Characterization of the small RNA transcriptome using the bcbio-nextgen python framework

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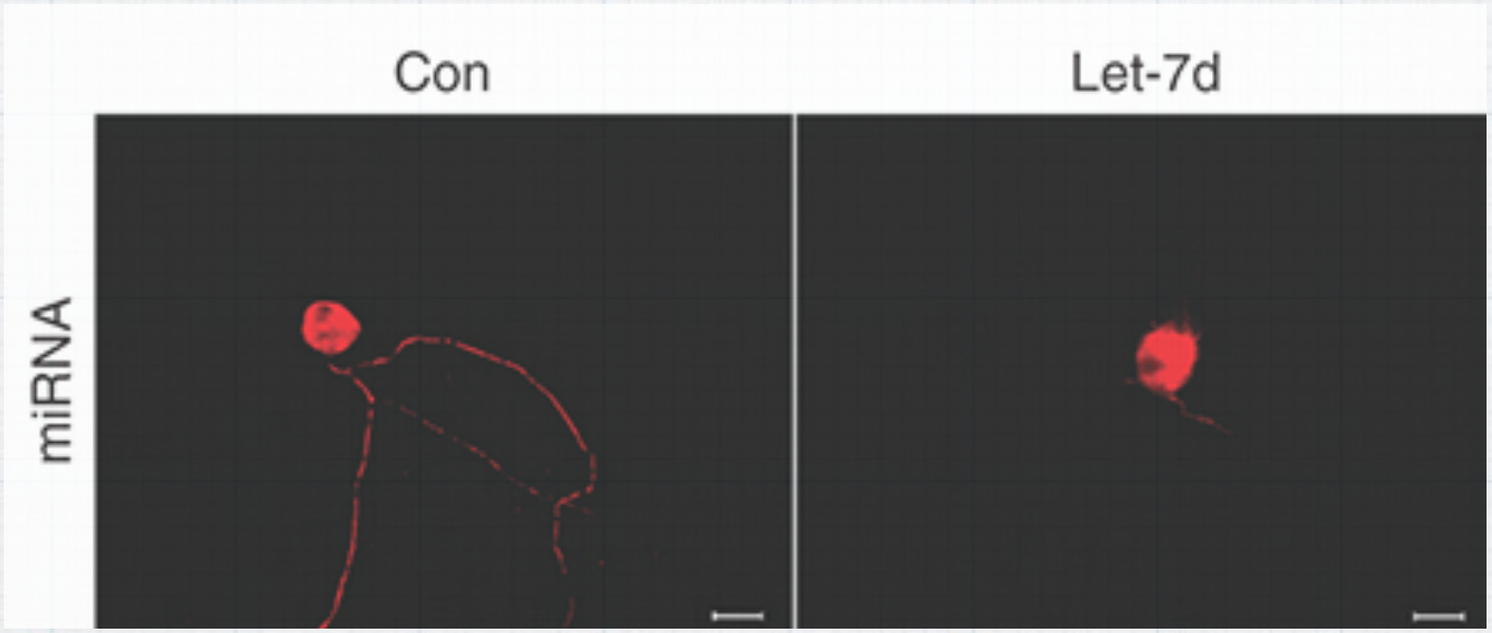
2016-07-14

small RNA

RNA molecules of 18-36 nts long with regulation function

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

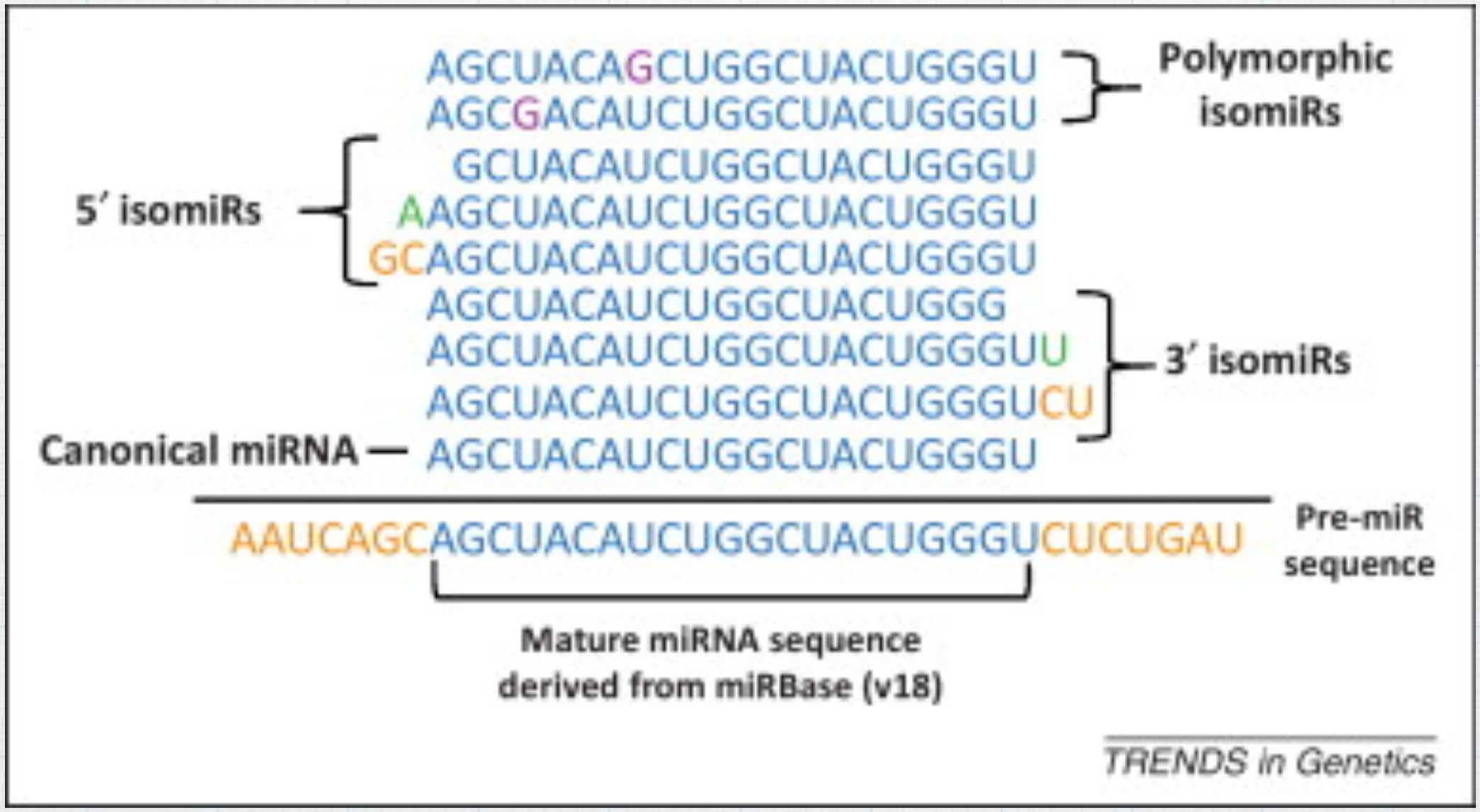
isomiRs

```
     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

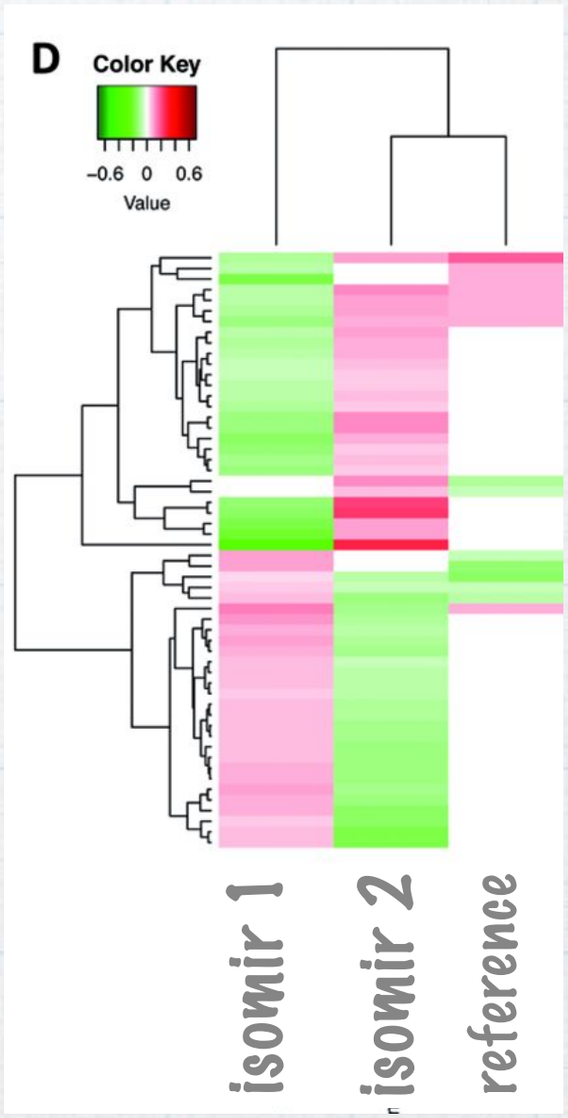
reference

isomir +2 nt

isomir -2 nt

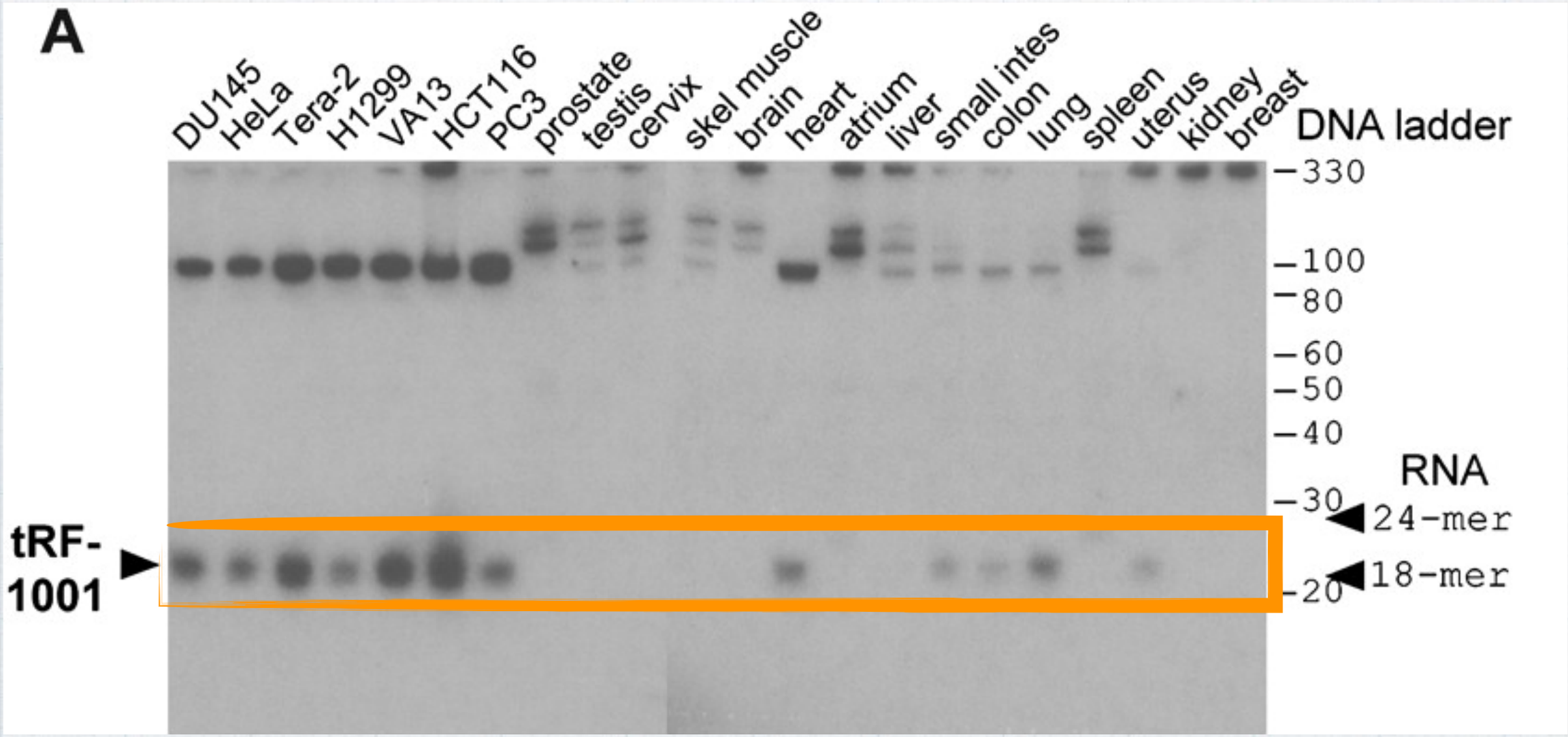
transfected mammary cells line
derived from metastatic site

Gene expression



Aristeidis G. Telonis et al. Nucl. Acids Res. 2015;nar.gkv922

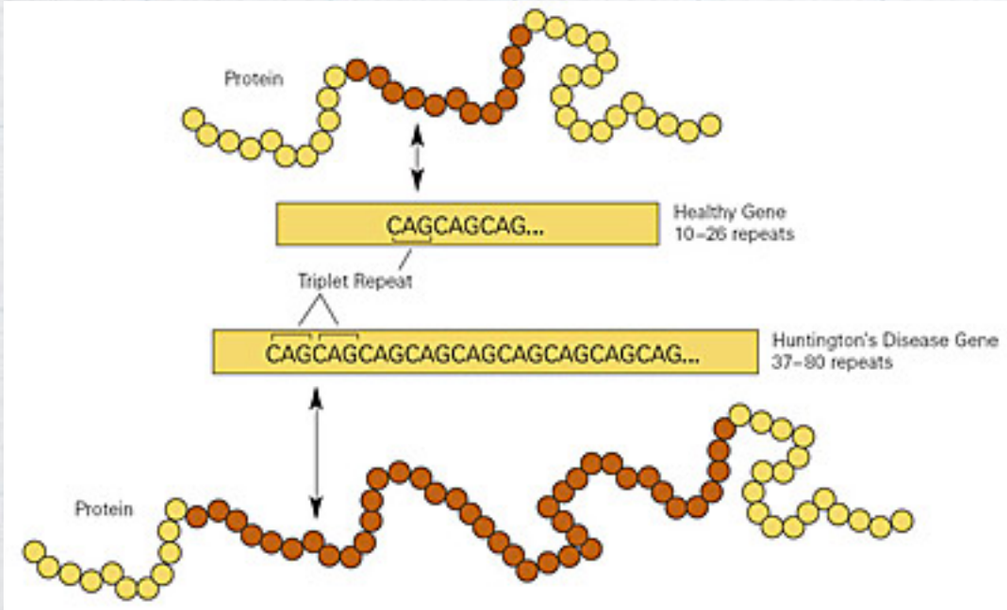
small tRNAs

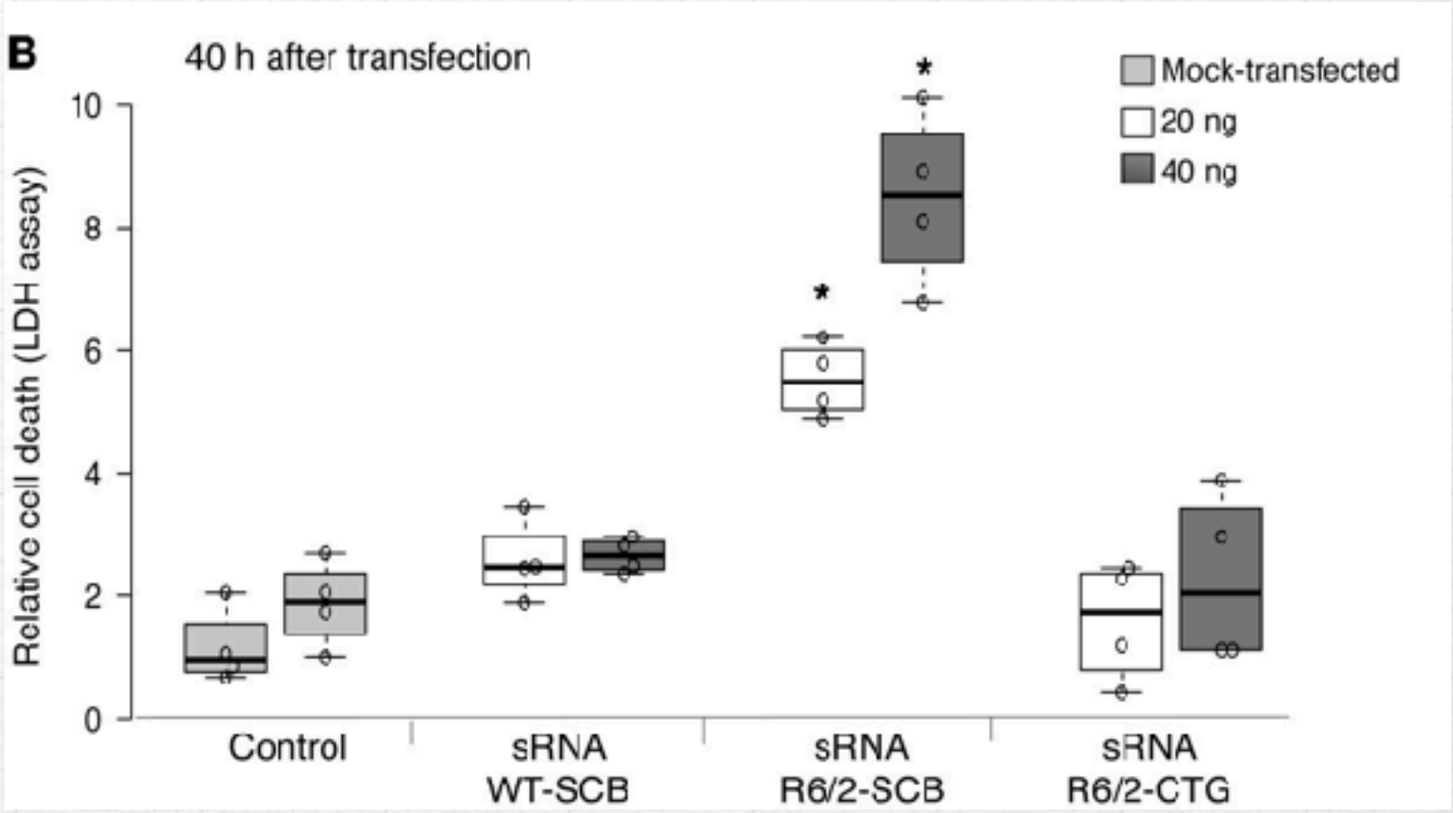


Yong Sun Lee et al. Genes Dev. 2009;23:2639-2649

small RNA

Huntington disease therapy





synthetic small RNA

CTGCTGCTGCTGCTGCTGCT

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 29, 2016 – June 29, 2016

Period: **1 month**

Overview

2 Active Pull Requests

74 Active Issues

2 Merged Pull Requests

0 Proposed Pull Requests

62 Closed Issues

12 New Issues

Excluding merges, **5 authors** have pushed **66 commits** to master and **66 commits** to all branches. On master, **68 files** have changed and there have been **1,085 additions** and **393 deletions**.

small RNA-seq analysis

processing & QC

cutadapt
fastqc
qualimap
multiqc

detection & annotation

miraligner
tdrmapper

de-novo

seqcluster
mirdeep2 for mirna
protac for pirna (next)

detection & annotation

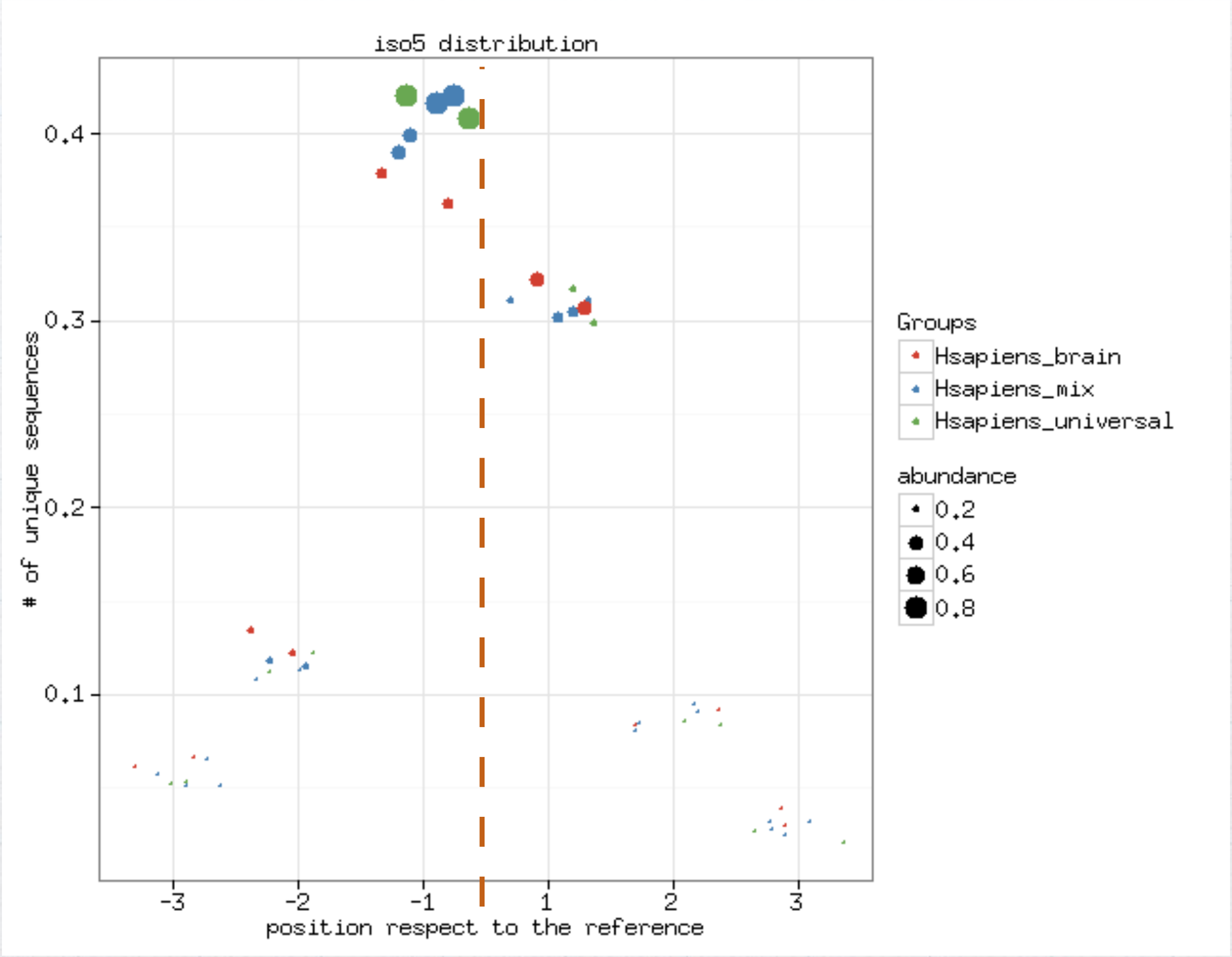
trimmed and collapsed reads

miraligner
(miRBase)

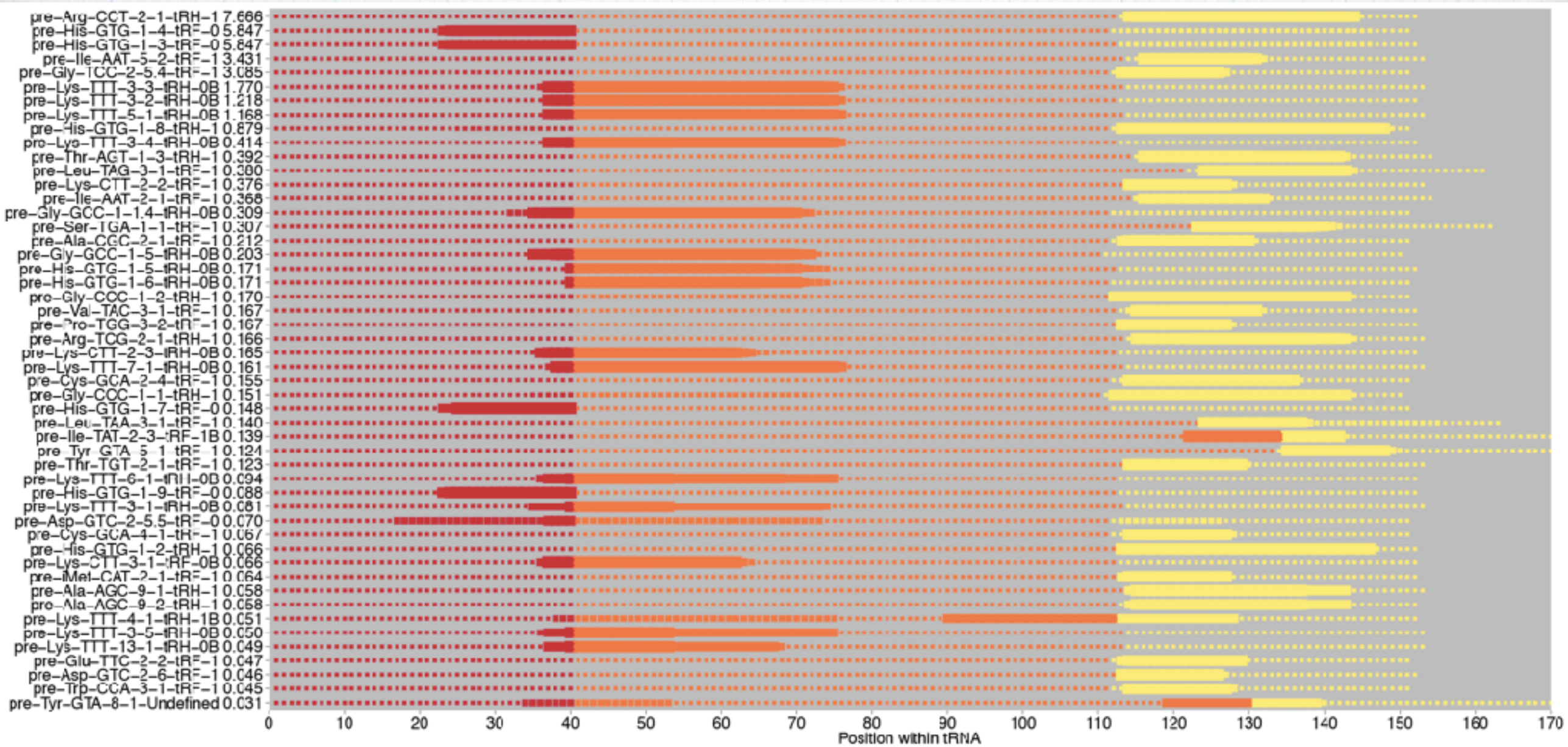
tdrMapper
(tRNA reference)

isomiRs package

isomiRs at 5' end of the miRNAs



tRNA analysis



**Pre-tRNA coverage map from NIH roadmap H1 derived mesendoderm cells, accession ID: GSM1296464*

de-novo detection

trimmed and collapsed reads

collapsing samples into one

align to genome

seqcluster (genome and annotation)

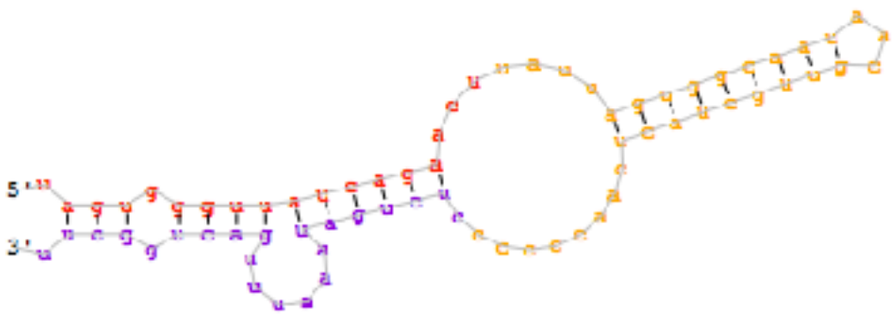
mirdeep2 (genome and annotation)

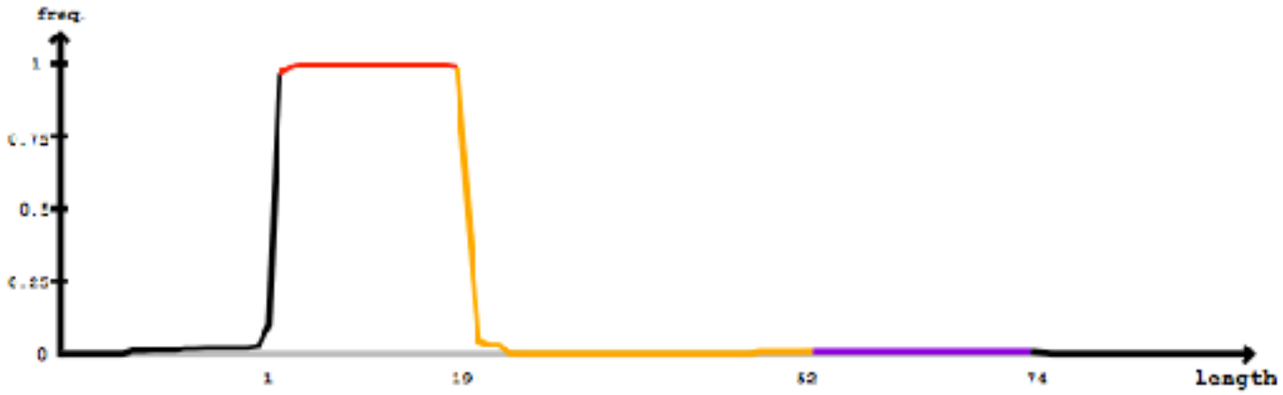
visualization & quantification

expression matrix

miRDeep2 output

Provisional ID	:	chr12_16160
Score total	:	18694
Score for star read(s)	:	39
Score for read counts	:	18666
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

seqcluster

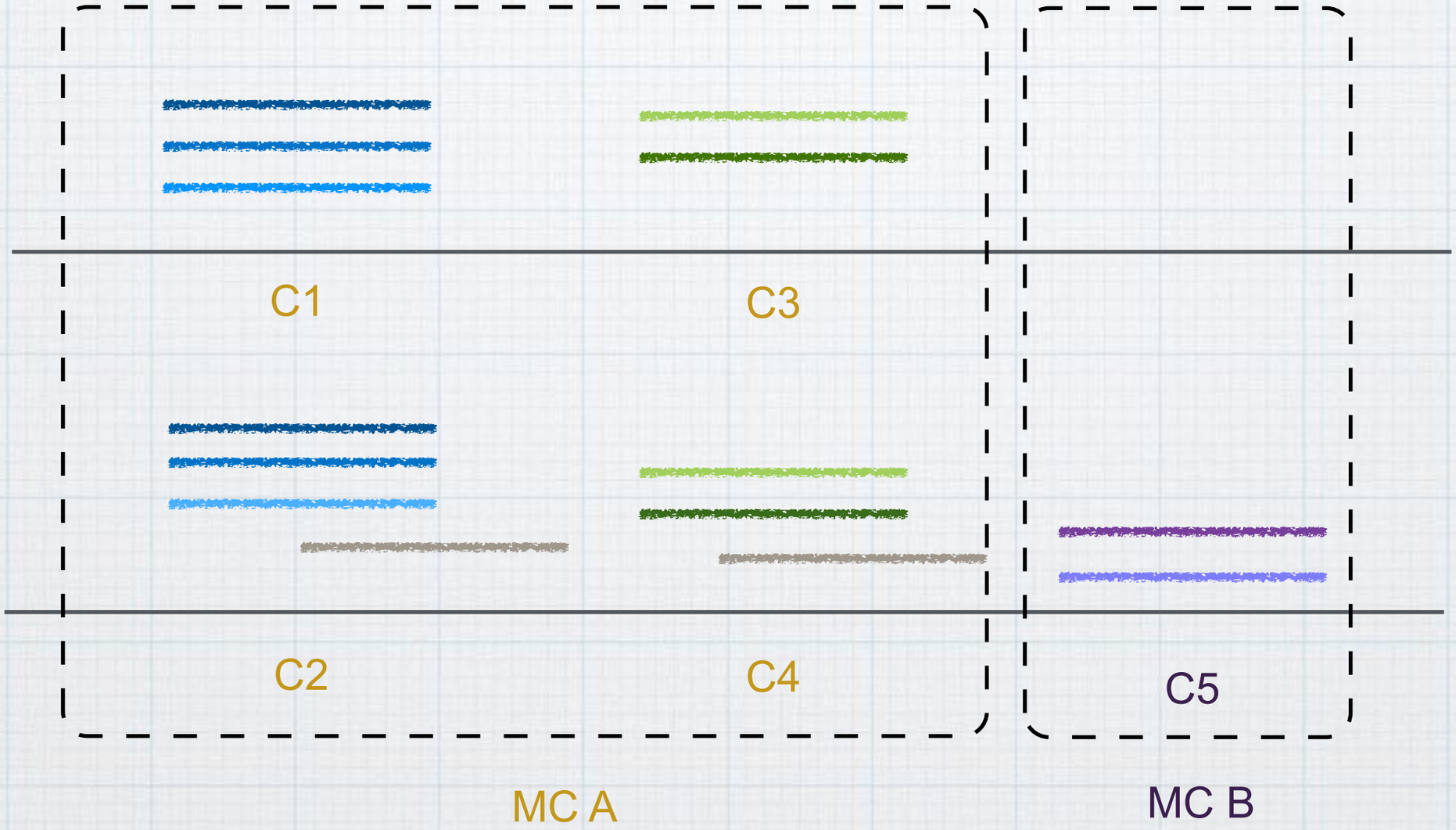
cluster at position 1

cluster at position 2

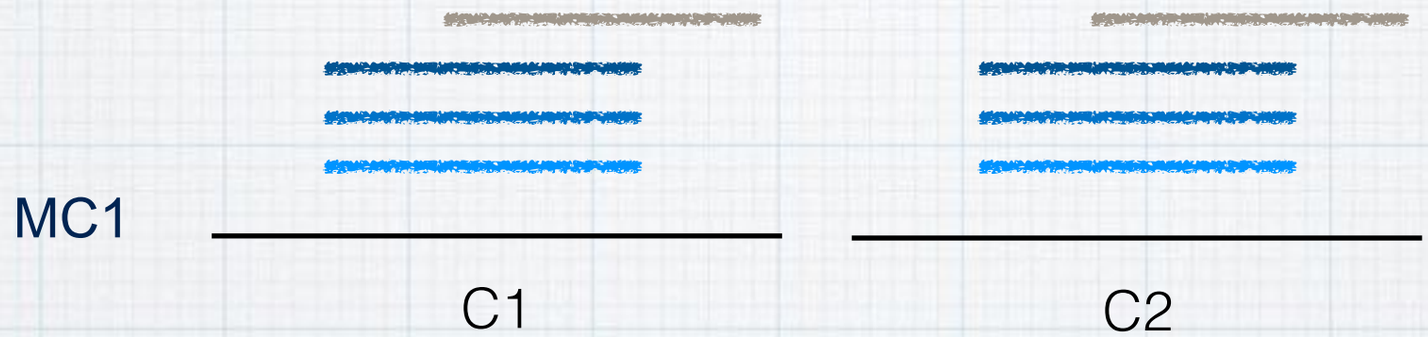
meta-cluster

seqcluster deals with multi-mapped reads

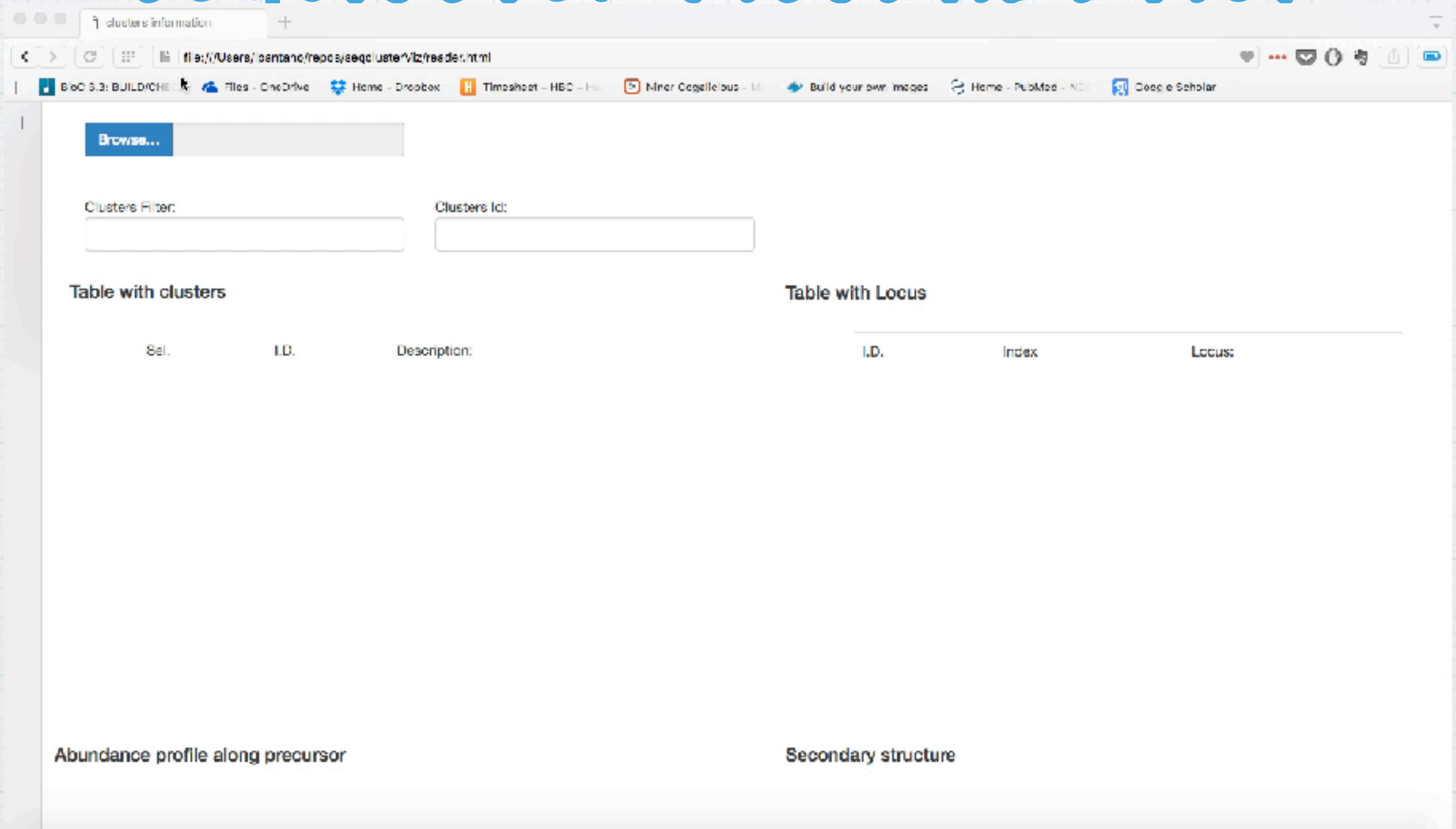
Step 1: clustering



Step 2: cleaning



seqcluster visualization



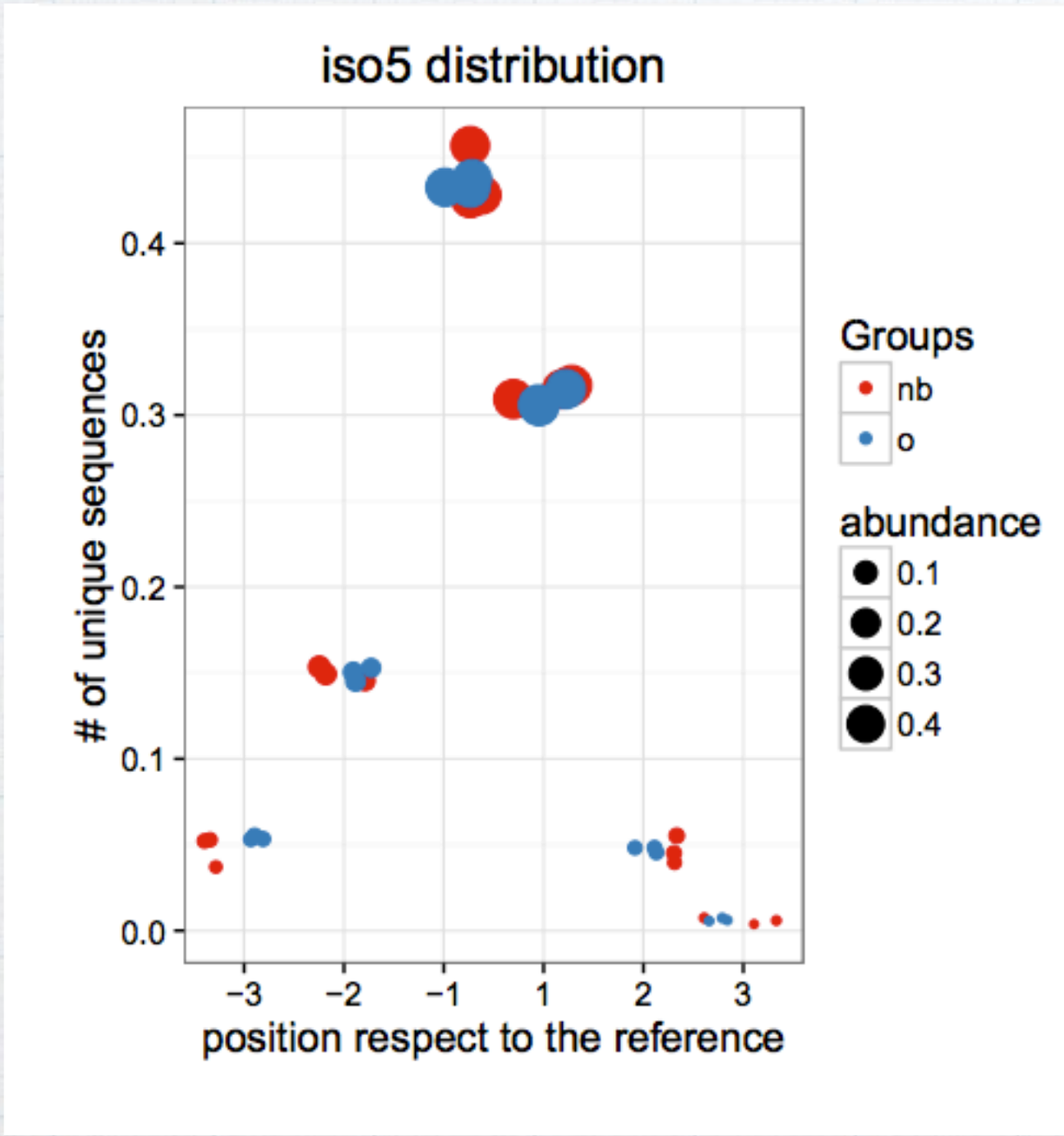
https://github.com/lpantano/seqclusterViz

isomiRs: R package

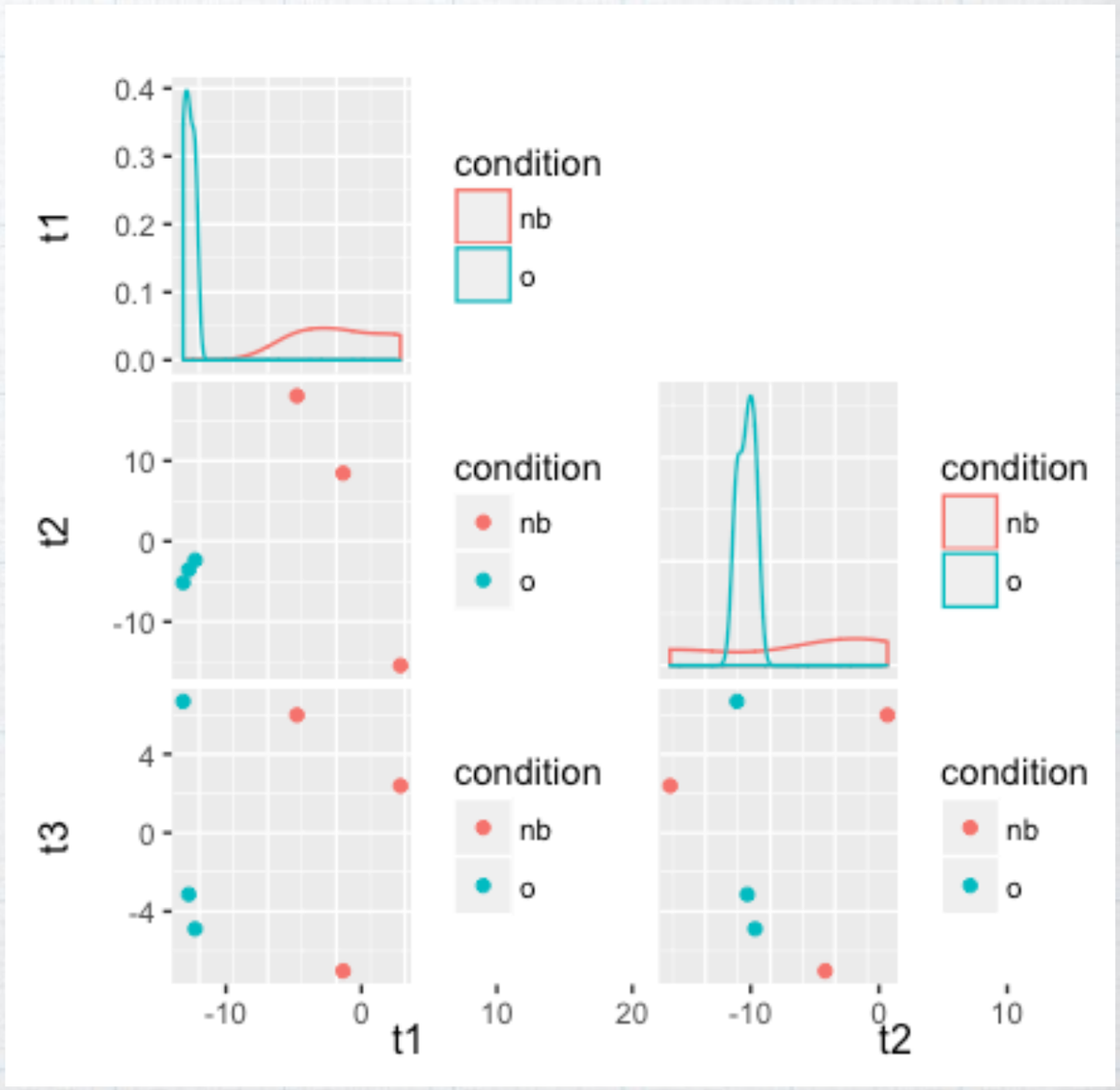
* General Characterization of isomiRs
* Collapsing isomiRs in different ways
* Supervised clustering analysis to detect important miRNAs (PLS-DA)
* RNAseq and miRNA time serie data

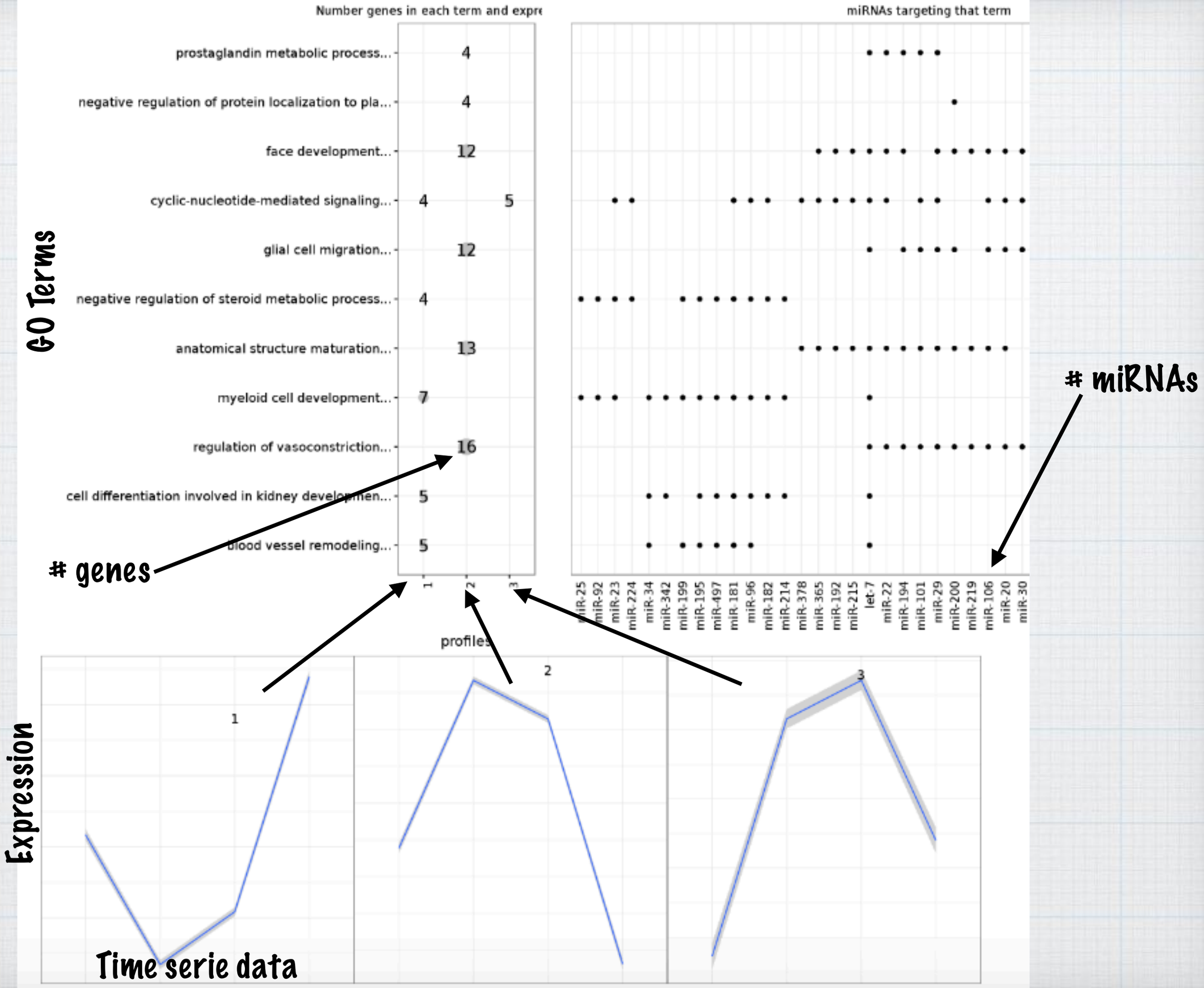
http://bioconductor.org/packages/release/bioc/html/isomiRs.html

isomiRs: R package



PLS-DA




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
genes
miRNAs
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
profiles
Expression
1
2
3
Time serie data

MultiQC

Phil Ewels

ewels

Bioinformatician working with next generation sequencing data.

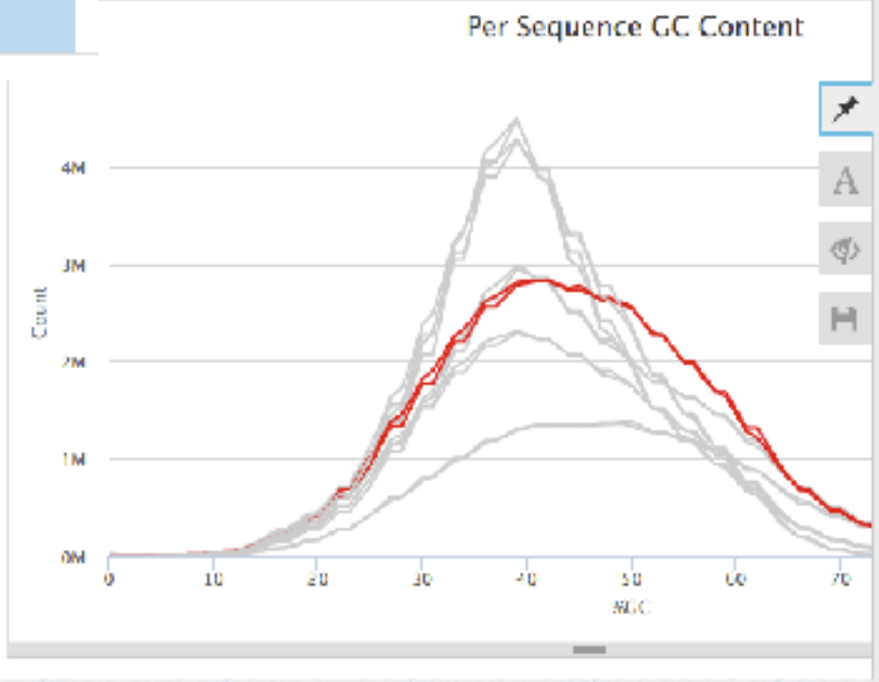
- Science for Life Laboratory
- Stockholm, Sweden
- phil.ewels@scilifelab.se
- http://phil.ewels.co.uk
- Joined on Nov 3, 2010

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



MultiQC Toolbox

Highlight Samples

Regex mode

miRQC project

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
```

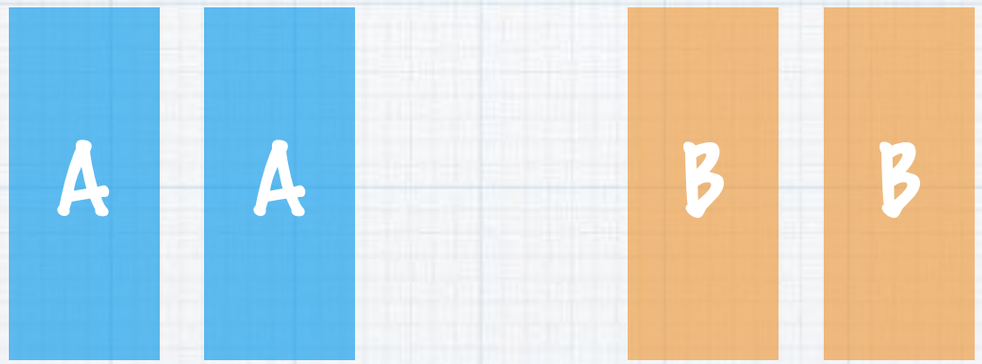
test-merged.csv
fastq/*fastq.gz

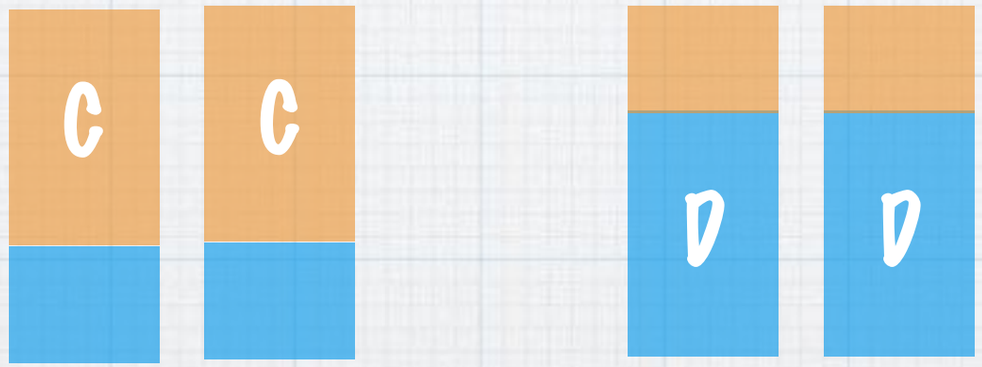
```
bcbio_nextgen.py -w template ….

bcbio_nextgen.py config.yaml ….
```

bcbio_setup_genome.py

```
Current genomes

index   mm10    /groups/bcbio/bcbio/genomes/Mmusculus/mm10/seq/mm10.fa
index   Zv9     /groups/bcbio/bcbio/genomes/Drerio/Zv9/seq/Zv9.fa
index   GRCh37  /groups/bcbio/bcbio/genomes/Hsapiens/GRCh37/seq/GRCh37.fa
index   WBcel235        /groups/bcbio/bcbio/genomes/Celegans/WBcel235/seq/WBcel235.fa
index   hg19    /groups/bcbio/bcbio/genomes/Hsapiens/hg19/seq/hg19.fa
index   hg19-mt /groups/bcbio/bcbio/genomes/Hsapiens/hg19-mt/seq/hg19-mt.fa
index   mm9     /groups/bcbio/bcbio/genomes/Mmusculus/mm9/seq/mm9.fa
index   greenberg-mm9   /groups/bcbio/bcbio/genomes/Mmusculus/greenberg-mm9/seq/greenberg-mm9.fa
index   MB2409  /groups/bcbio/bcbio/genomes/Ecoli/MB2409/seq/MB2409.fa
index   MG1655  /groups/bcbio/bcbio/genomes/Ecoli/MG1655/seq/MG1655.fa
index   MB0009  /groups/bcbio/bcbio/genomes/Ecoli/MB0009/seq/MB0009.fa
index   NC_912.3        /groups/bcbio/bcbio/genomes/Ecoli/NC_000913.3/seq/NC_000913.3.fa
index   MG1655_v2       /groups/bcbio/bcbio/genomes/Ecoli/MG1655_v2/seq/MG1655_v2.fa
index   MG1655_virus    /groups/bcbio/bcbio/genomes/Ecoli/MG1655_virus/seq/MG1655_virus.fa
index   MB2455  /groups/bcbio/bcbio/genomes/Ecoli/MB2455/seq/MB2455.fa
index   k12     /groups/bcbio/bcbio/genomes/Ecoli/k12/seq/k12.fa
index   UMD3.1  /groups/bcbio/bcbio/genomes/Btaurus/UMD3.1/seq/UMD3.1.fa
index   ASM294v2        /groups/bcbio/bcbio/genomes/spombe/ASM294v2/seq/ASM294v2.fa
index   ASM284v2.25     /groups/bcbio/bcbio/genomes/Spombe/ASM284v2.25/seq/ASM284v2.25.fa
index   DQ900900.1      /groups/bcbio/bcbio/genomes/haD37/DQ900900.1/seq/DQ900900.1.fa
index   hg19-ercc       /groups/bcbio/bcbio/genomes/Hsapiens/hg19-ercc/seq/hg19-ercc.fa
index   FGSC_A4 /groups/bcbio/bcbio/genomes/Anidulans/FGSC_A4/seq/FGSC_A4.fa
index   loxAfr3 /groups/bcbio/bcbio/genomes/Lafricana/loxAfr3/seq/loxAfr3.fa
index   BDGP6   /groups/bcbio/bcbio/genomes/Dmelanogaster/BDGP6/seq/BDGP6.fa
index   rn6     /groups/bcbio/bcbio/genomes/Rnorvegicus/rn6/seq/rn6.fa
```

Quality Control samples

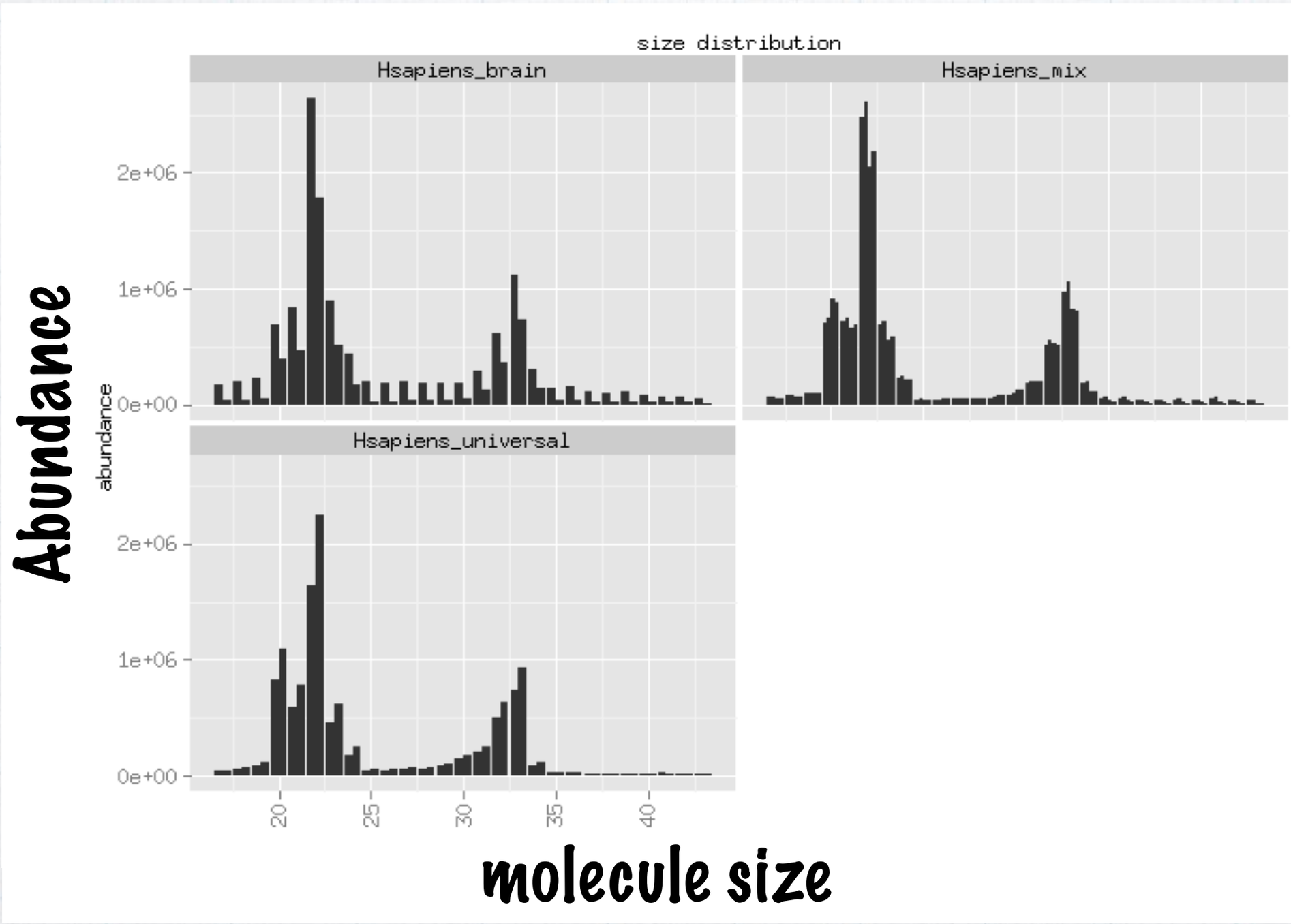




For each molecule:

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

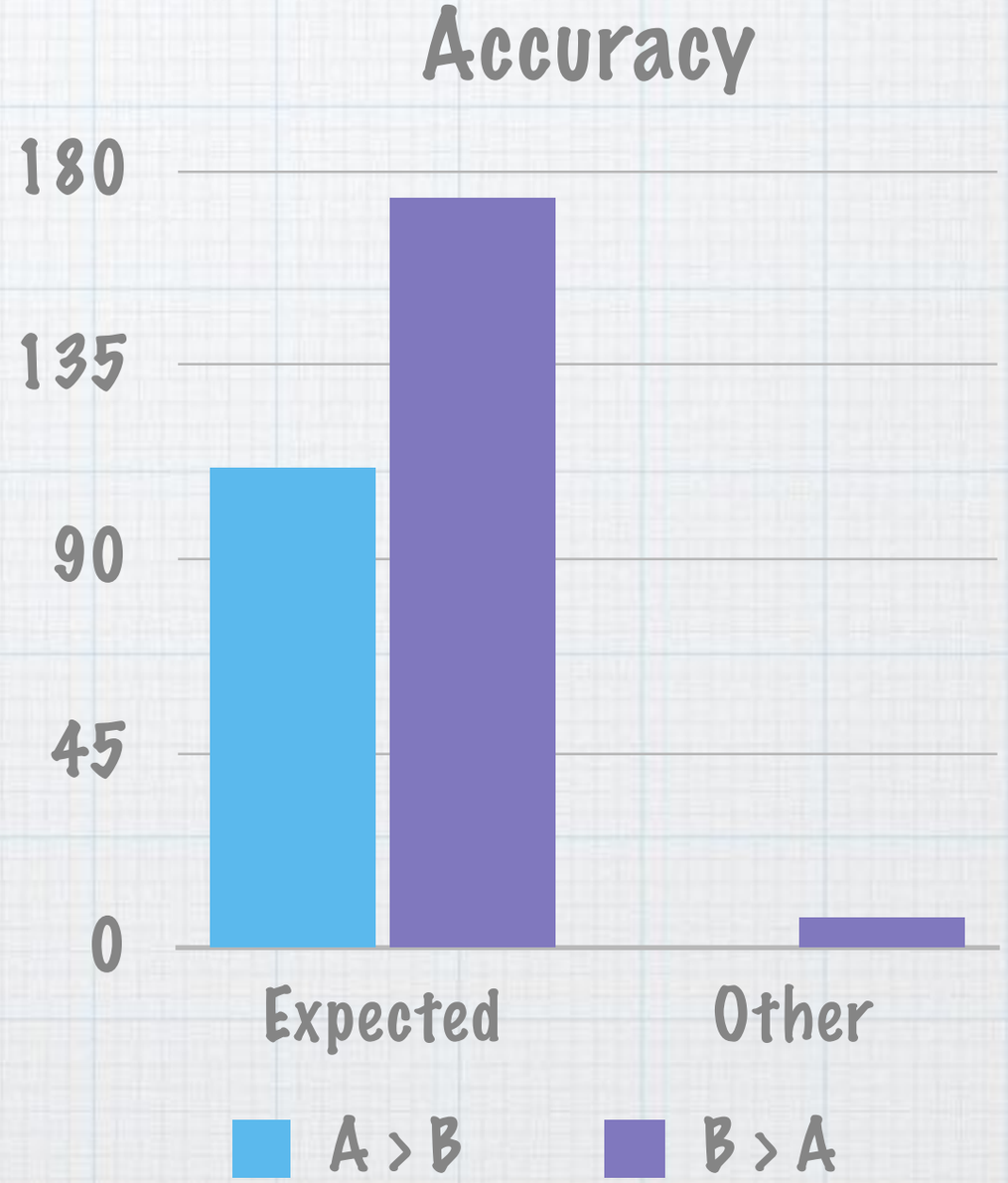
Good samples



miRNA quantification

miRNAs > 5 counts in average

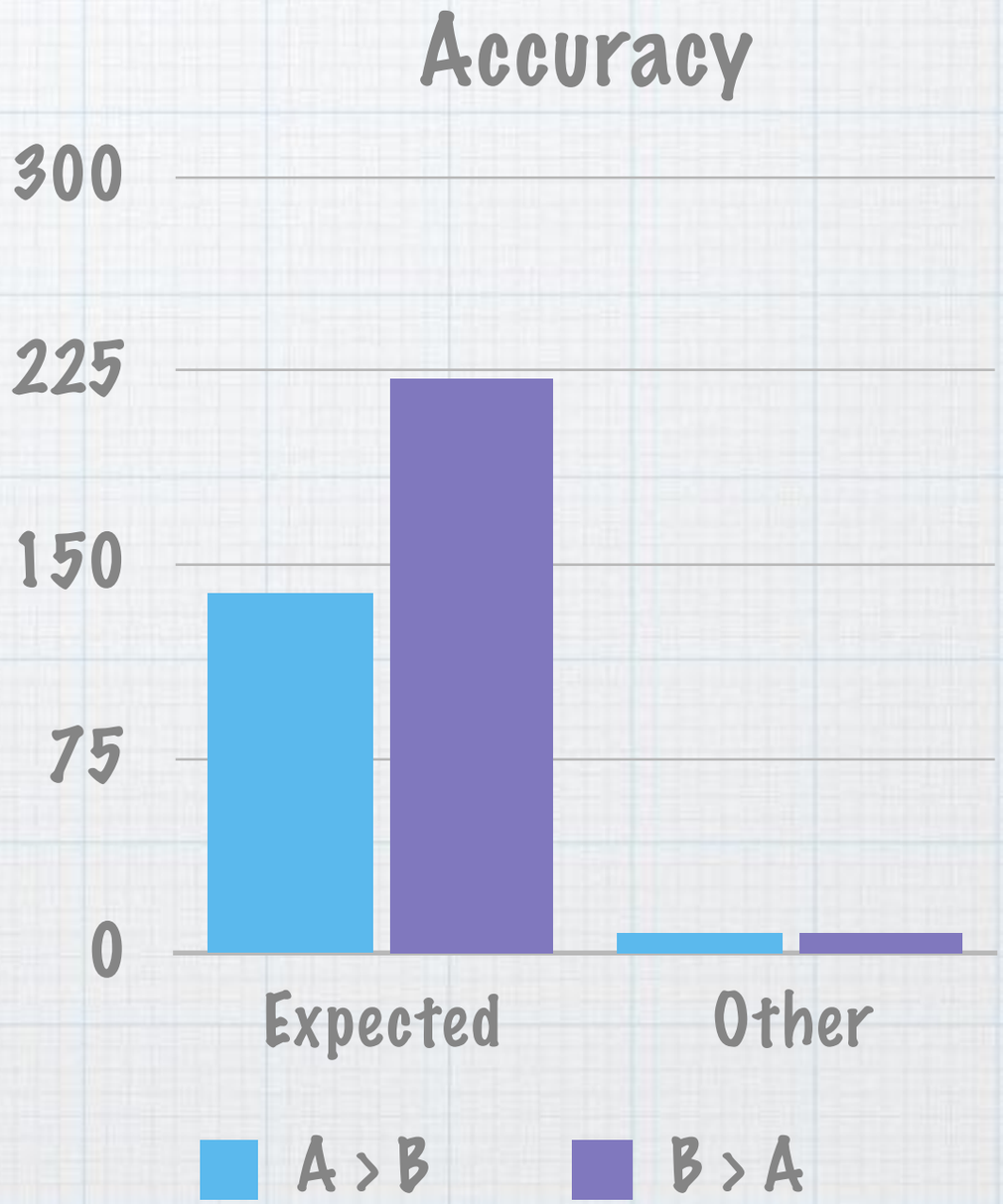
upper quantile normalization



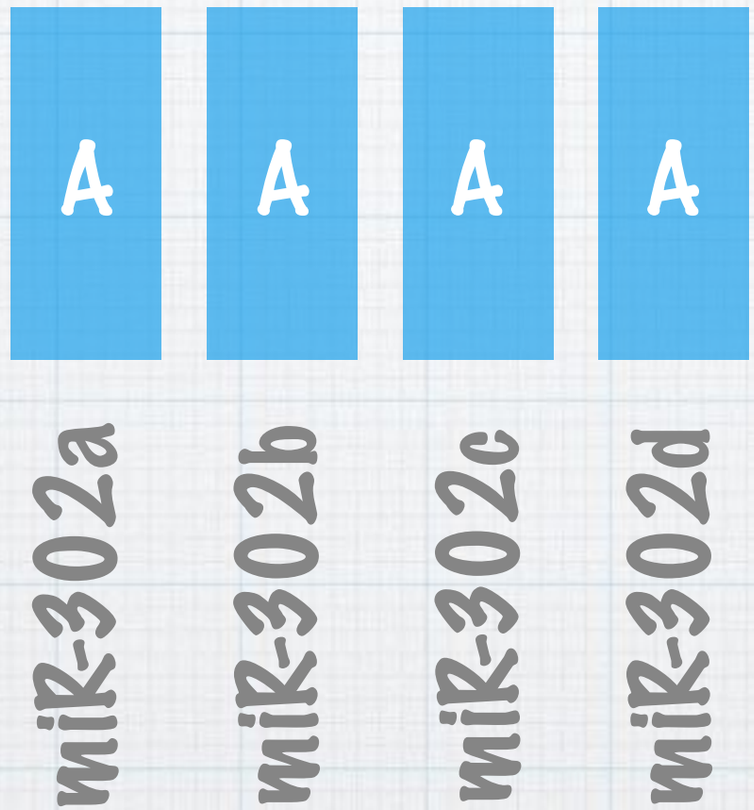
clusters quantification

expression > 5 counts in average

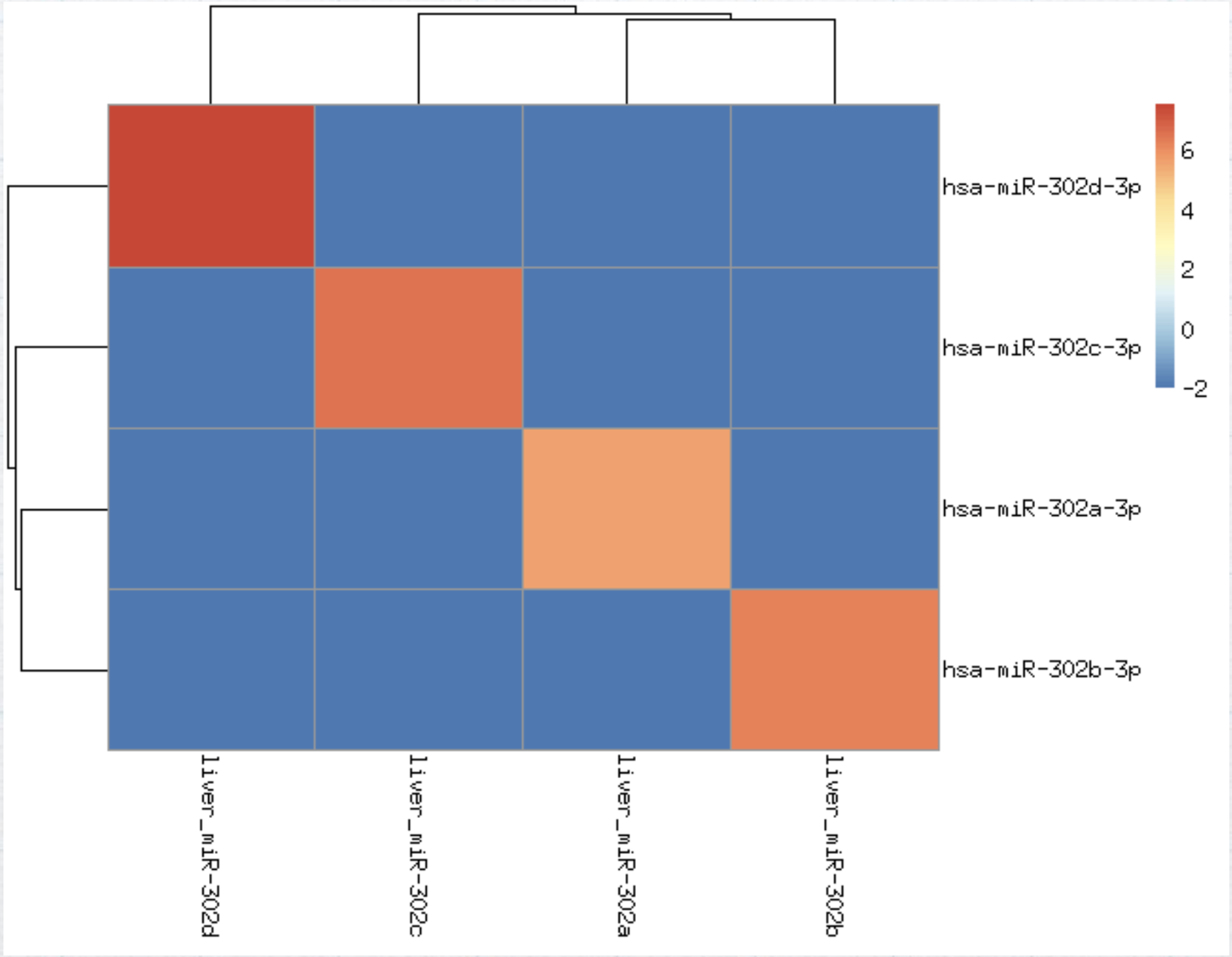
upper quantile normalization



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

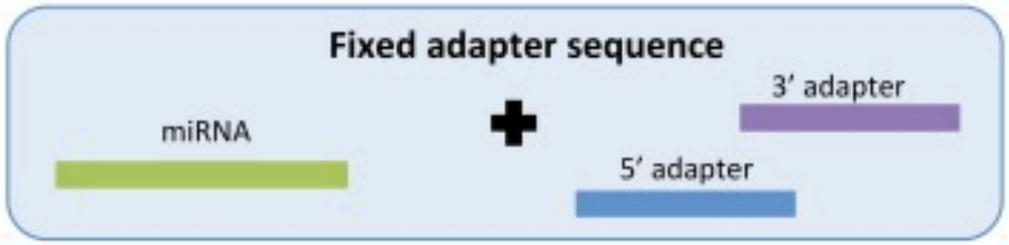
	Time (h)
organize	0:01
adapter	0:27
alignment	0:26
annotation	3:43
cluster + mirdeep2	4:15
qc	0:04

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

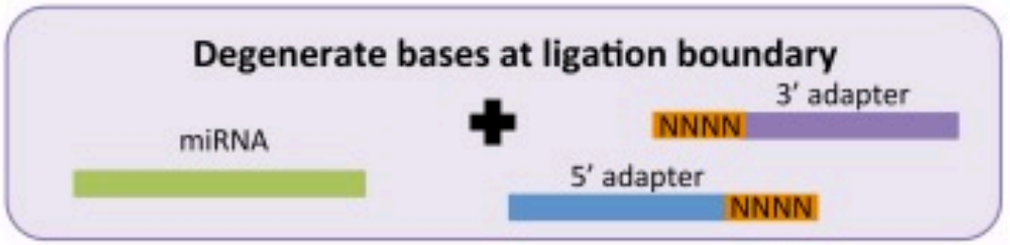
Protocols

Critical differences in small RNA library preparation protocols

Issue 1: Adapter ligation introduces bias



OR



Issue 2: Adapter dimers compete with small RNAs, reducing effective sequencing depth

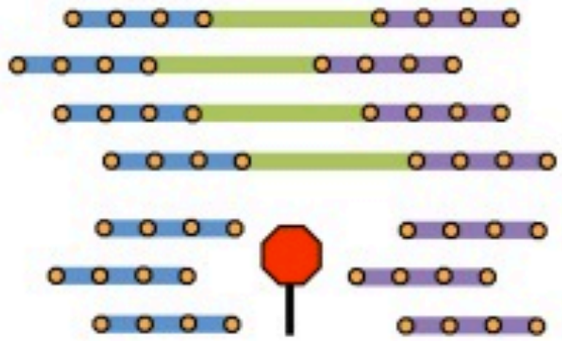
No removal

OR

Removal of excess adapters / dimers

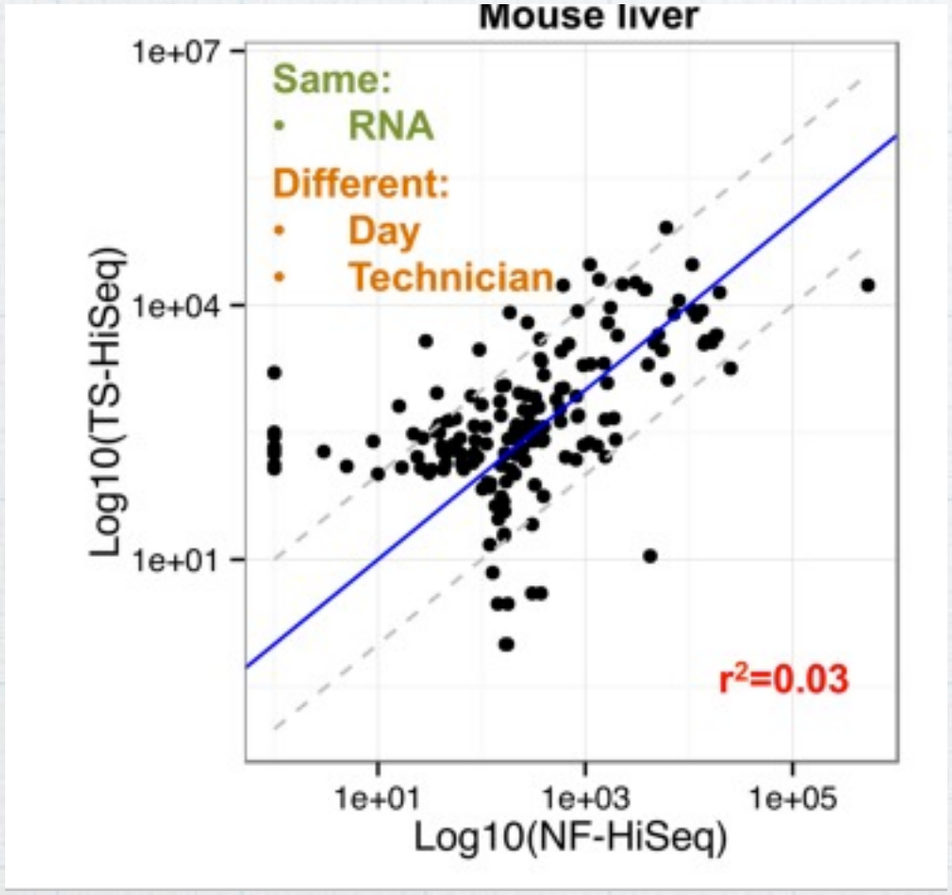
OR

Chemically modified adapters block dimer formation

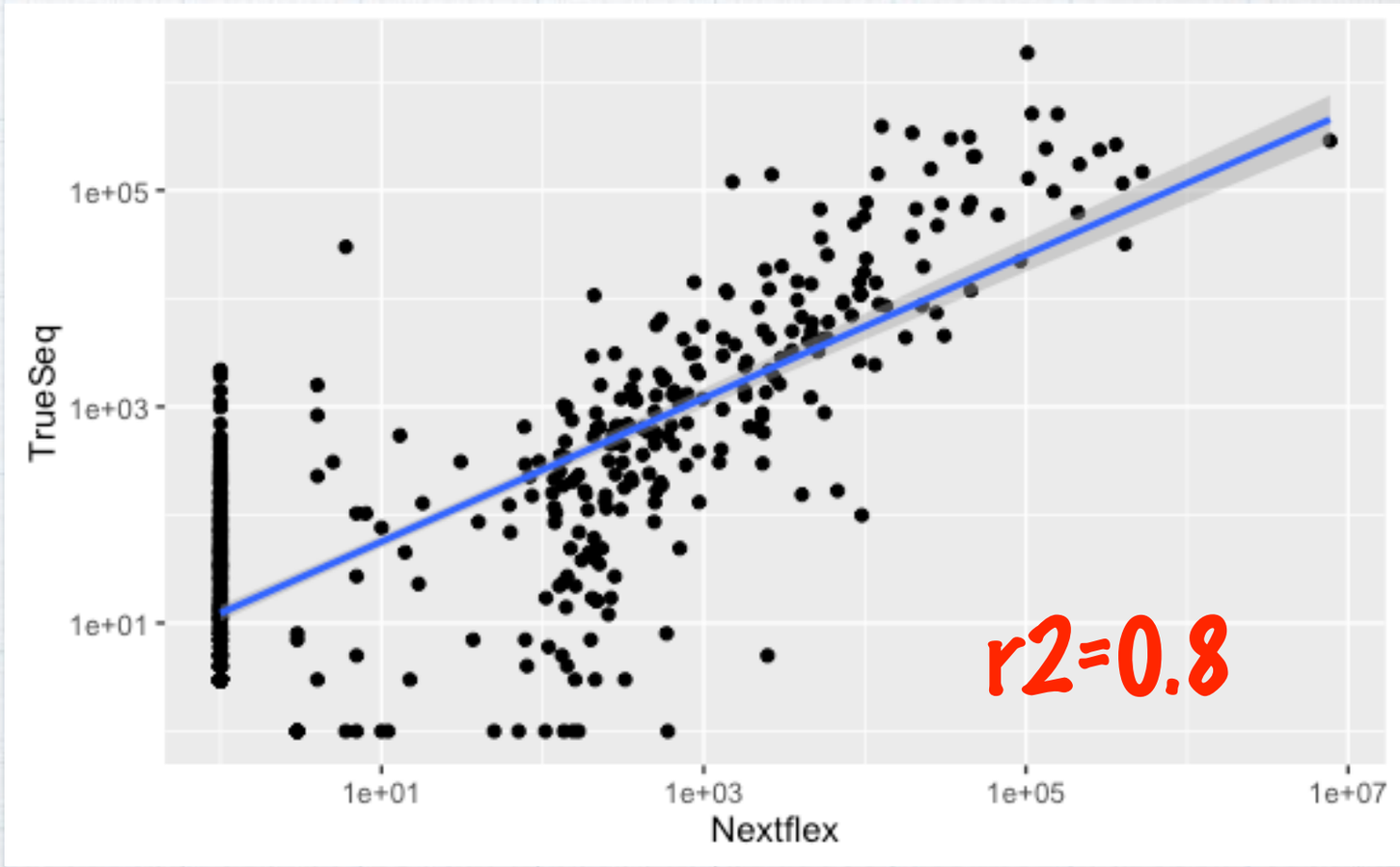


Protocol correlation

Paper figure

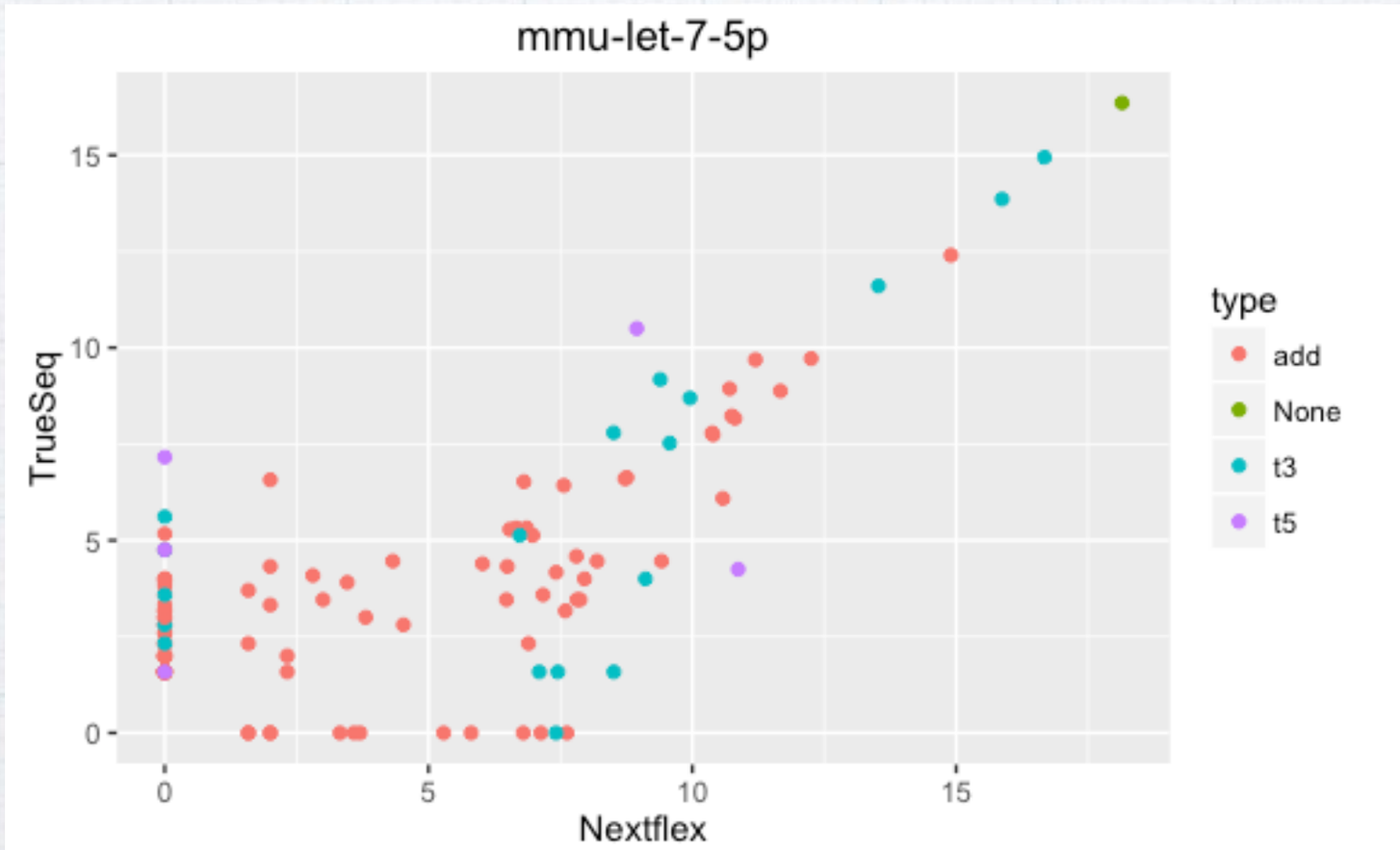


bcbio pipeline



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

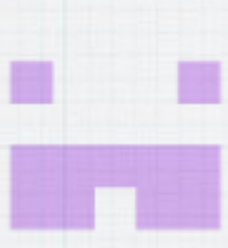
let7-a-5p miRNA



Caveats

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

open project for small RNA annotation and analysis



mirTOP

miRNA transcriptome open project

http://mirtop.github.io

http://mirtop.github.io

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incubator

★ 1 ⑂ 1

Where all ideas and discussions happen to lead to new repositories

Updated 3 days ago

standard formats
naming rules

best-practices

mirtop

Python ★ 0 ⑂ 0

command lines tool to annotate miRNAs with a standard mirna/isomir naming

Updated 3 days ago

miRNAs, tRNAs ...

miRTOP.github.io

CSS ★ 0 ⑂ 0

project for small RNA standard annotations

Updated on Mar 29

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Founded Mar 27, 2015

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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.