modified Adapters

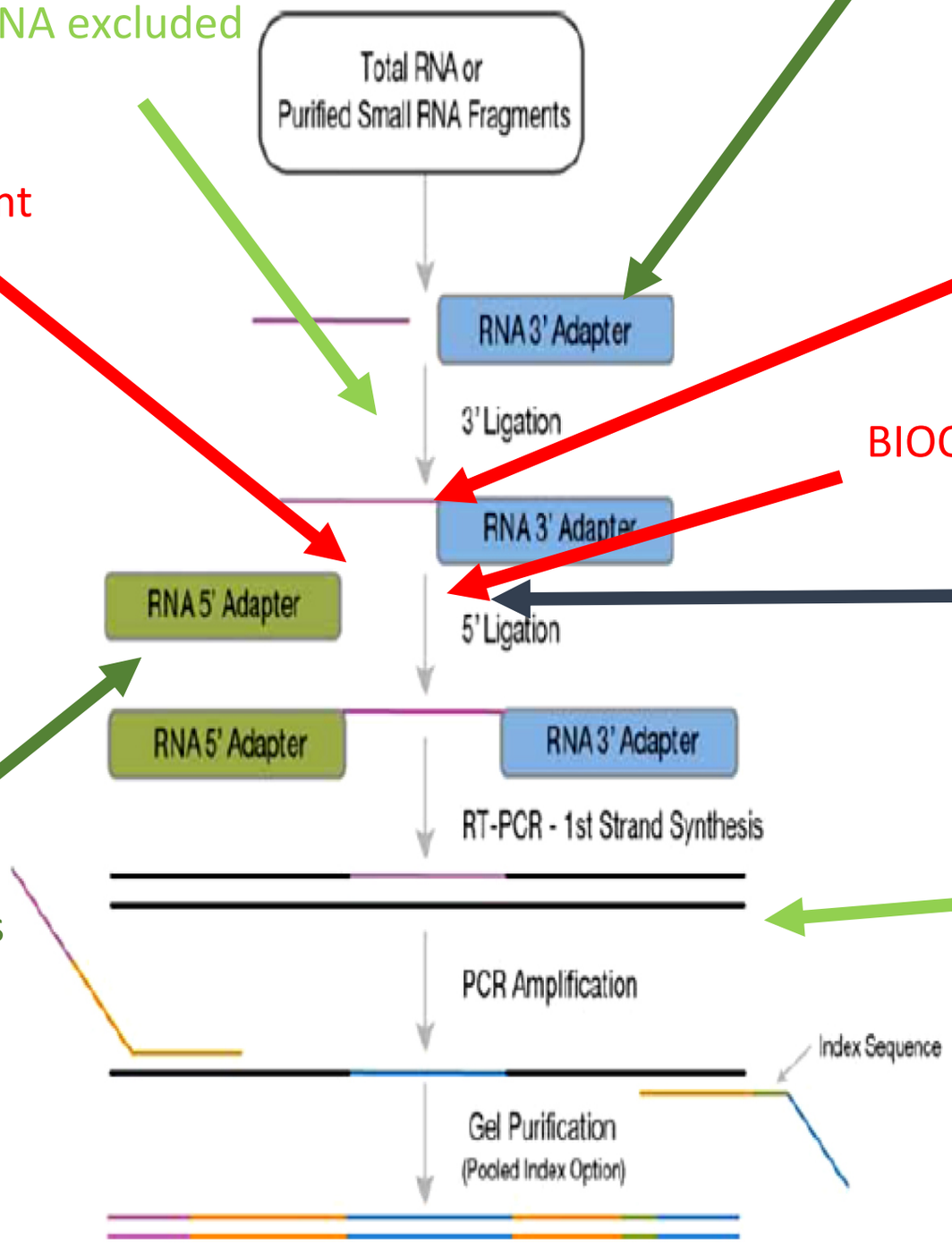
QIAGEN:
Large RNA excluded

BIOO: N4 at ligation point

BIOO: N4 at ligation point

BIOO: Adapter bocking

NEB: Add primer to block primer-dimer



TRILINK:
Modified Adapters

QIAGEN: UMI

Systematic comparison of small RNA library preparation protocols for next-generation sequencing

Cloelia Dard-Dascot[1], Delphine Naquin[1], Yves d'Aubenton-Carafa[1], Karine Alix[2], Claude Thermes[1] and Erwin van Dijk[1*]

Comprehensive multi-center assessment of small RNA-seq methods for quantitative miRNA profiling

Maria D Giraldez, Ryan M Spengler [...] Muneesh Tewari

Nature Biotechnology **36**, 746–757 (2018) | Download Citation

Caveats

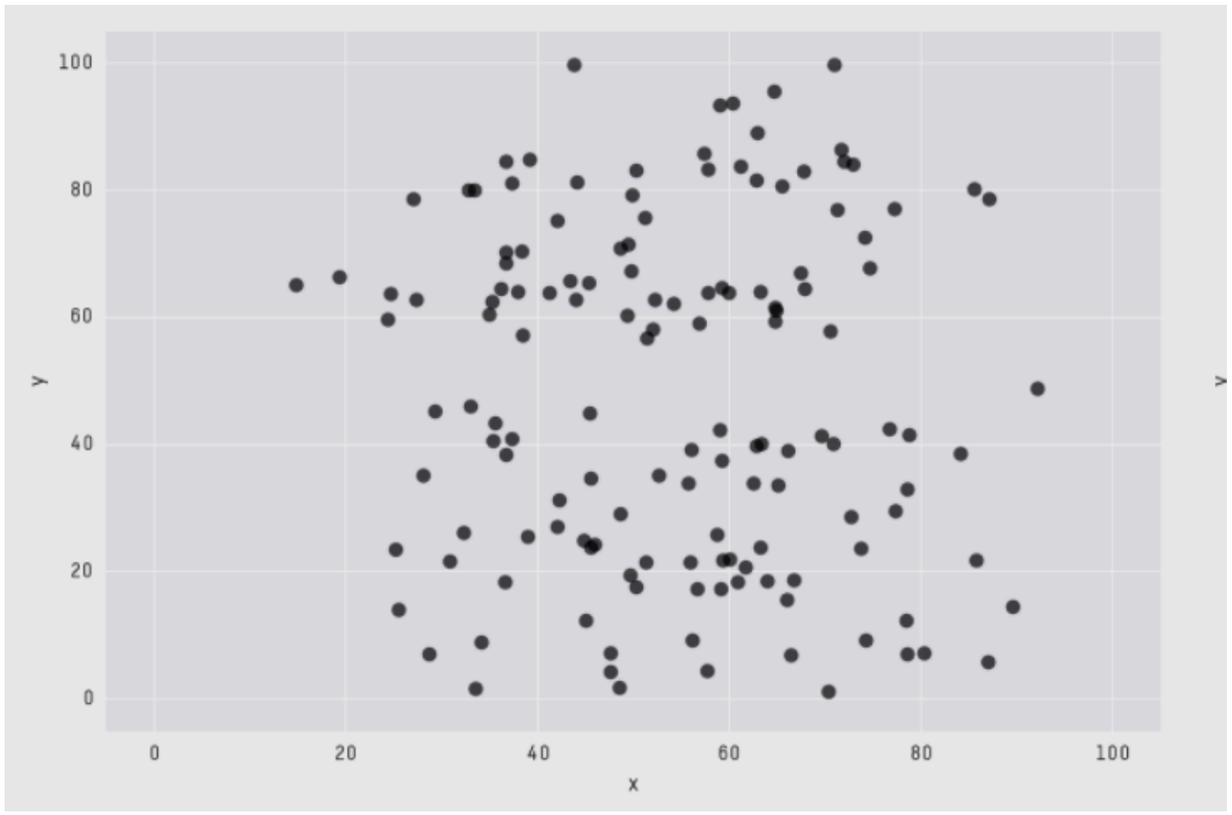
- TrueSeq Illumina: ligation bias
- NEBNext: ligation bias (potentially less)
- 4N + (NextFlex): generation of random sequences. We lose the accuracy to detect isomiRs. Partial ligation bias.
- 2Om small RNA like piRNA, or plant miRNA needs protocol modifications to be detected.

Visualization

“Visualization gives you answers to questions you didn’t know you had.” – Ben Schneiderman

“There is no such thing as information overload. There is only bad design.” – Edward Tufte

Same Stats, Different graphs



X Mean: 54.26

Y Mean: 47.83

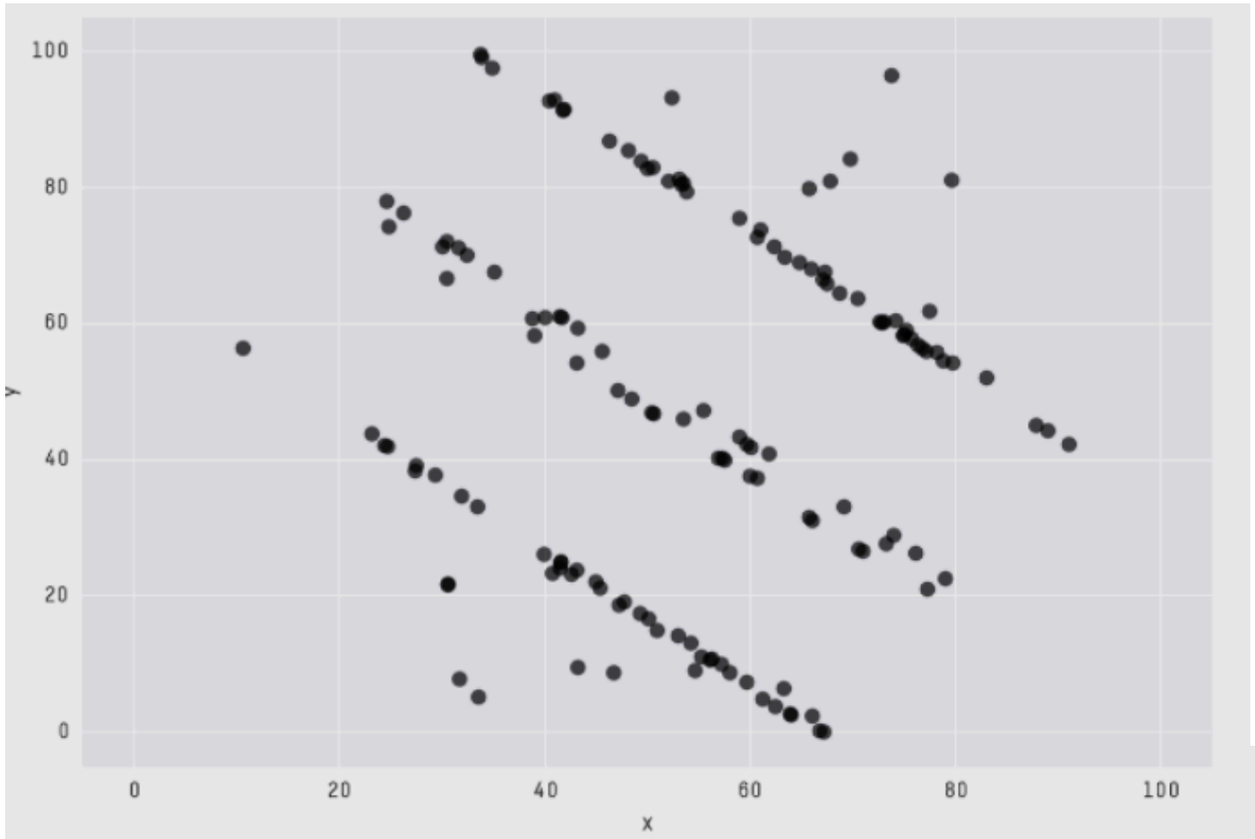
X SD : 16.76

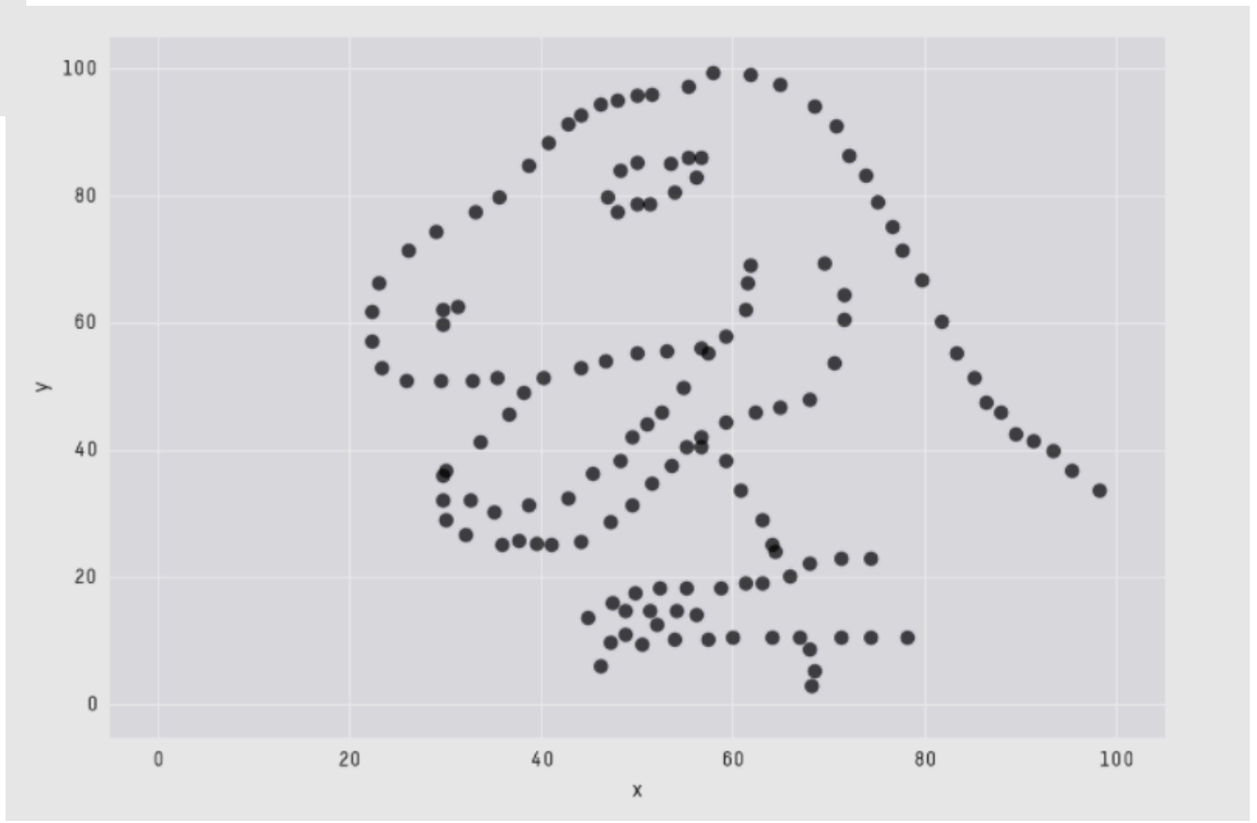
Y SD : 26.93

Corr. : -0.06

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

A TRex in your data

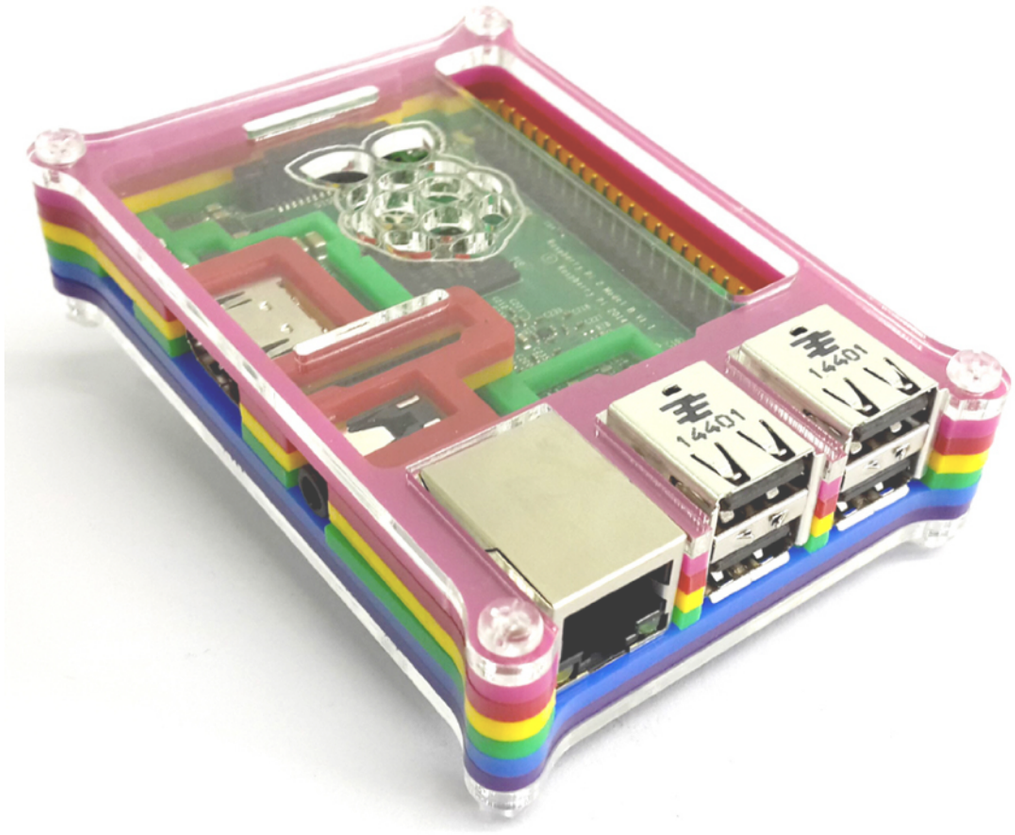




challenges

- 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

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)

mirGFF3

A proxy for miRNA/isomiR data analysis where all tools meet with the idea to create an ecosystem of data analysis promoting community collaboration.

repo status Active | gff3 definition | gff3 example | fairsharing accepted | edam accepted

Introduction

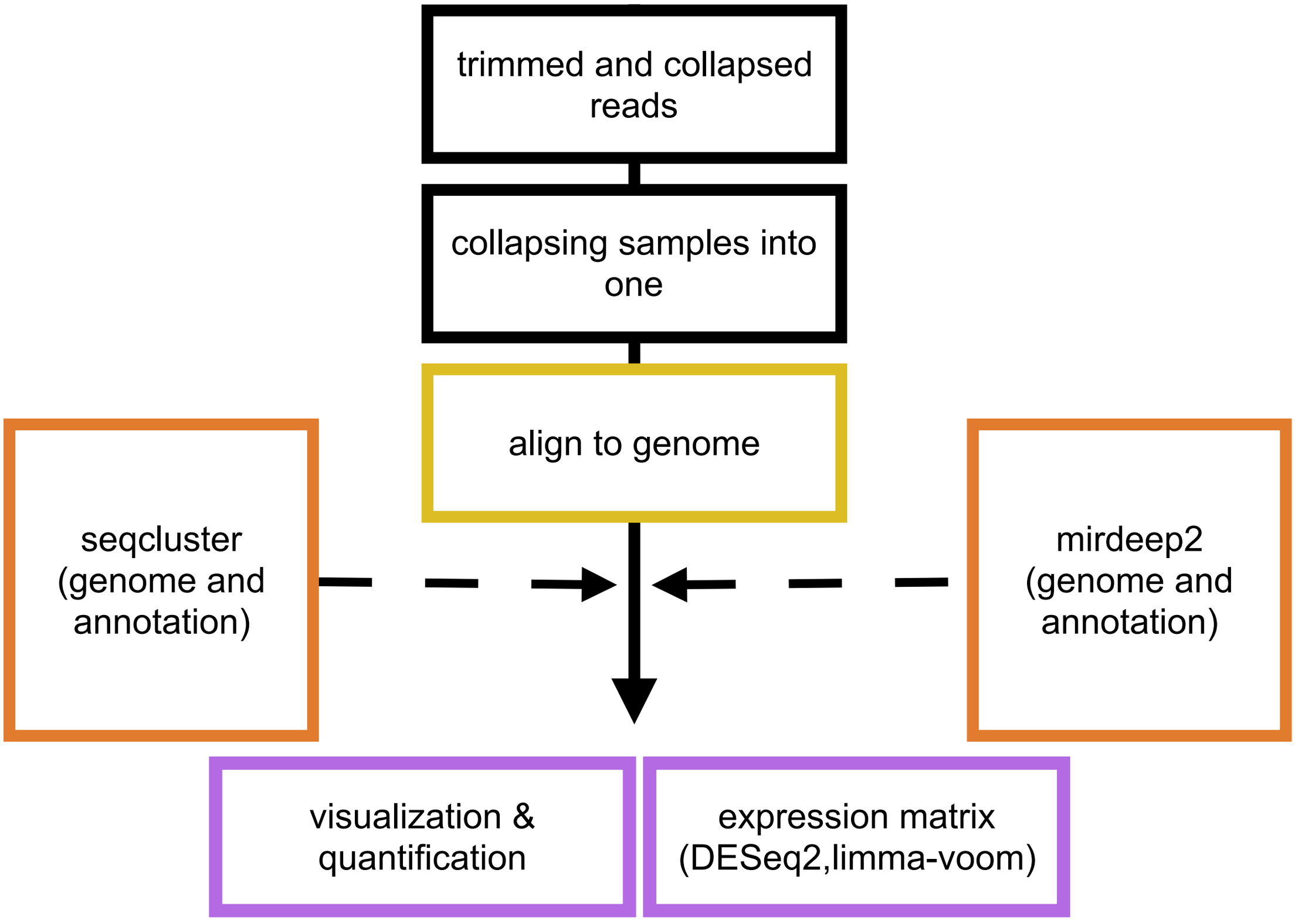
As discussed here: https://github.com/miRTop/incubator/issues/10 we've defined a GFF3 format for output of small RNA pipelines focused on miRNA data currently. We is an open community project (read more) and joint us!

This output is based on the current GFF3 definition: https://github.com/The-Sequence-Ontology/Specifications/blob/master/gff3.md

Note: Keep in mind this is for the output of a pipeline, so we know there will be bias toward methodology, but the idea is to put enough information to be able to re-analyze or filter sequences using information described here. As well, it would be a proxy for downstream analysis or packages.

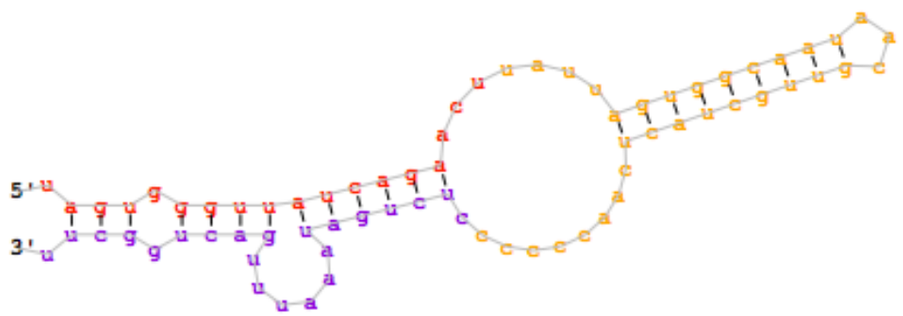
Advantages

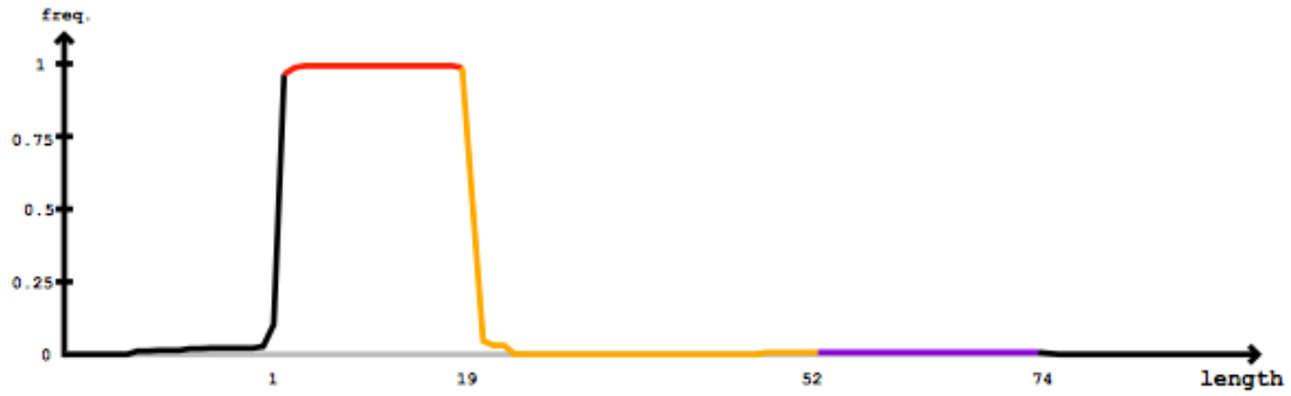
De-novo detection



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

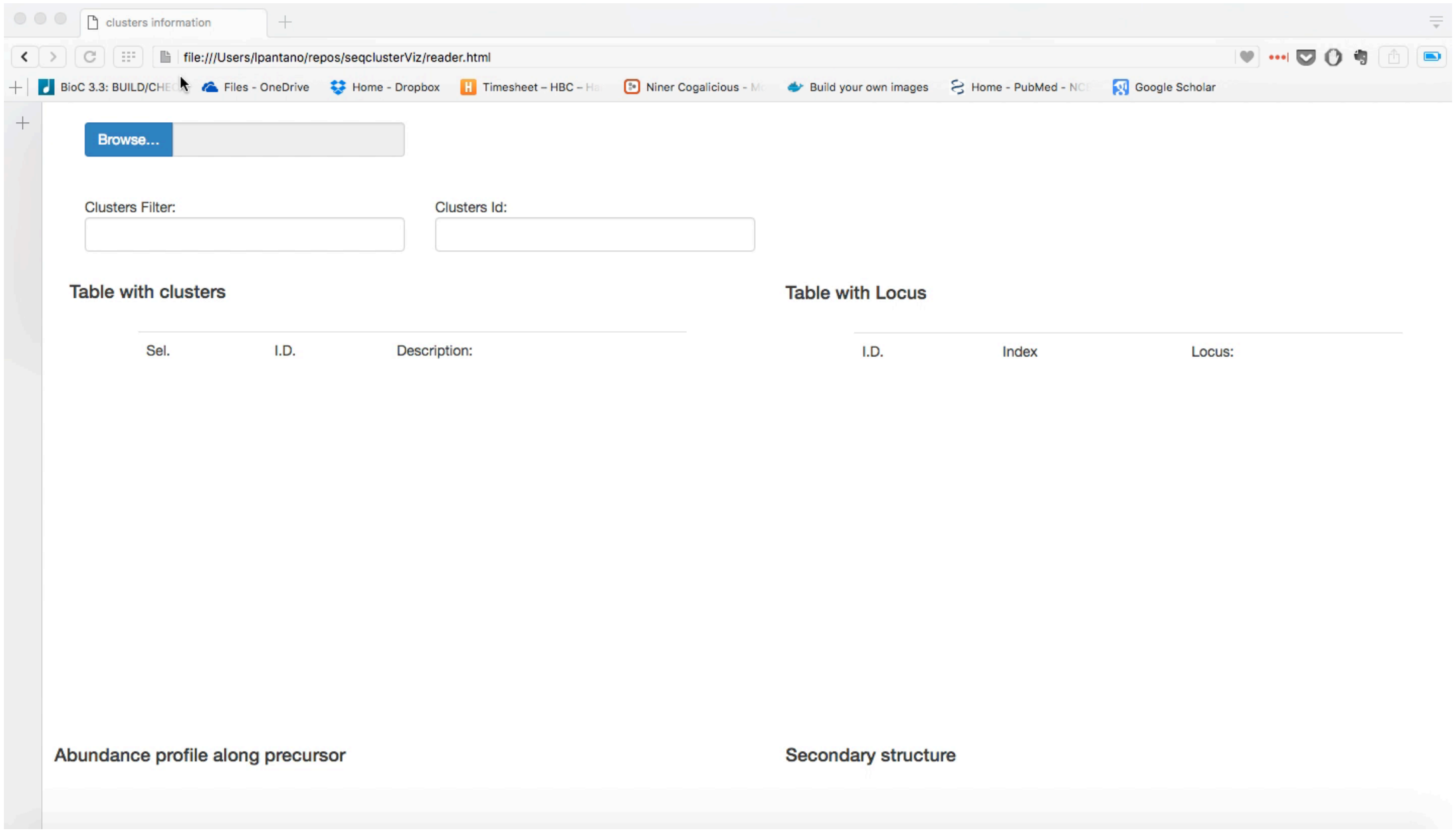




Mature Star

```
5'- gaaaugccuggguccaaugguagugggguuaucagaacuuauuagugggcaauaacguugcuacucaaccccccucugauaaaauuugacuggcuuaaaaaaaaaaaaaaucaggaa  -3'   obs
    gaaaugccuggguccaaugguagugggguuaucagaacuuauuagugggcaauaacguugcuacucaaccccccucugauaaaauuugacuggcuuaaaaaaaaaaaaaaucaggaa        exp
    ......((((((.....(.((((.((((((((((.......(((((((((...)))))))))........)))))))).))).))))).).............))))))..
    .....gccuggguccGaugguaguggguuaucagaacu.....................................................................  38        1     seq
    .....gccuggguccGaugguaguggguuaucagaacuua....................................................................  3         1     seq
    .....gccuggguccGaugguaguggguuaucagaacuuauu..................................................................  3         1     seq
    .......cuggguccGaugguaguggguuaucagaacuu.....................................................................  3         1     seq
    .......cuggguccGaugguaguggguuaucagaacuua....................................................................  3         1     seq
    .......cuggguccGaugguaguggguuaucagaacuuauu..................................................................  3         1     seq
    .........guccGaugguaguggguuaucagaacuu.......................................................................  16        1     seq
```

seqcluster visualization



https://github.com/lpantano/seqclusterViz

Bcbio-nextgen done,

Now what?

bcbioSmallRna: R package

- Load all the data from bcbio pipeline
  - `miRNA`
  - `isomiRs`
  - `clusters`
- QC figures

https://lpantano.github.io/bcbioSmallRna

isomiRs: R package

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

- Collapsing isomiRs in different ways

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

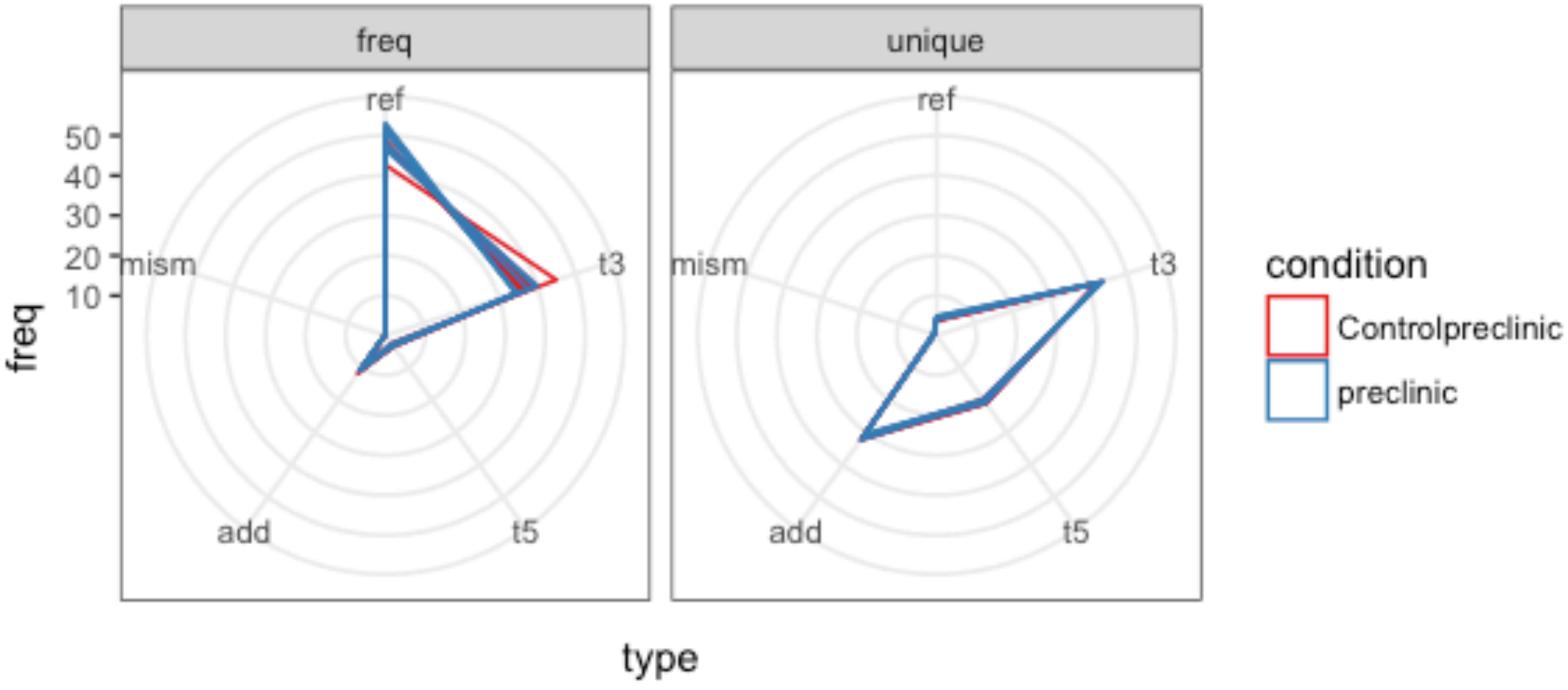
- RNAseq and miRNA time serie data

- Help with DE analysis

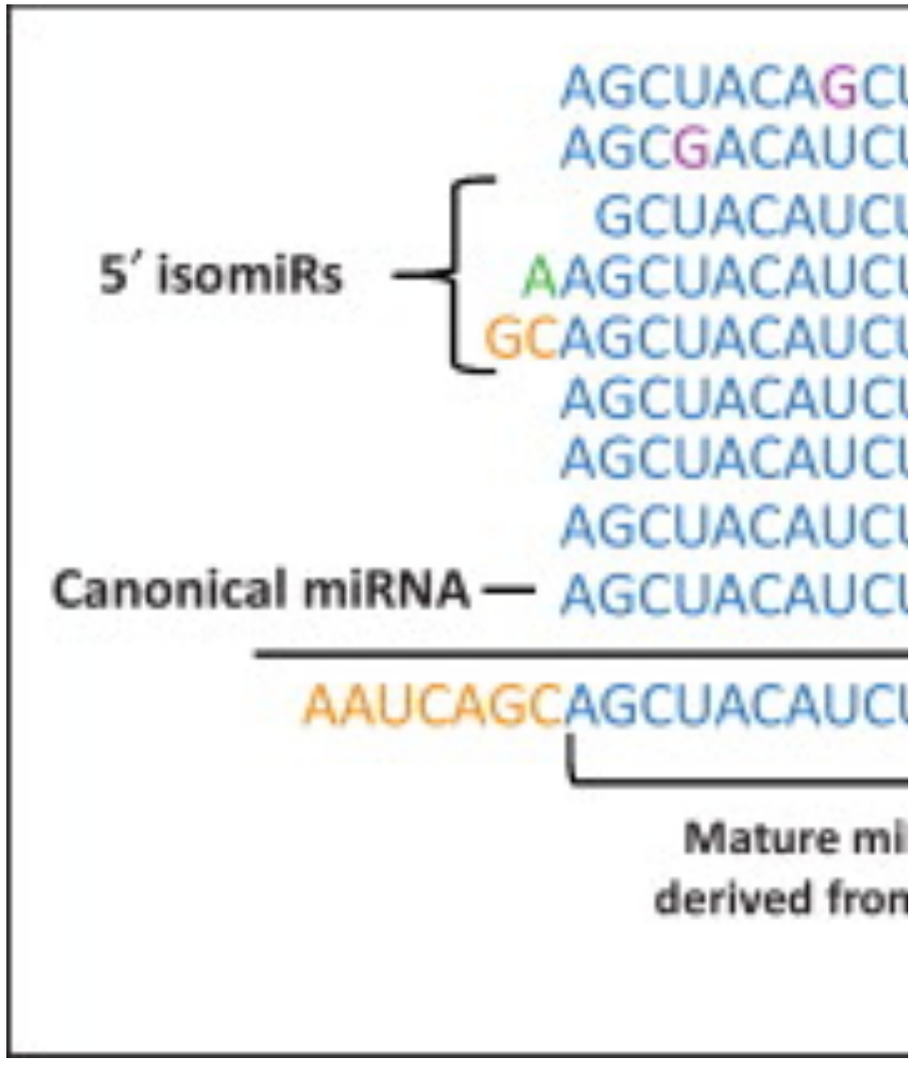
http://bioconductor.org/packages/release/bioc/html/isomiRs.html

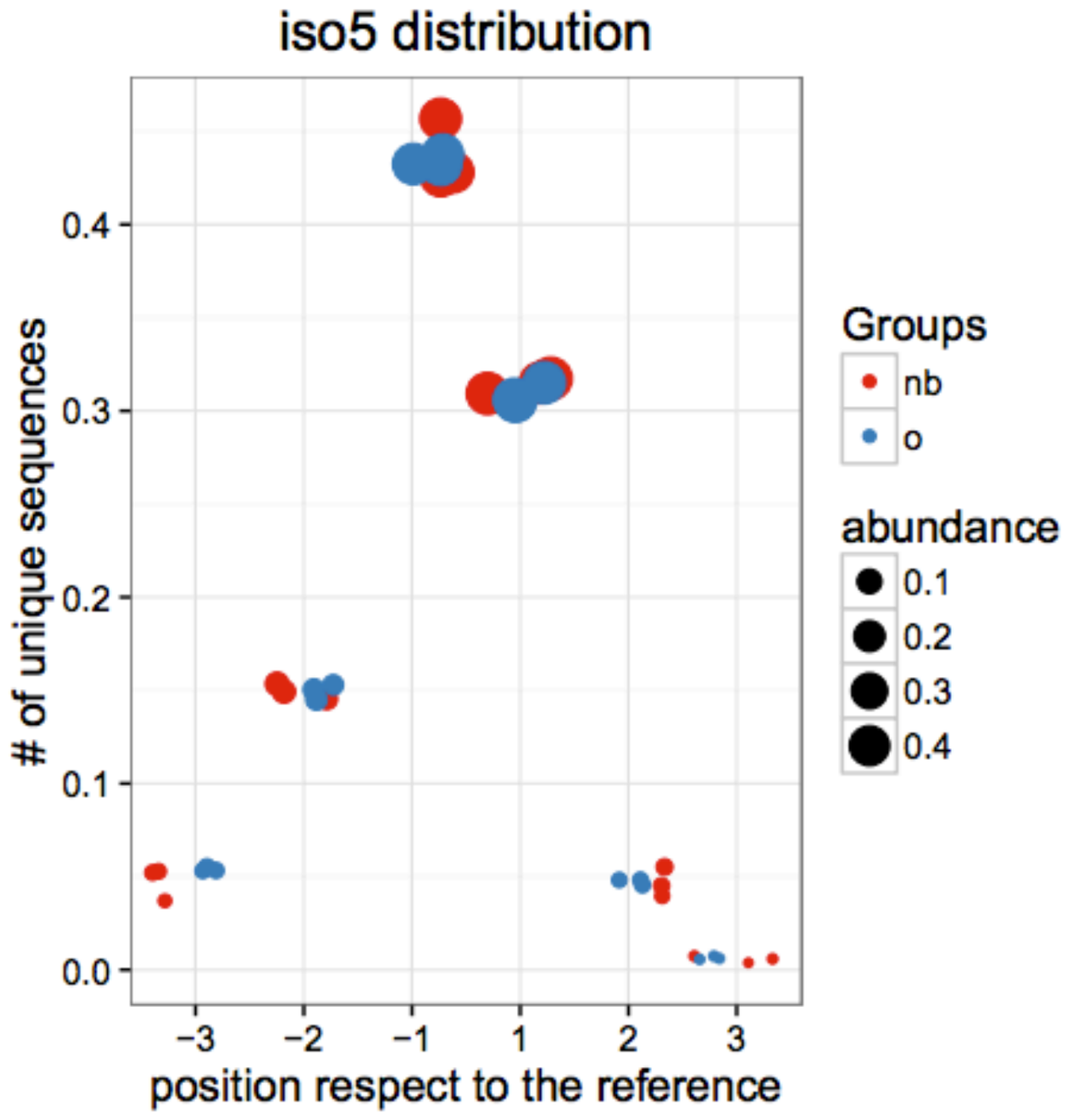
isomiRs: R package

Spider plots

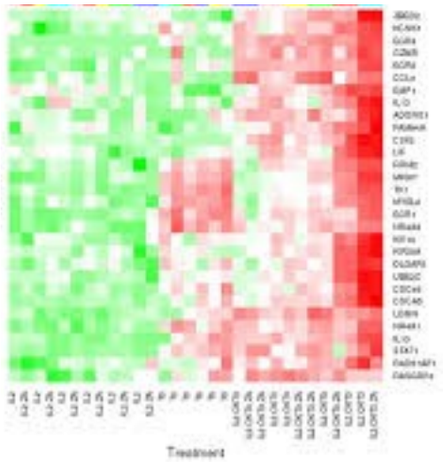


isomiRs: R package

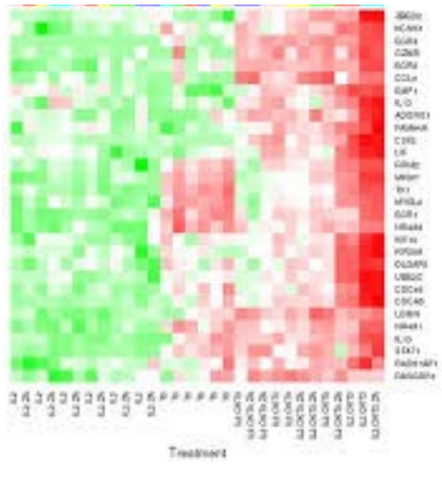




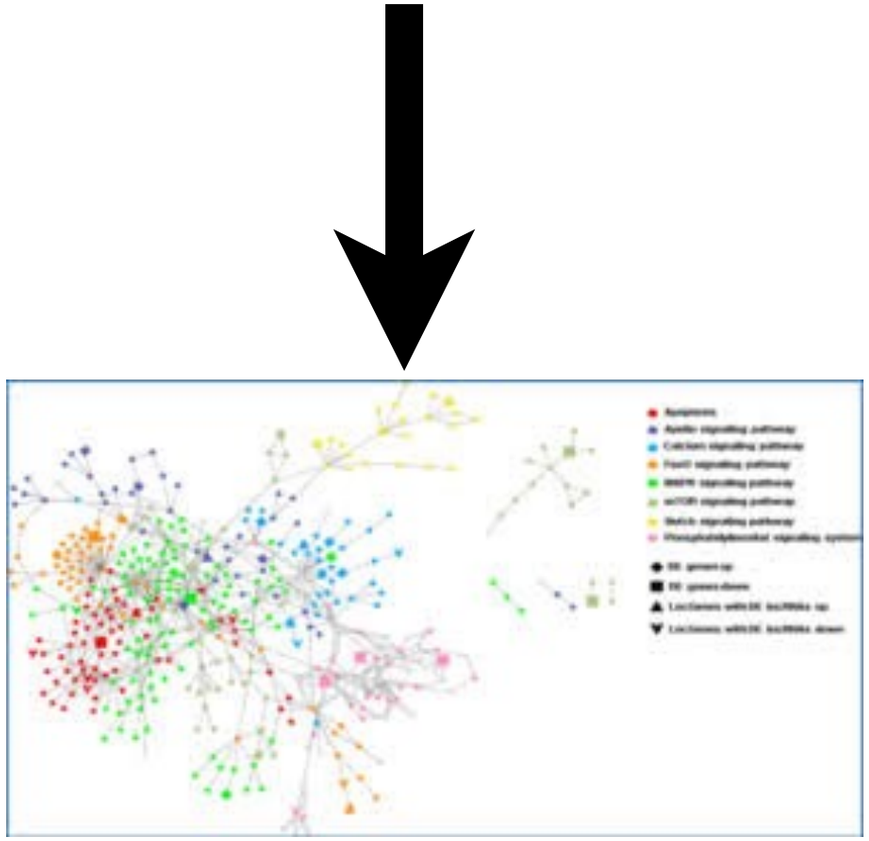
Omics integration



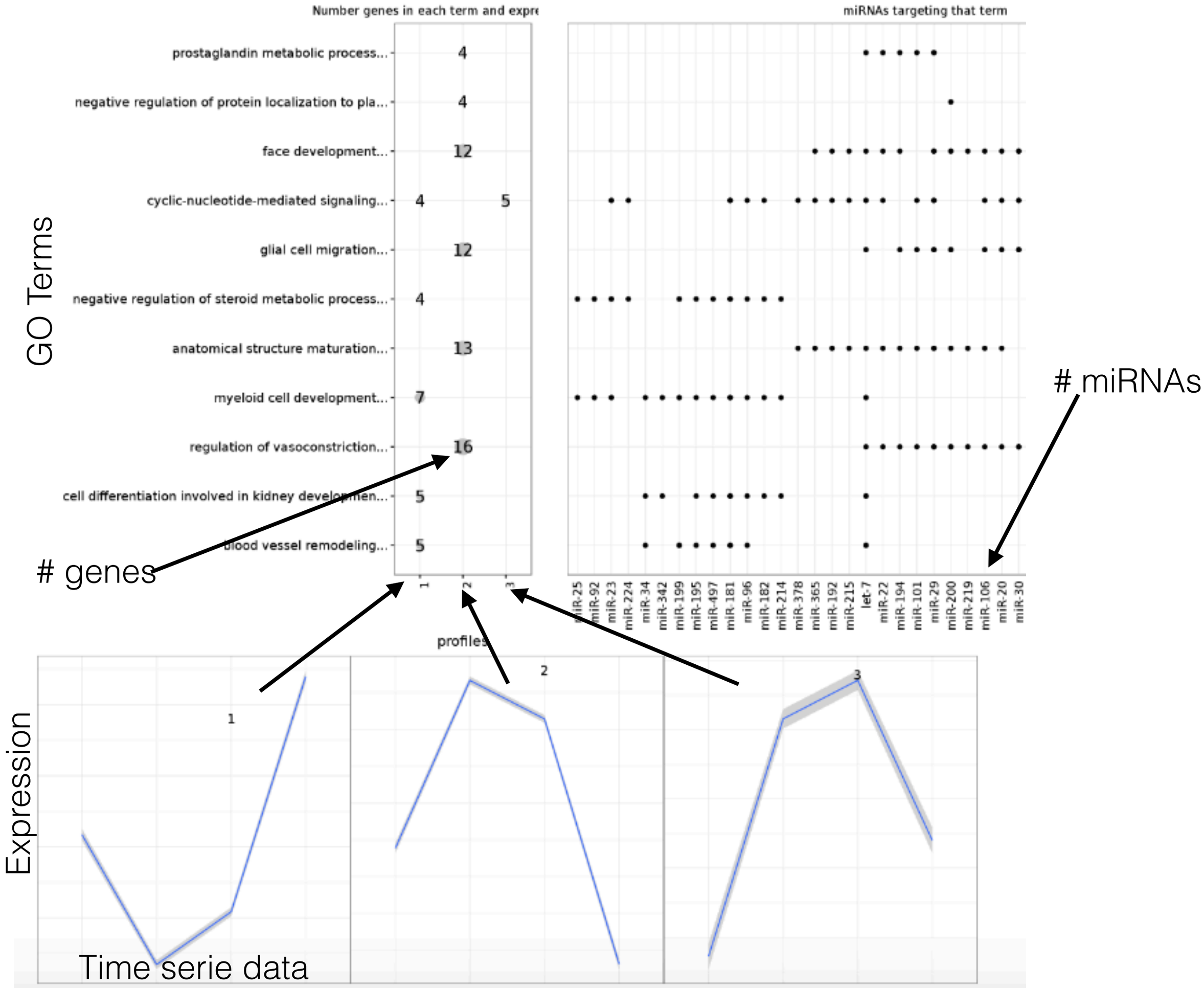
Gene expression



miRNA expression



Functional enrichment


Number genes in each term and expre
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
Expression
Time serie data
1
2
3
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

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...

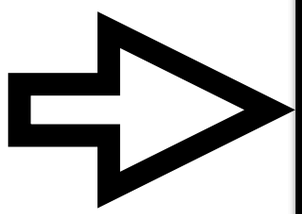
GSM1207643	miRQC A
GSM1207644	miRQC A repeat
GSM1207645	miRQC B
GSM1207646	miRQC B repeat
GSM1207647	miRQC C
GSM1207648	miRQC C repeat
GSM1207649	miRQC D
GSM1207650	miRQC D repeat

```
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

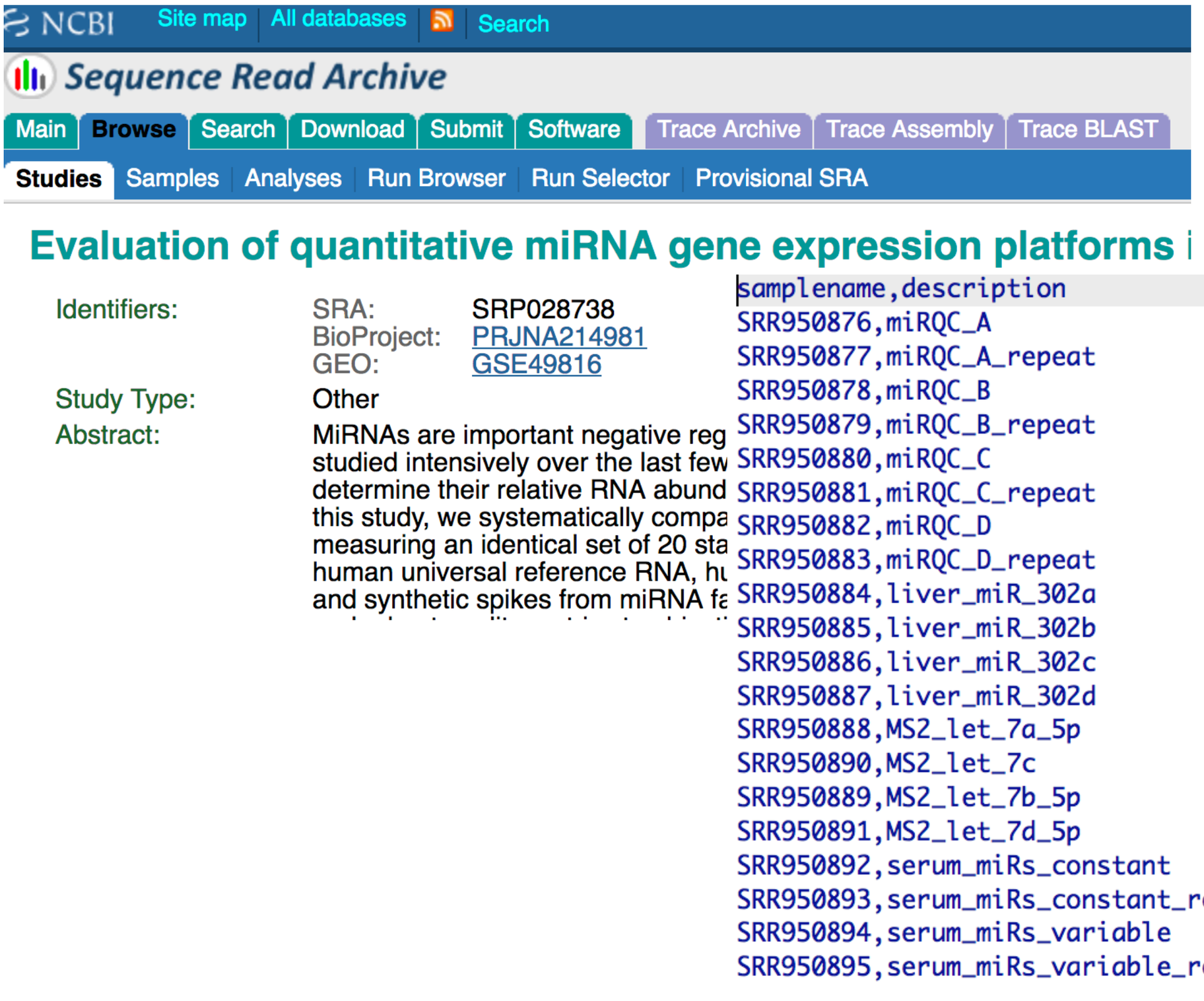
fastq/*fastq.gz



```
bcbio_nextgen.py -w template ….

bcbio_nextgen.py config.yaml ….
```

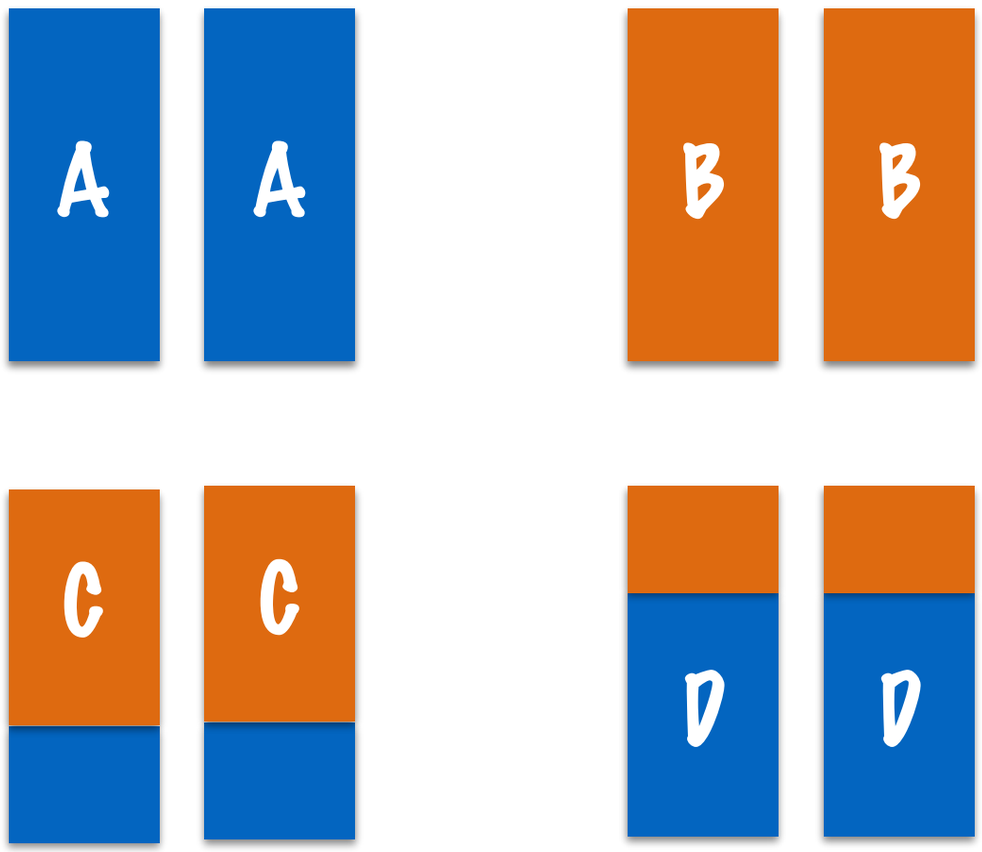
Analyze Public Dataset



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
```

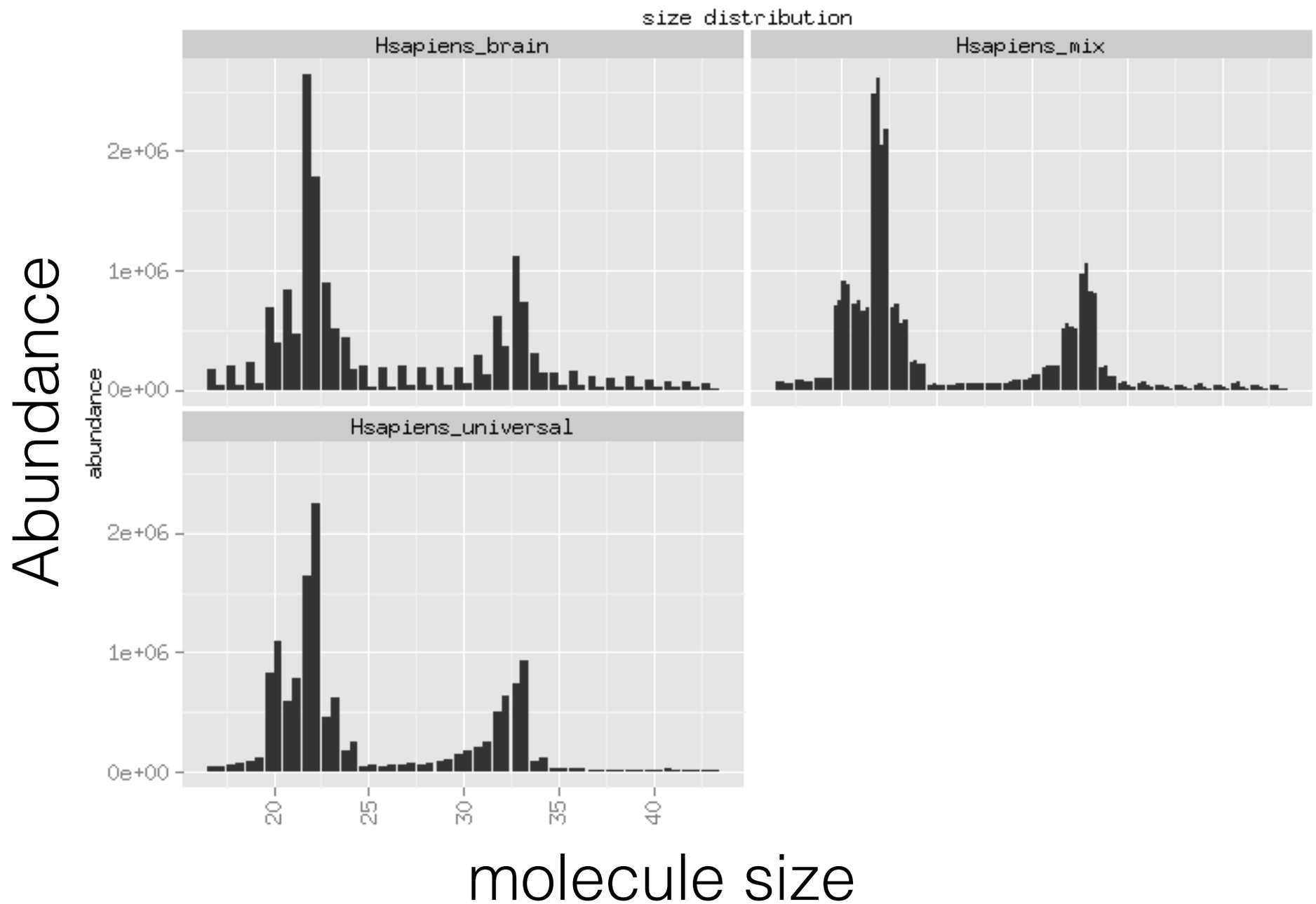
Quality Control samples



For each molecule:

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

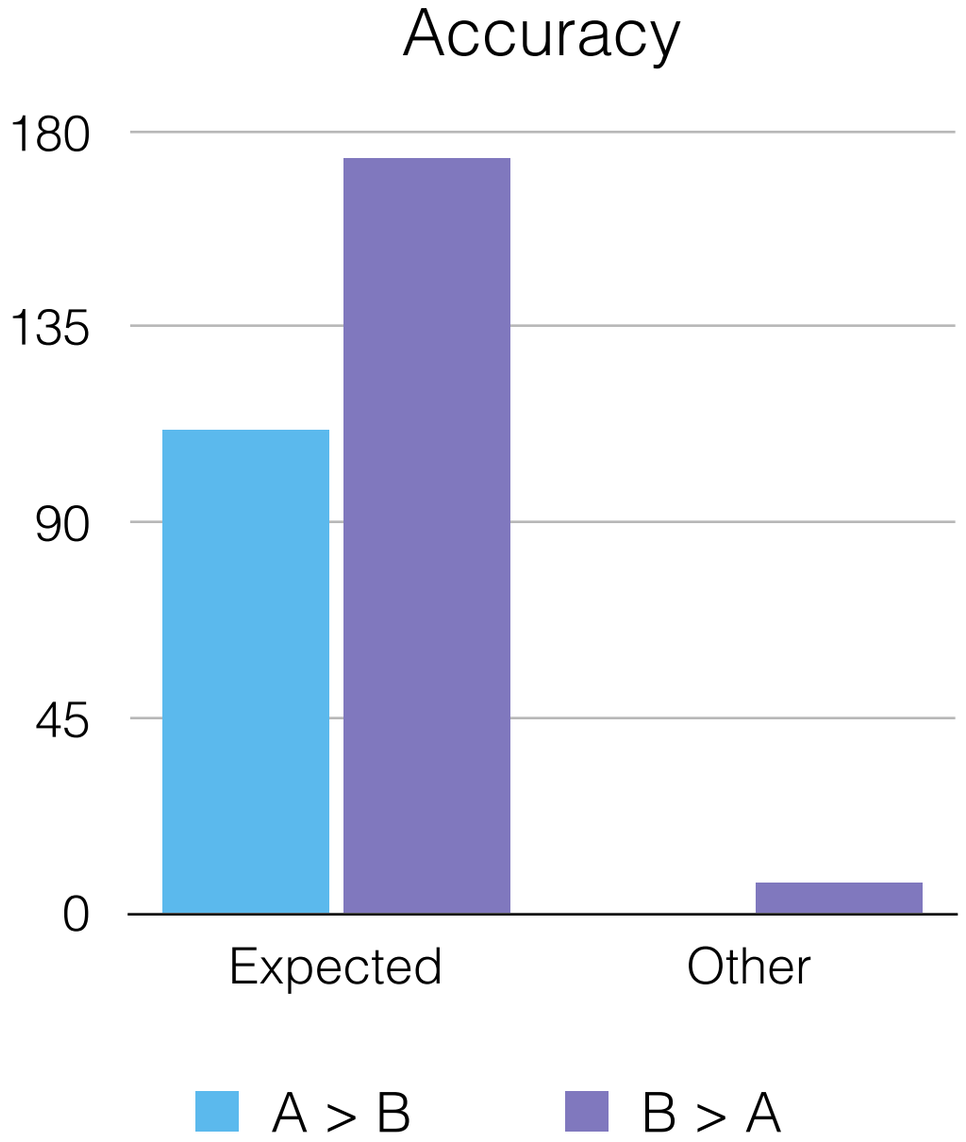
Good samples



miRNA quantification

miRNAs > 5 counts in average

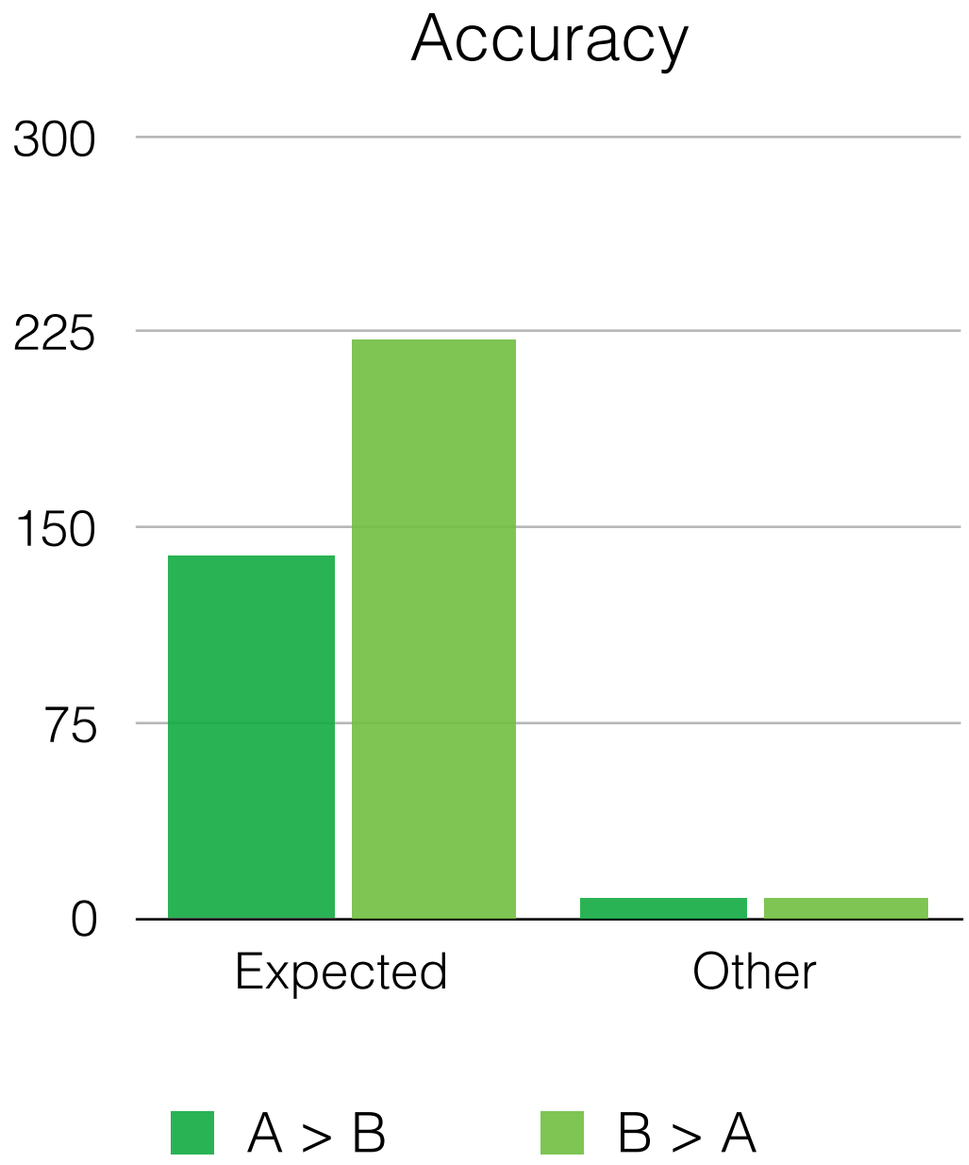
upper quantile normalization



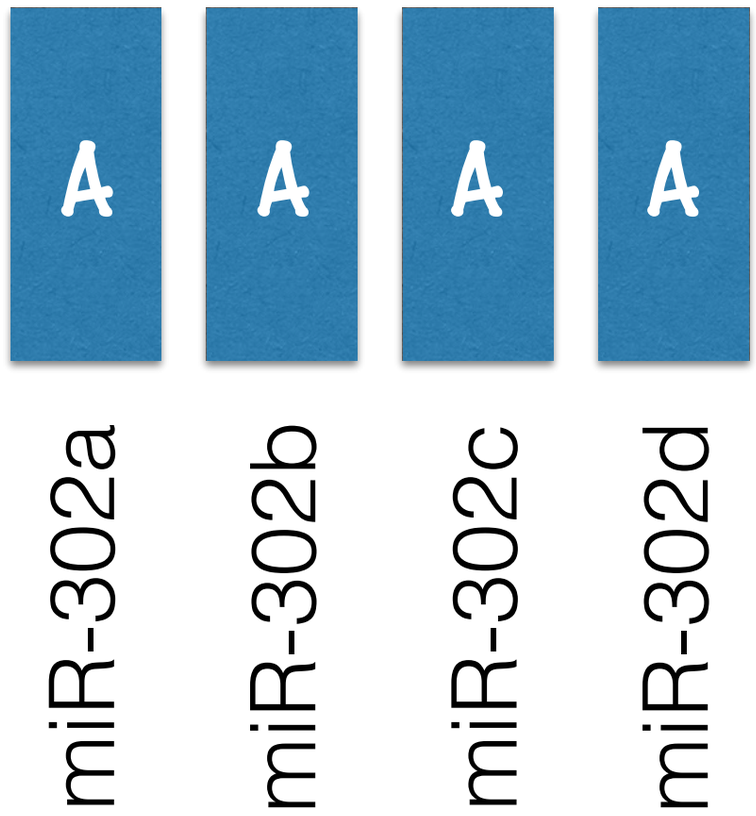
clusters quantification

expression > 5 counts in average

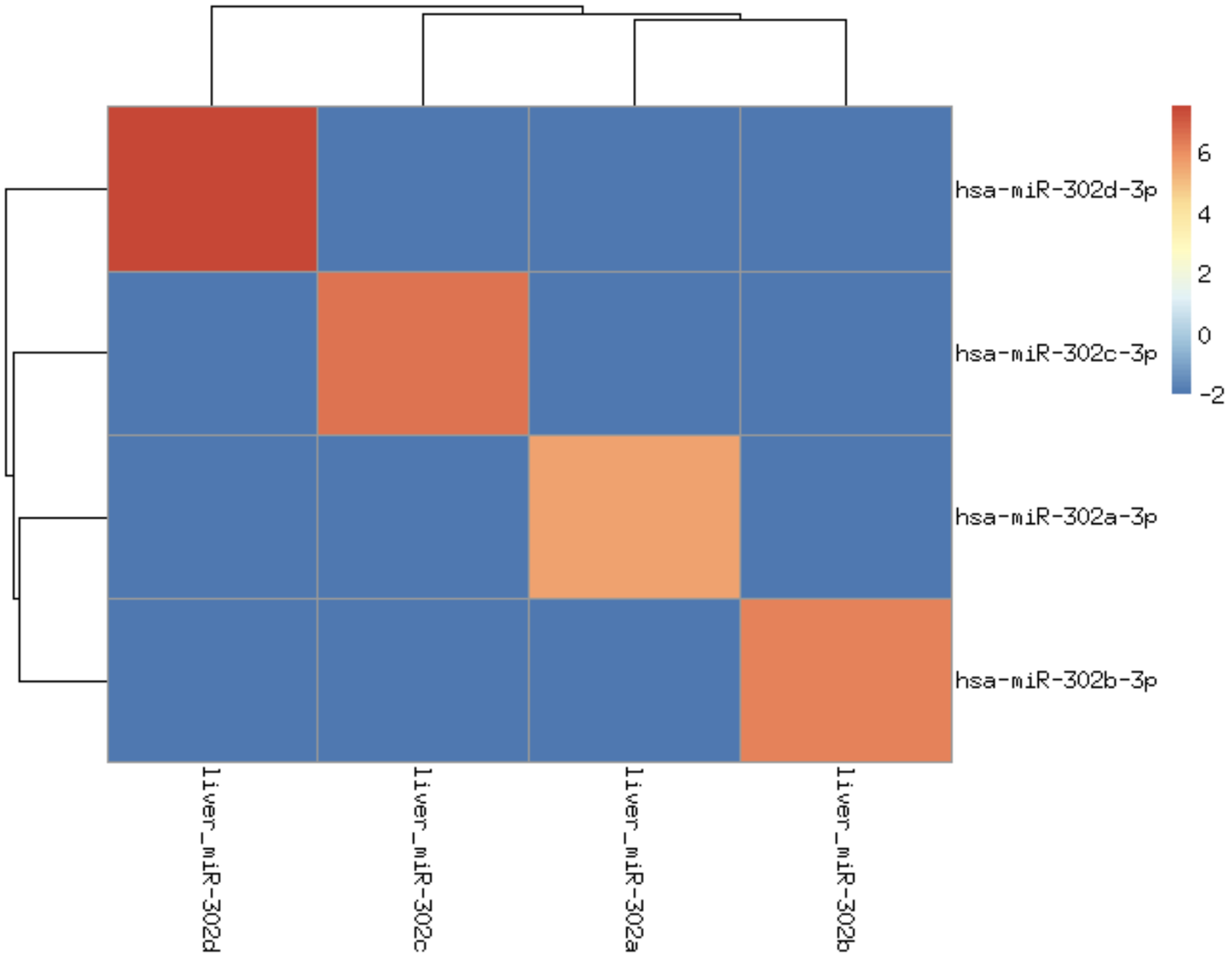
upper quantile normalization



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.

Boston-area Women's Bioinformatics Meetup

★★★★★ 35 ratings

Cambridge, MA

500 members · Private group

Organized by
Lorena Pantano and **3 others**

Share:

Next Meetup

See all

2 OCT

Tuesday, October 2, 2018, 6:00 PM

Develop your Bioinformatics Shiny App - day 2 out of 4

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.

miRToP consortium