miRNA and isomiR annotation

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Agenda

- miRNA and isomiR definition
- miRNA mapping comparison
- isomiR annotation strategy
- isomiR annotation from BAM files
- isomiR analysis in R

miRNA

RNA molecules of 18-36 nts long with regulation function

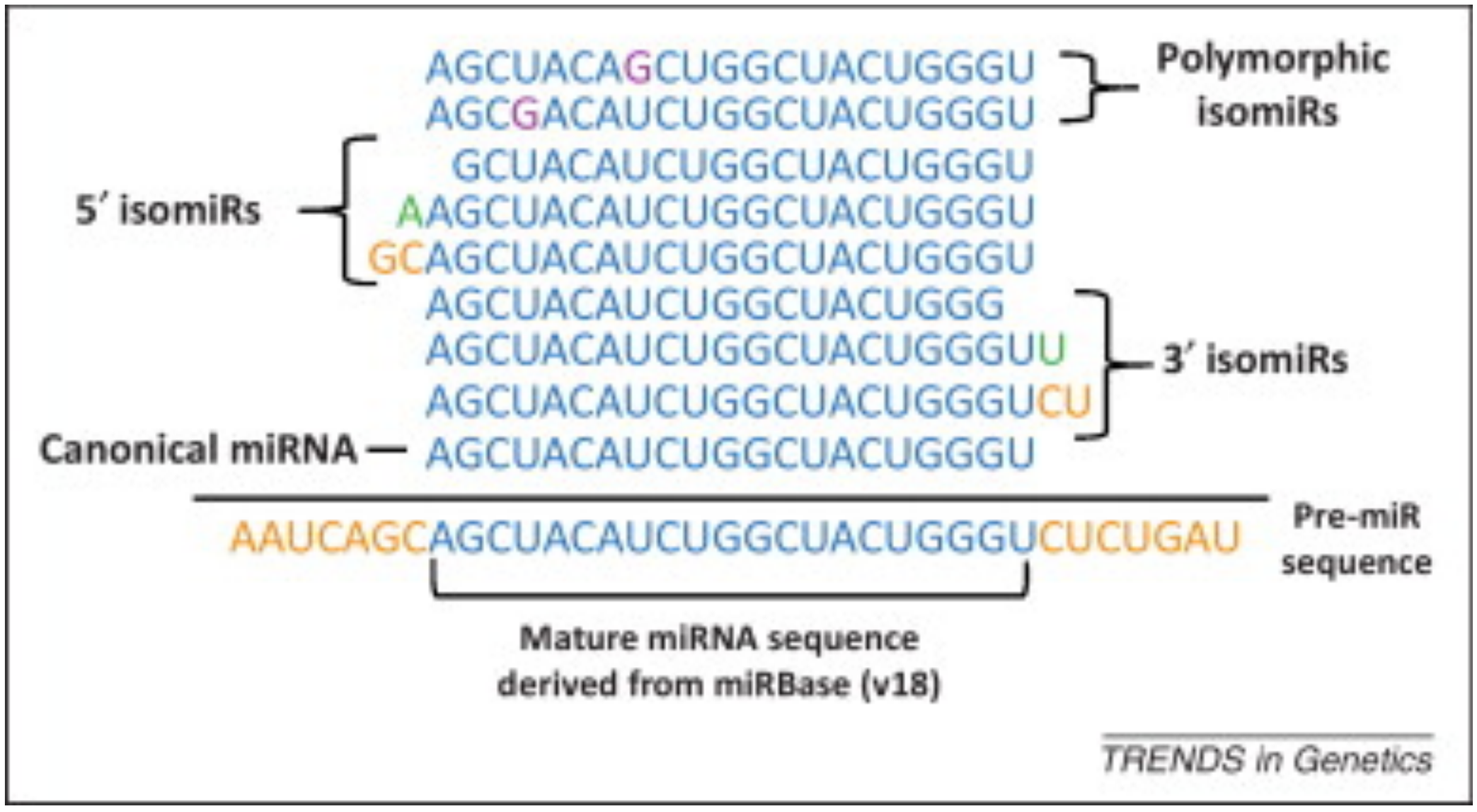
isomiR

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

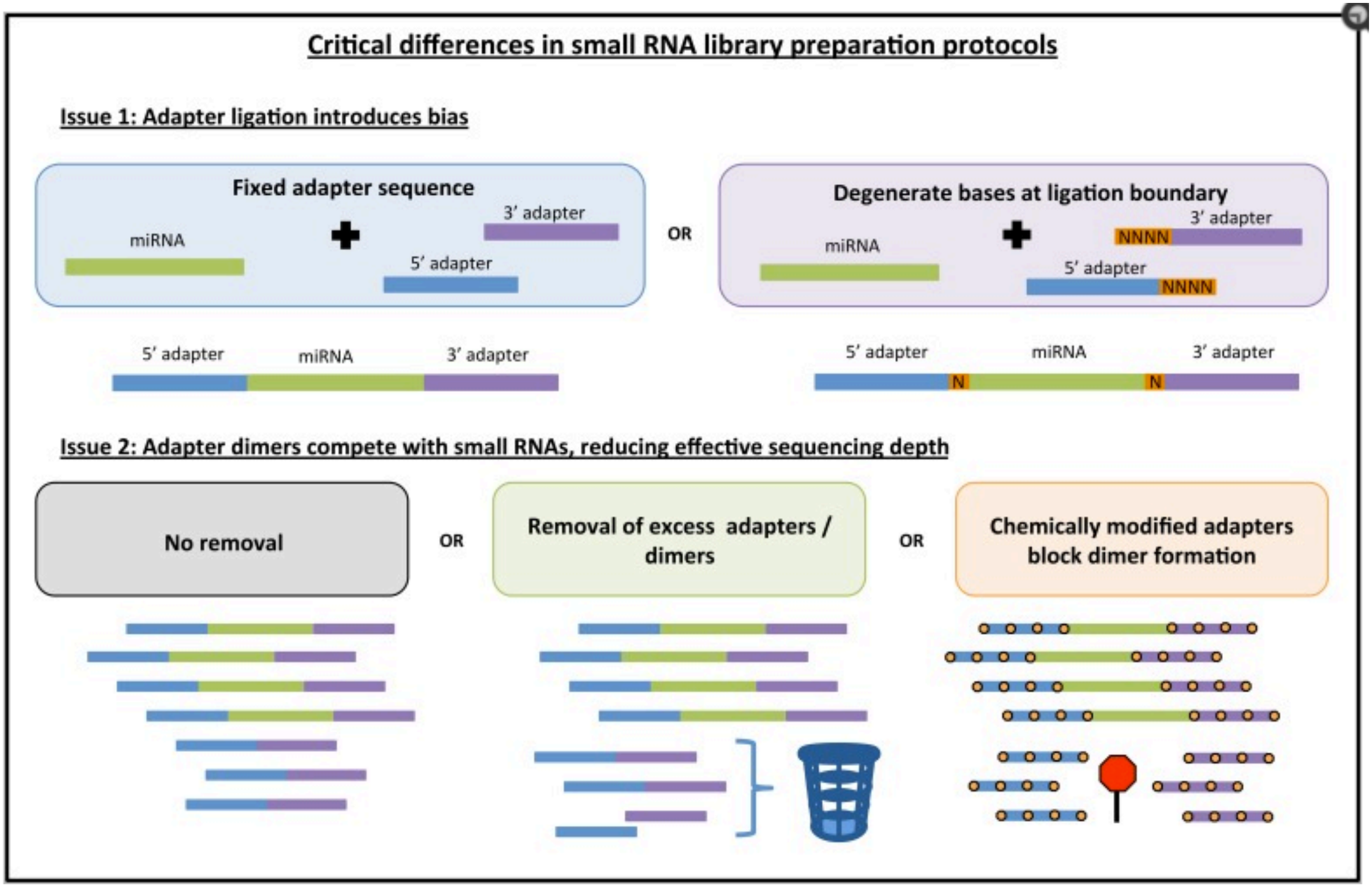
```
CUCCGGUGCCUACUGAGCUGAUAUCAGUUCUCAUUUUACACACUGGCUCAGUUCAGCAGGAACAGGAG
((((.((.(((.((((((((((..(((((.............))))).)))))))))).))).)).)))) (-26.32)
```

precursor

Types of isomiRs

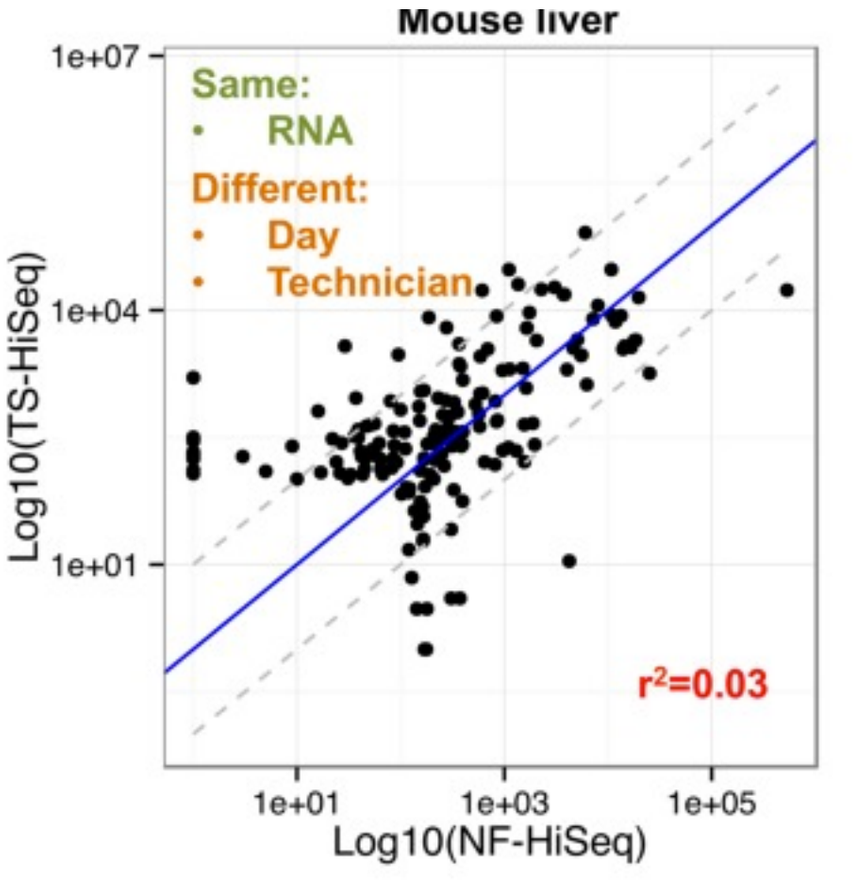


Protocols

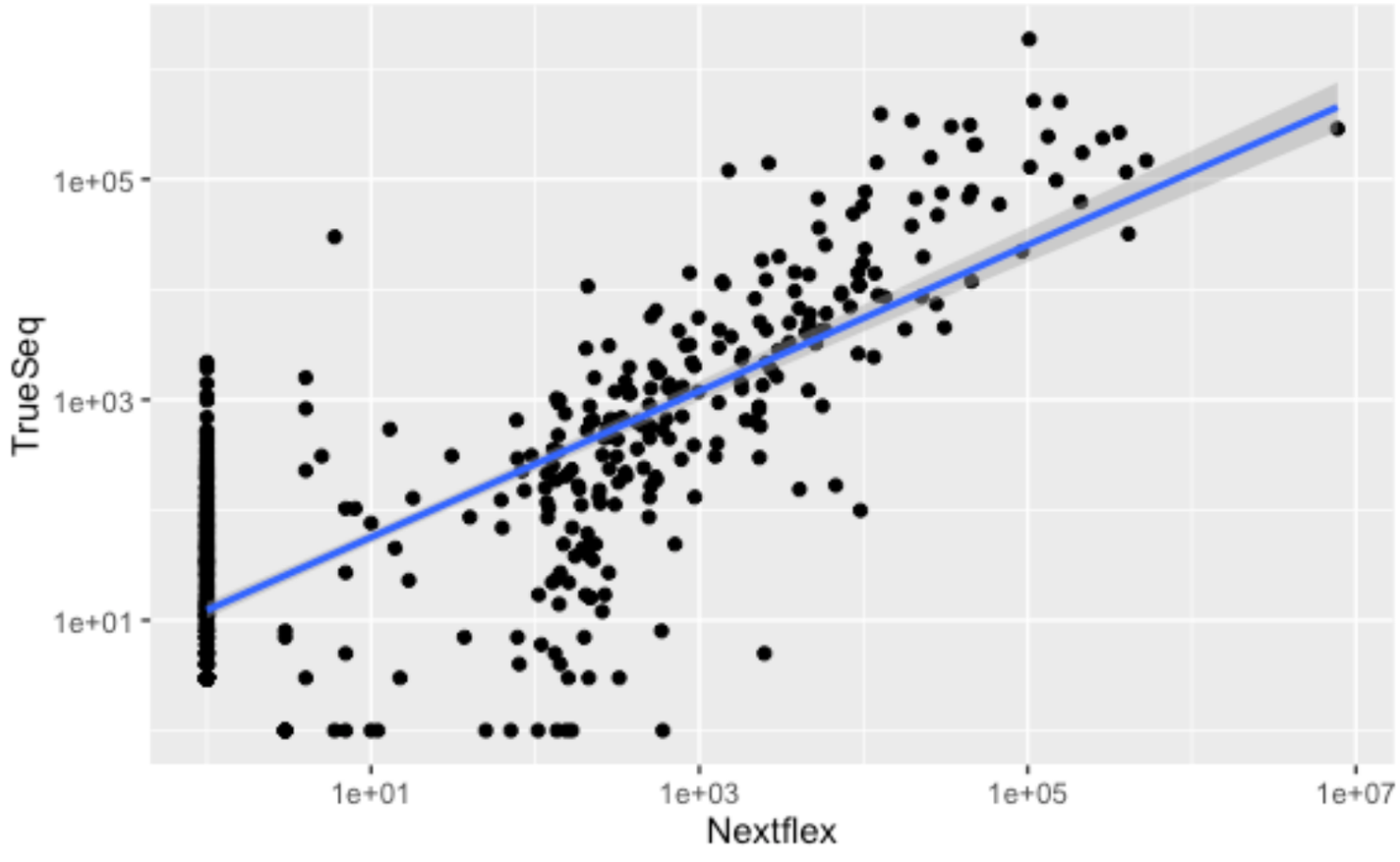

Critical differences in small RNA library preparation protocols
Issue 1: Adapter ligation introduces bias
Fixed adapter sequence
miRNA
3' adapter
5' adapter
OR
Degenerate bases at ligation boundary
miRNA
3' adapter
NNNN
5' adapter
NNNN
5' adapter
miRNA
3' adapter
5' adapter
N
miRNA
N
3' adapter
Issue 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 comparison

Paper figure

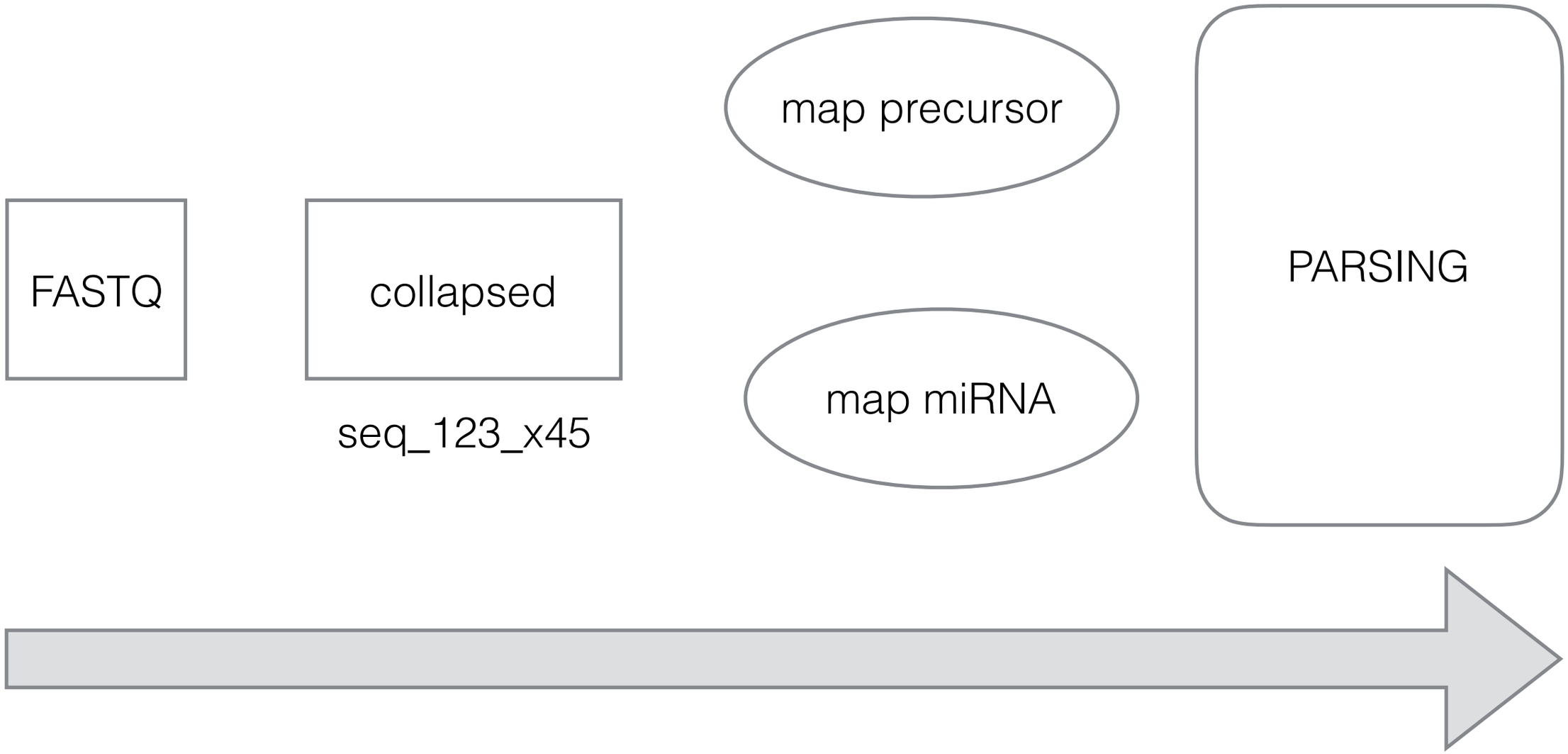


bcbio pipeline



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

miRNA mapping



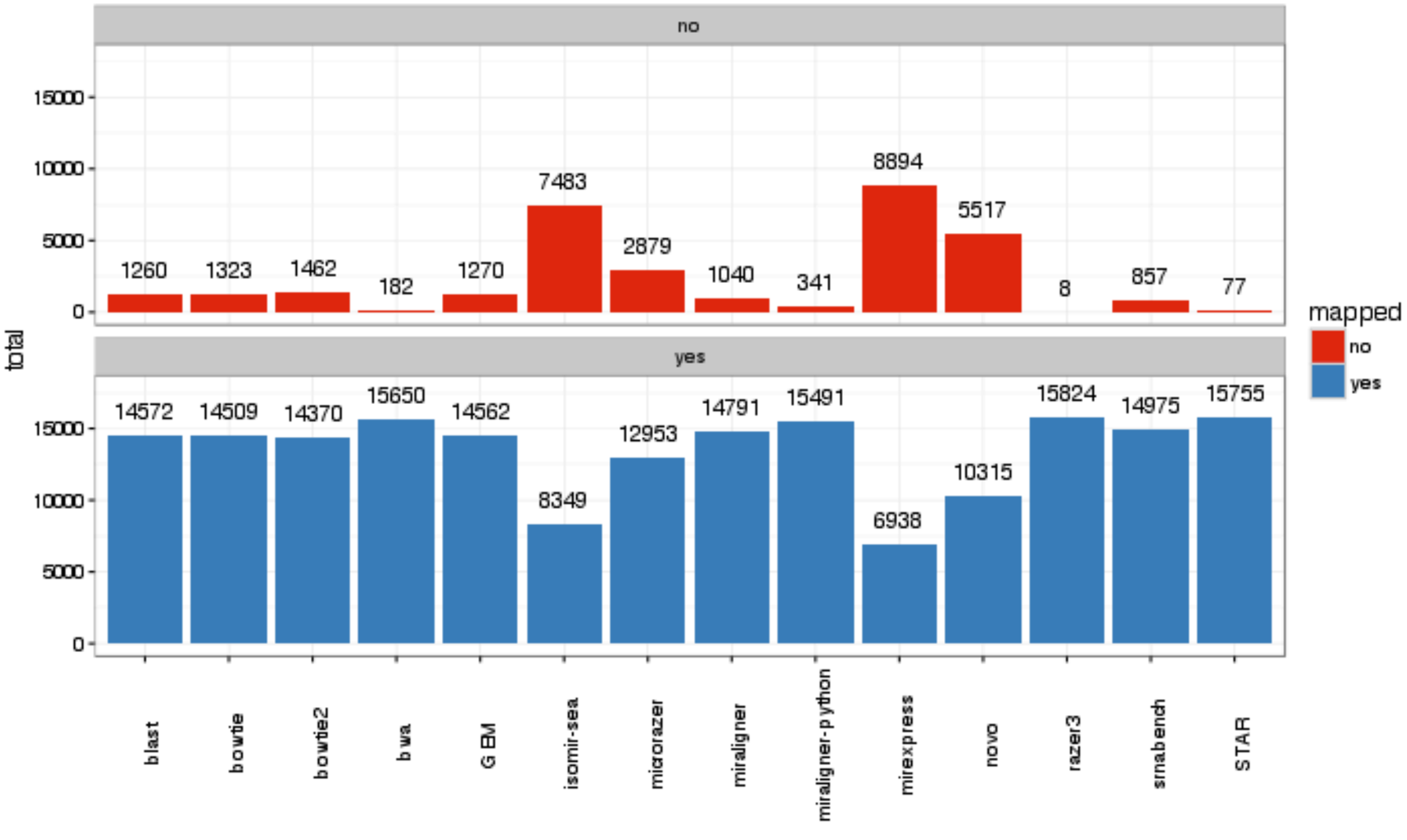
Benchmark

- simulation of miRNAs/isomiRs (~ 16000)
- mapping with different tools
- compare miRNA detection and accuracy

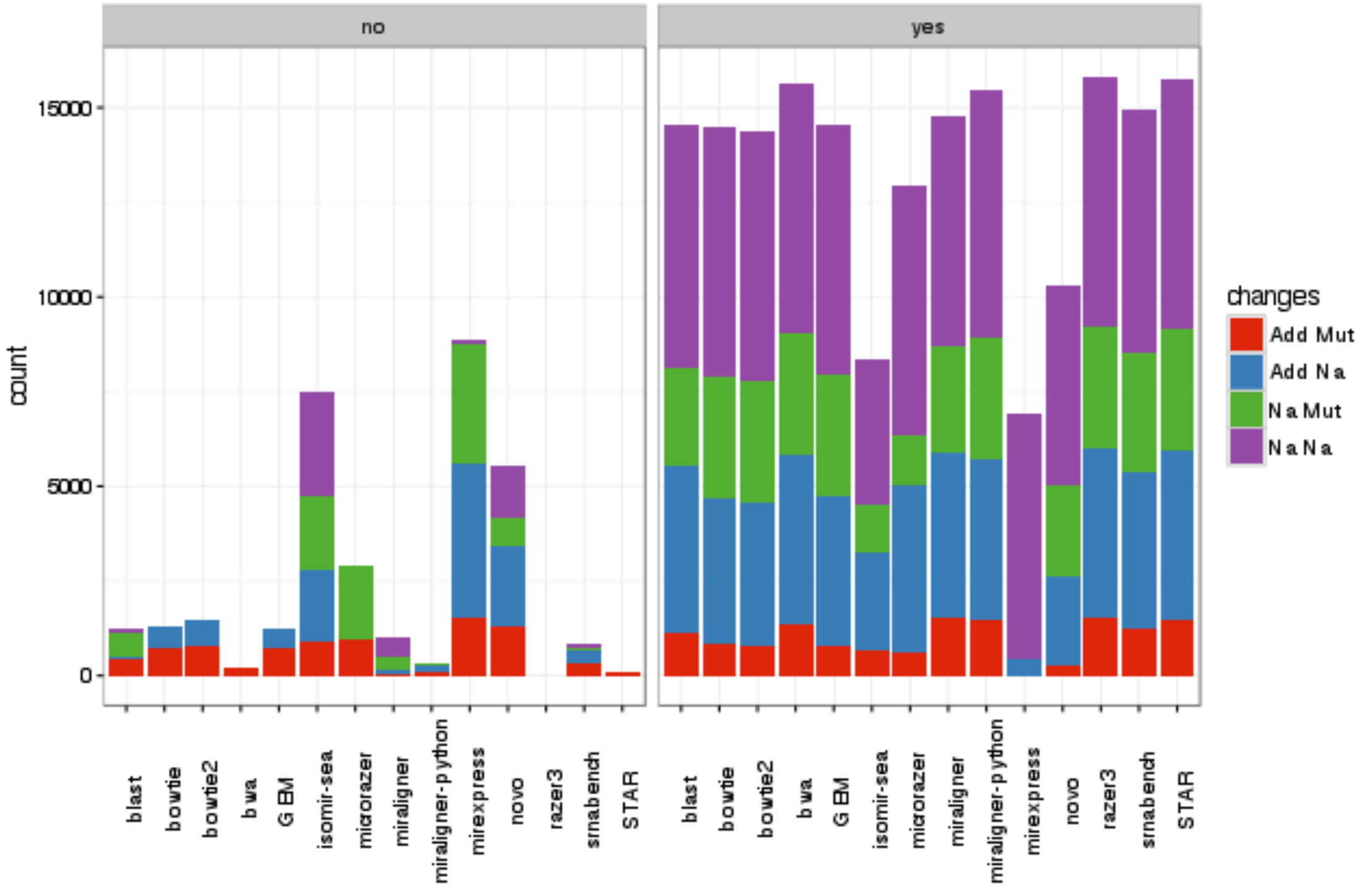
tools compared

bowtie, bowtie2, blast, GEM, microzer, miraligner, miraligner-python, novoaling, razer3, STAR, megablast

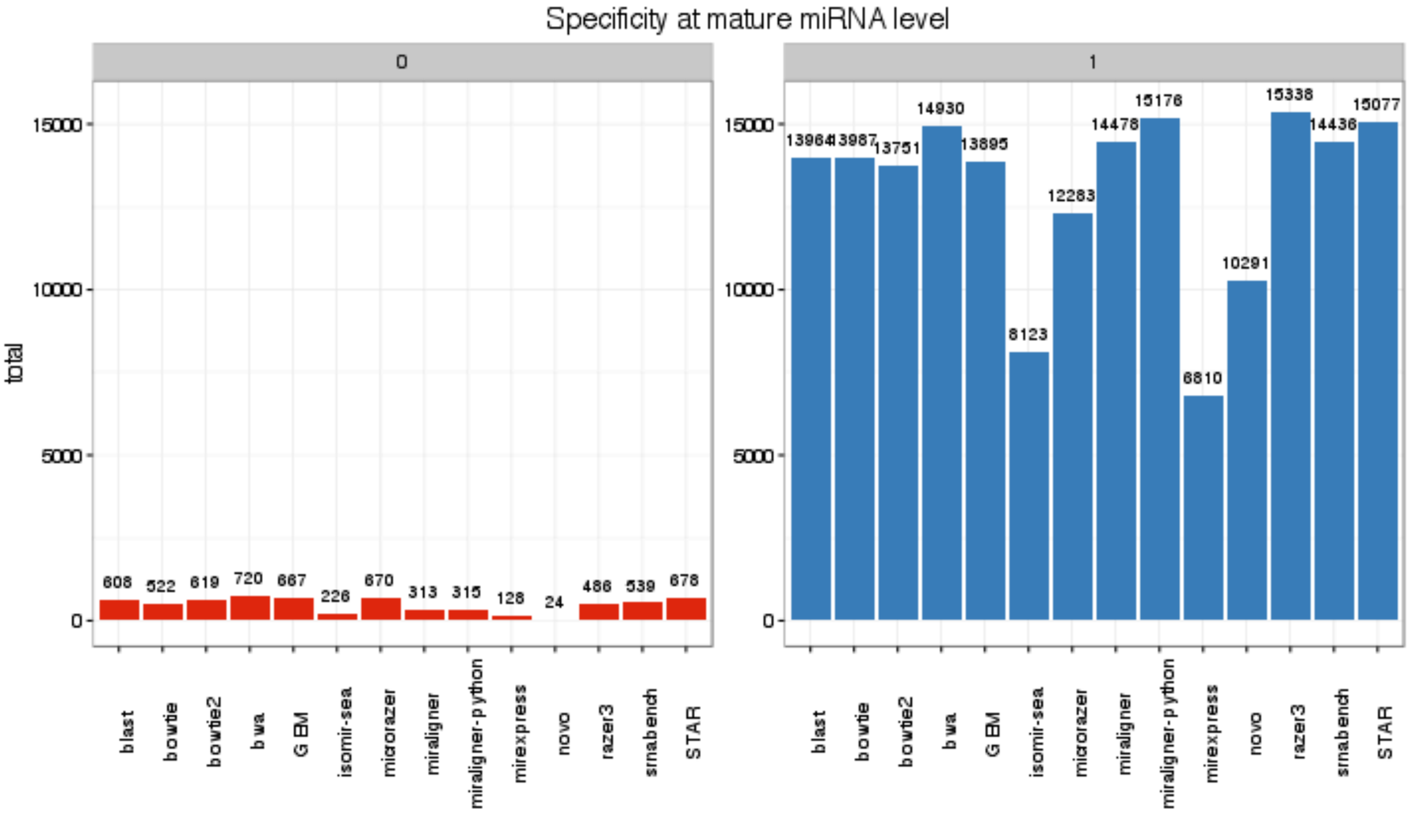
miRNA detection



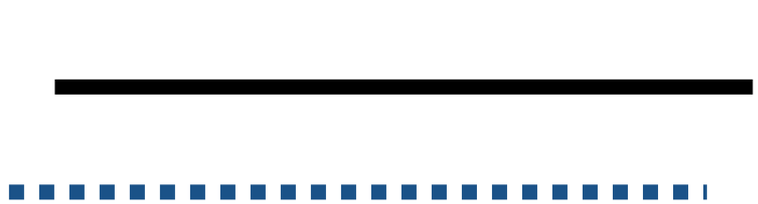
Cause of missing



miRNA accuracy



isomiR annotation



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

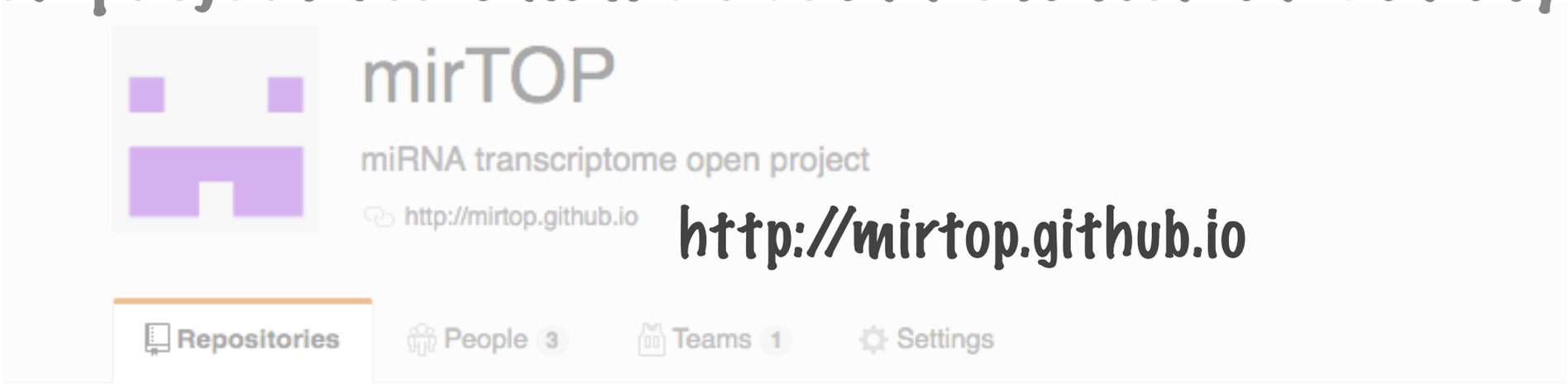
tools compared

bowtie, bowtie2, GEM, miraligner, novoaling, razer3, STAR

```
mirtop annotate --sps hsa
--hairpin ../hairpin.hsa.fa
--mirna ../miRNA.str
-o  gem_out ../gem/sim.21.hsa.sam
```

You can input multi-bam files at the same time

open project for small RNA annotation and analysis



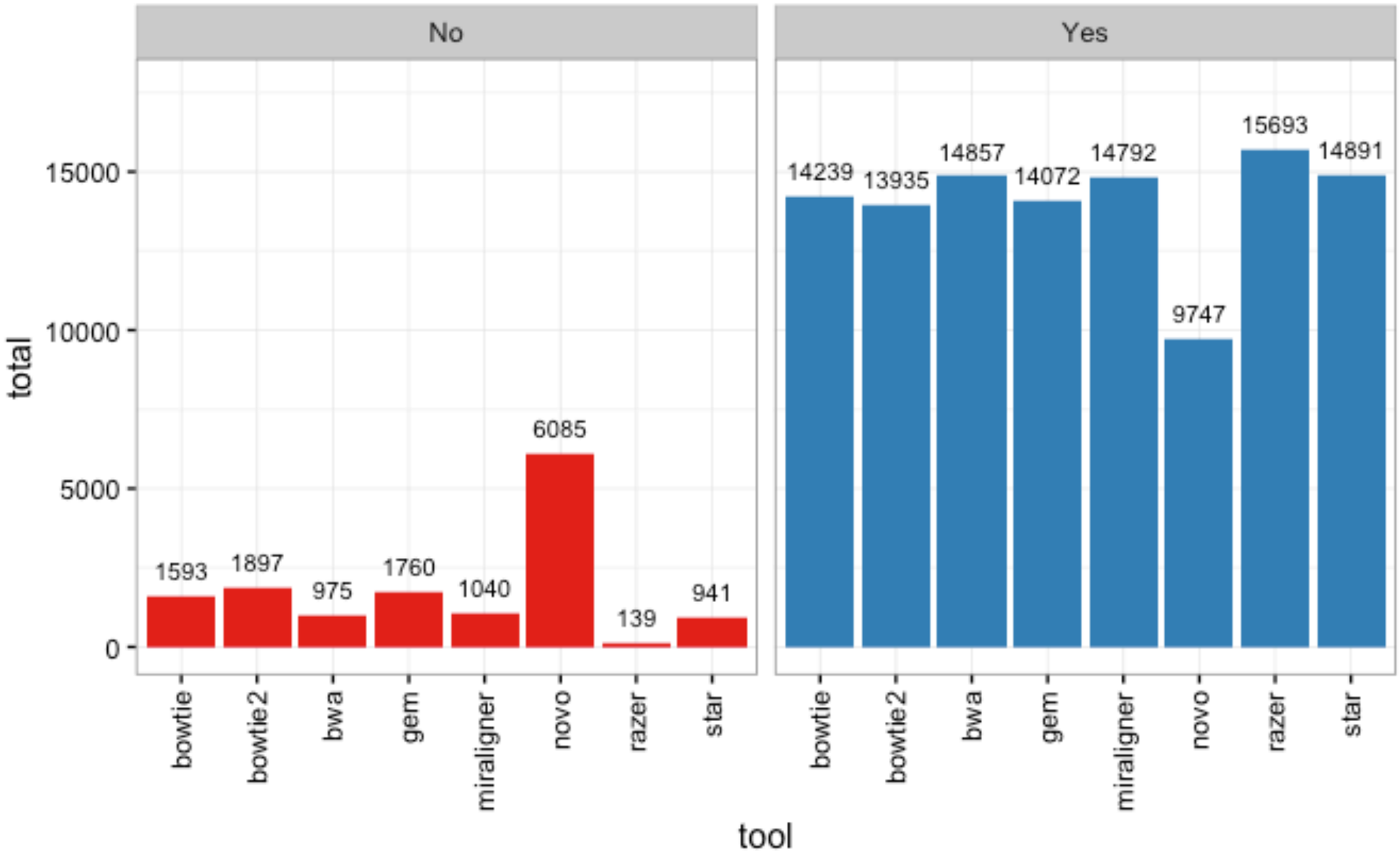
http://mirtop.github.io

standard formats
naming rules

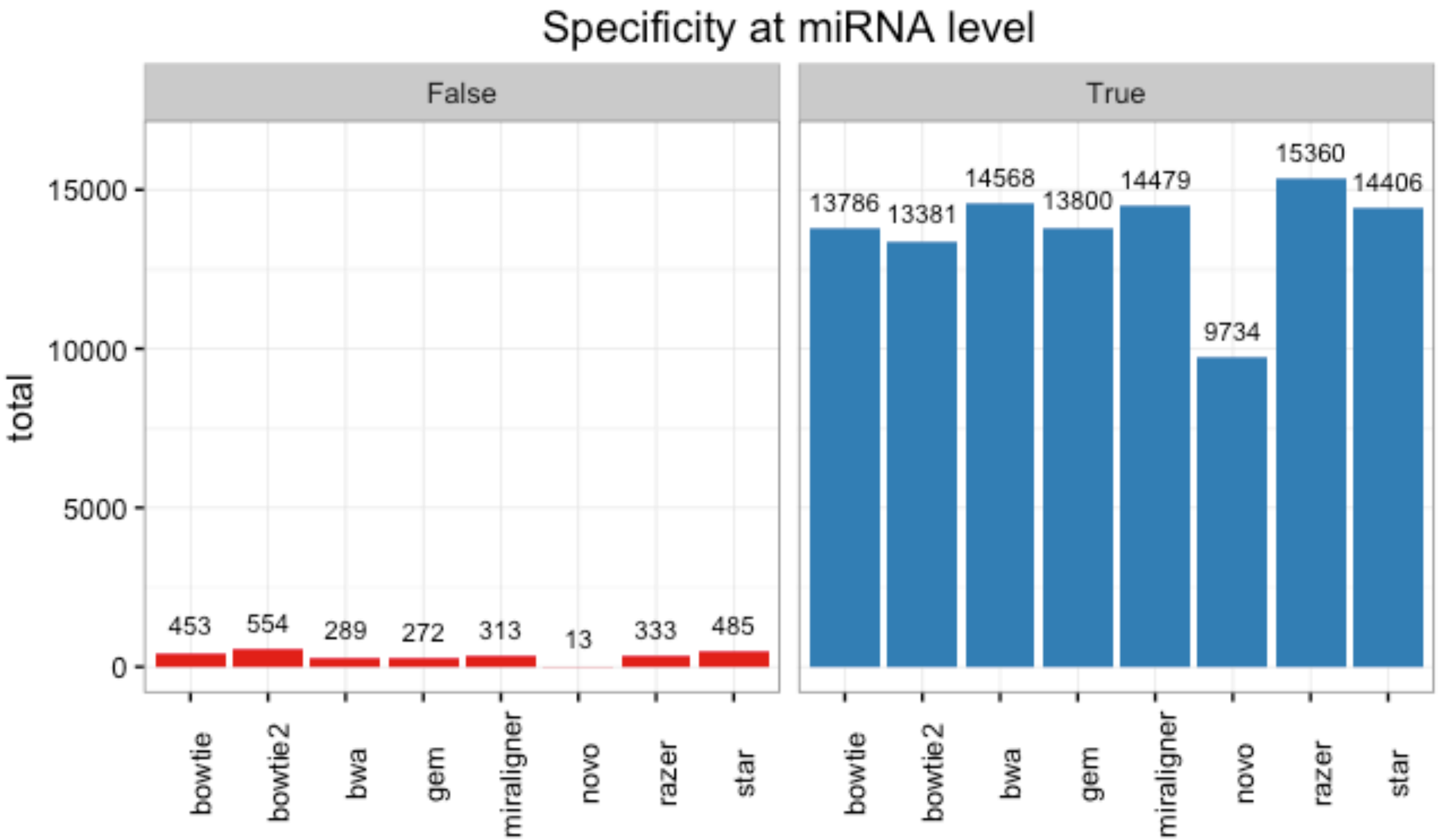
best-practices

miRNAs, tRNAs ...

isomiR from BAM file



isomiR comparison



miRNA with R

- what to consider as input for the DE tools
- isomiR characterization
- query the data
- Supervised clustering with feature selection

Input

seq	name	freq	mir	start	end	mism	add	t5	t3	s5	s3	DB	precu
AGGTCGACCGTGTTATATTCG			seq_100056_x3	3		rno-miR-369-5p		14	34	3GA	0	0	c
TTGAAAGGCTGTTTCTTGGTT			seq_100058_x15	15		rno-miR-488-3p		49	68	0	T	0	c
TACTGCACTCGTCCCGGCCT			seq_100063_x3	3		rno-miR-92b-3p		52	71	3CT	0	0	cc
TTGAAAGGCTGTTTCTTGGTG			seq_100069_x33	33		rno-miR-488-3p		49	68	0	G	0	c
CTACTTCACAACACCAGGGTTA			seq_10011_x13	13		rno-miR-138-1-3p			64	83	0	TA	cgg
TGAGGTAGTAGTTTGTGCTGAT			seq_100122_x3	3		rno-let-7i-5p		6	25	0	AT	0	tt
TCTACAGTGCACGTGCCTCCA			seq_100131_x5	5		rno-miR-139-5p		7	27	16CT	0	0	g
ACGTCATCGTCGTCATCGTTA			seq_100132_x5	5		rno-miR-598-3p		49	69	0	0	t	0
TGTGACAGATTGATAACTGAAAG			seq_100147_x11	11		rno-miR-542-3p		49	71	0	0	0	G
CTGGCCCTCTCTGCCCTTCCGCAT			seq_100148_x9	9		rno-miR-328a-3p		48	68	0	CAT	0	
NGAATTGTGGCTGGACATCTGT			seq_100185_x4	4		rno-miR-219a-2-3p		62	83	1NA	0	0	
GGAAGACTAGTGATTTTATTGT			seq_100227_x5	5		rno-miR-7a-5p		20	41	18AG	0	t	0
AACATTTATTGCTGTCGGTGGGT			seq_100277_x8	8		rno-miR-181b-5p		15	37	7TC	0	0	0

Processing annotation

```
<<package-plot-iso,message=FALSE,eval=FALSE>>=
ids <- IsomirDataSeqFromFiles(fn_list, design=de)
@
```

Order in fn_list should be the same than in the design data.frame

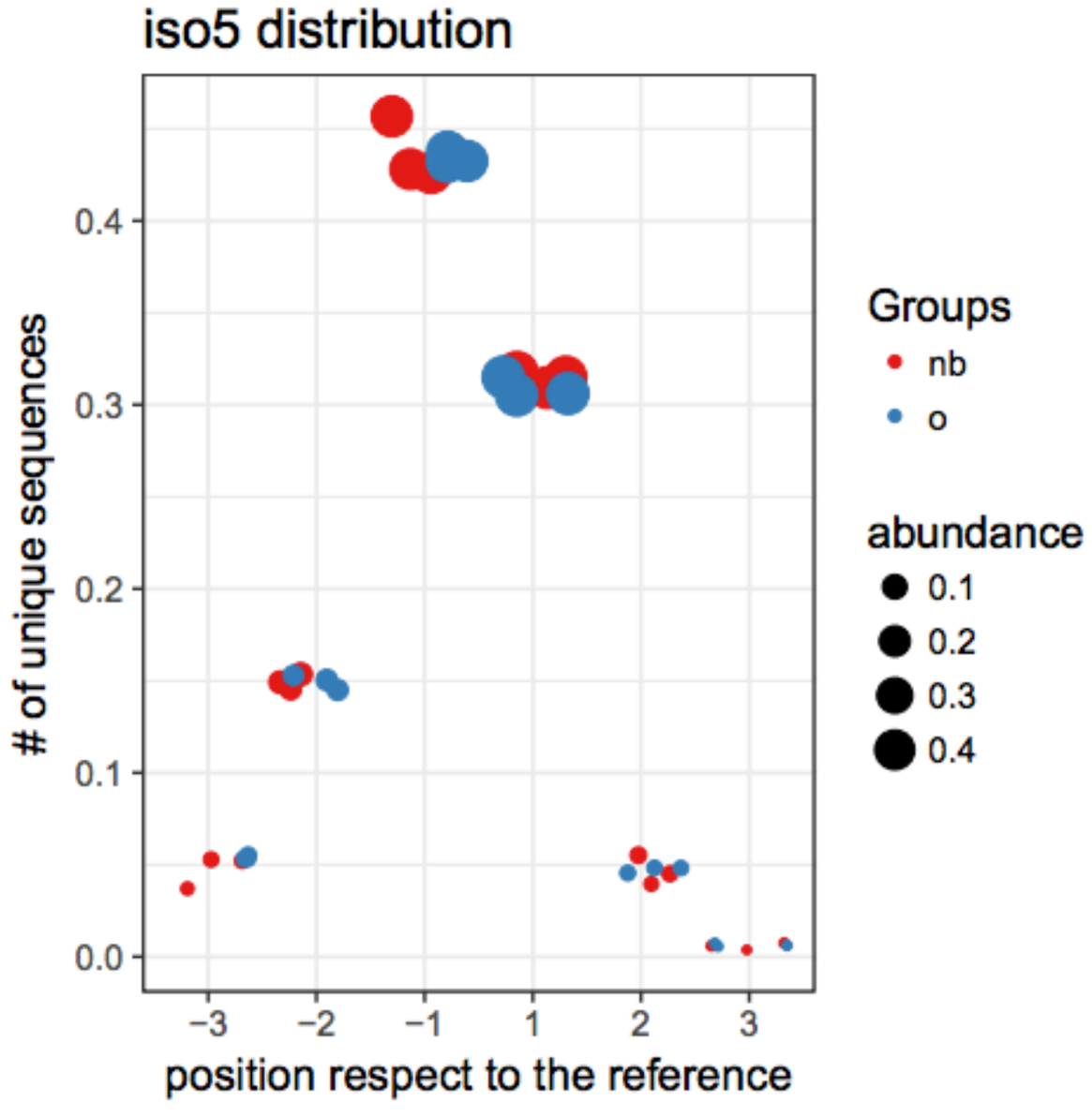
```
> fn_list
[1] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/isomiRs/extra/sample1.m
irna"
[2] "/Library/Frameworks/R.framework/Versions/3.3/Resources/library/isomiRs/extra/sample2.m
irna"
```

```
> de
   condition
f1   newborn
f2   newborn
```

isomeR figures

Higher in figure means different sequences with that isomiR type

Bigger the size of the dot means expression of that isomiR type is higher

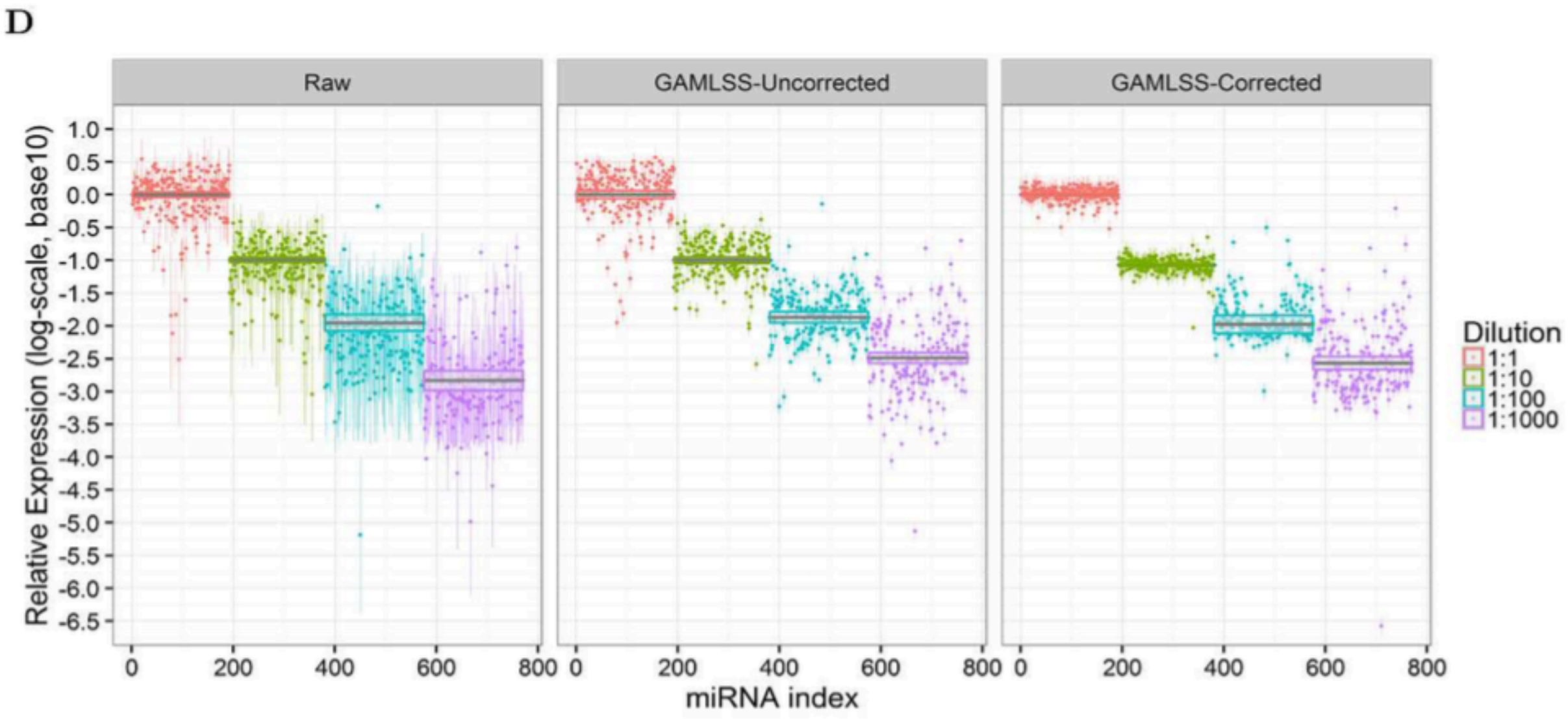


DE analysis

- DESeq2 as in RNAseq
- Sometimes filtering miRNA by group can help to increase power.
- limma-voom strategy should work equally

Correcting quantification

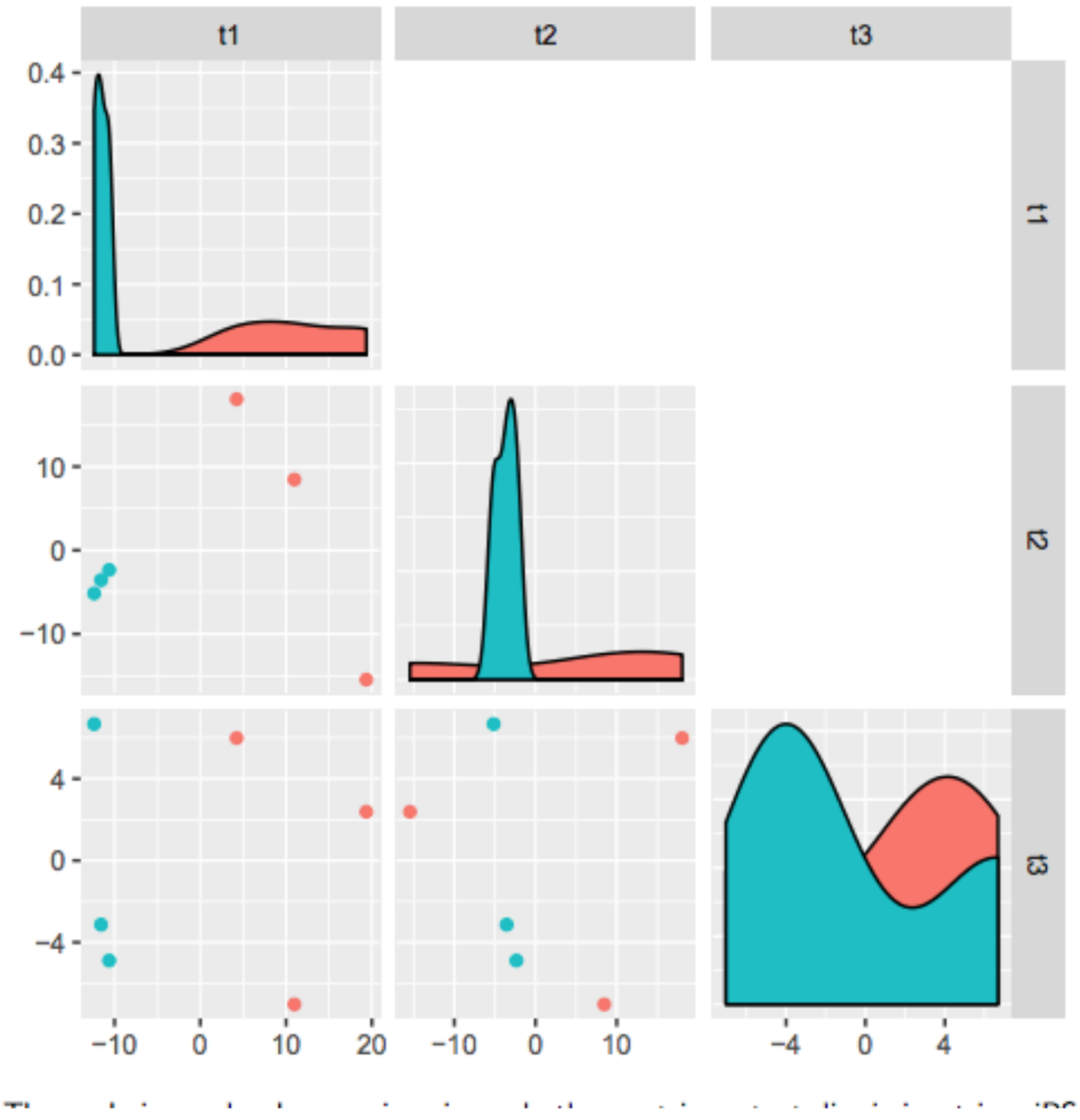
PCR amplification and ligase bias correction factors



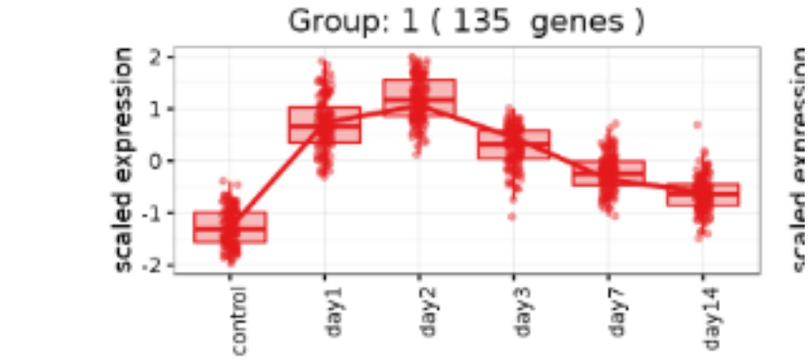
Clustering

```
ids = isoCounts(ids, iso5=TRUE, minc=10, mins=6)
ids = isoNorm(ids)
pls.ids = isoPLSDA(ids, "condition", nperm = 2)
df = isoPLSDAplot(pls.ids)
```

```
> head(pls.ids$vip)
                                variable      VIP
hsa-let-7c-5p.t5:GT hsa-let-7c-5p.t5:GT 1.518223
hsa-let-7d-5p.t5:0   hsa-let-7d-5p.t5:0 1.533554
hsa-let-7f-5p.t5:tg hsa-let-7f-5p.t5:tg 1.421619
hsa-let-7i-5p.t5:0   hsa-let-7i-5p.t5:0 1.356090
hsa-let-7i-5p.t5:t   hsa-let-7i-5p.t5:t 1.525162
hsa-miR-1.t5:0           hsa-miR-1.t5:0 1.383350
```



mRNA-miRNA interaction



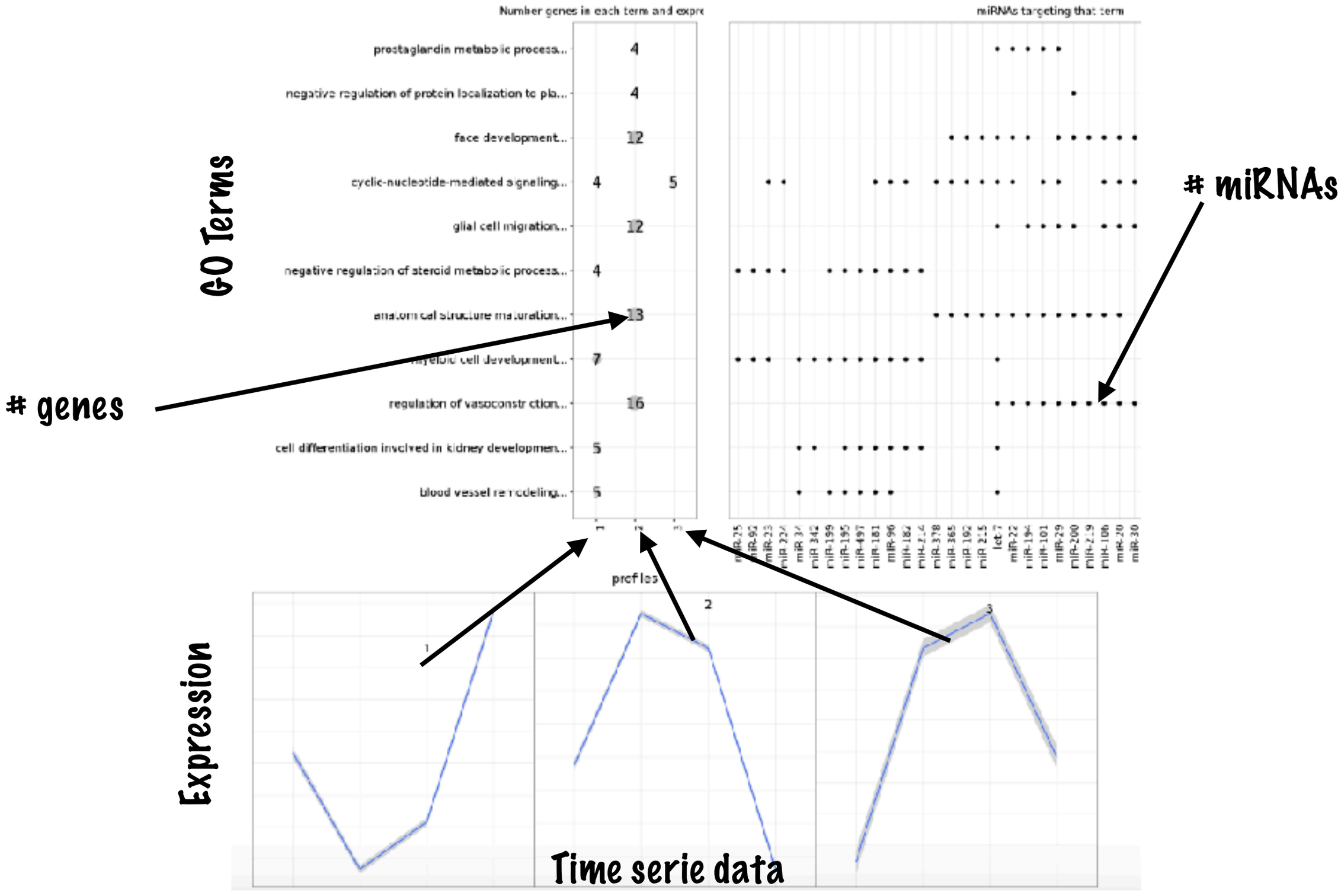
+

GO enrichment

targetscan targets

negative regulation

Output of target analysis



Conclusion

- mapping to precursor and parsing with mirtop
- participate in the open project for miRNA annotation
- analyze isomiRs as well (isomiRs)
- DESeq2 for differential expression (my experience)
- mRNA-miRNA paired data helps incredible for downstream analysis