

Small RNAs in Bull Fertility

Bull Fertility Assessment

“Classical” Evaluation

Common parameters

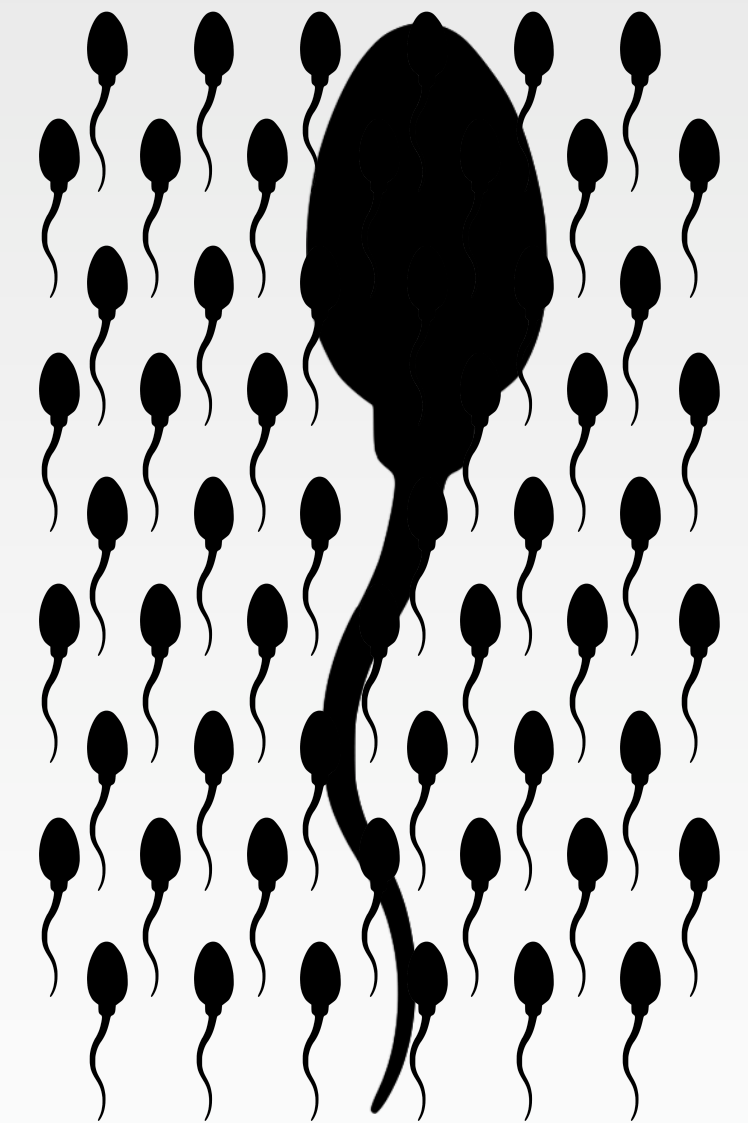
- Performed manually or with software
 - CASA (Computer Assisted Semen Analysis)
- Allow for quality estimation
 - **< 60% of variation in fertility identified with these methods**



Motility



Morphology



Concentration

Are there other features in sperm/semen that reflect fertility?

“Ideal” Sperm Biomarkers

Measurable differences that correlate with fertility



Low Fertility



High Fertility

Potential Biomarkers

Small Non-coding RNAs

- ~ 15 – 35 nt RNA sequences
- Usually associate with Argonaute (AGO) family proteins
- Post-transcriptionally regulate the transcriptome (somatic genes, transposons)
- Experimentally linked to spermatogenesis and sperm quality
- “Functional biomarkers” of many (human) diseases
- Present in sperm

Small Non-Coding RNAs (sncRNAs)

Classes

MicroRNAs (miRNAs)

- ~20 nt RNA
- Silence mRNAs by RNAi
 - Associate with AGO proteins

tRNA-derived

- 18 – 50n nt RNA
- Recently implicated in fertility and found in bovine sperm

PIWI-interacting RNA (piRNA)

- 24–32 nt RNA
- Silence TEs + mRNAs by RNAi
 - Associate with PIWI (AGO) proteins
- Also recruit histone and DNA methyltransferases – longer term changes in gene expression.

Hypothesis

Sperm-borne sncRNA are associated with differences in bovine fertility

Objectives

Use Illumina sequencing to identify fertility-linked differences in sncRNA expression patterns

Bovine Subfertility

Subtle, idiopathic fertility differences

High Fertility

- ~ Passed Corporate QC Standards.
- ~ Fertility: $+2.1 \pm 0.7$
 - ~ Sire Conception Rate

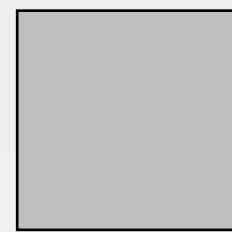
Low Fertility

- ~ Passed Corporate QC Standards.
- ~ Fertility: -2.1 ± 1.1
 - ~ Sire Conception Rate

Bovine Subfertility

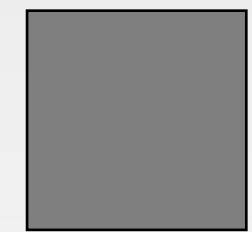
Subtle, idiopathic fertility differences

High Fertility

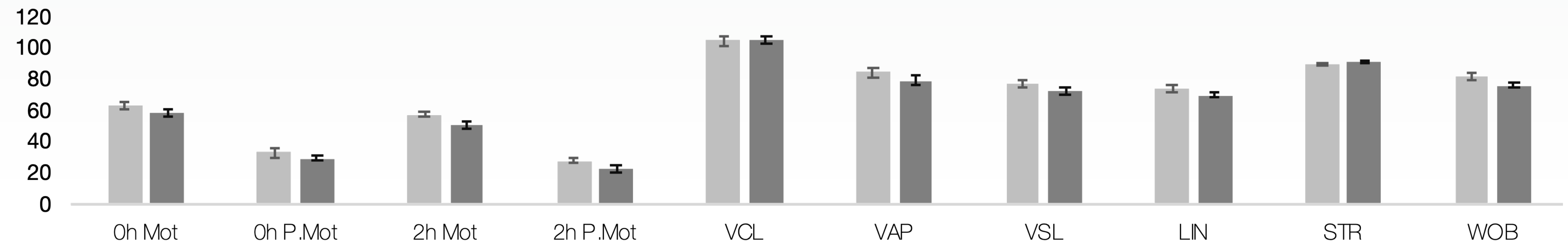


- ~ Passed Semex QC
- ~ Fertility: $+2.1 \pm 0.7$
- ~ Sire Conception Rate

Low Fertility



- ~ Passed Semex QC
- ~ Fertility: -2.1 ± 1.1
- ~ Sire Conception Rate

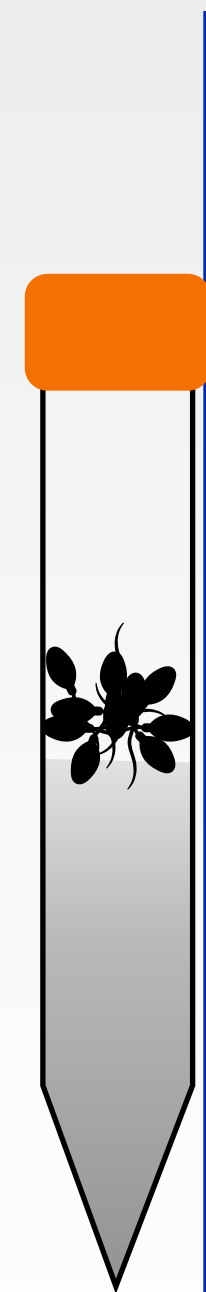


Next Generation Sequencing

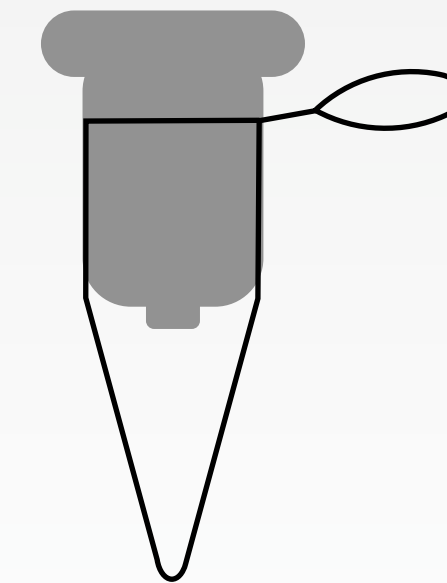
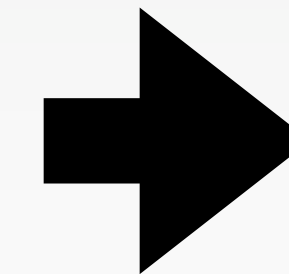
Sample Preparation

Isolate RNA

- Percoll gradient to purify sperm
- Column based total RNA extraction



Isolate sperm



Extract RNA

Next Generation Sequencing

Library prep

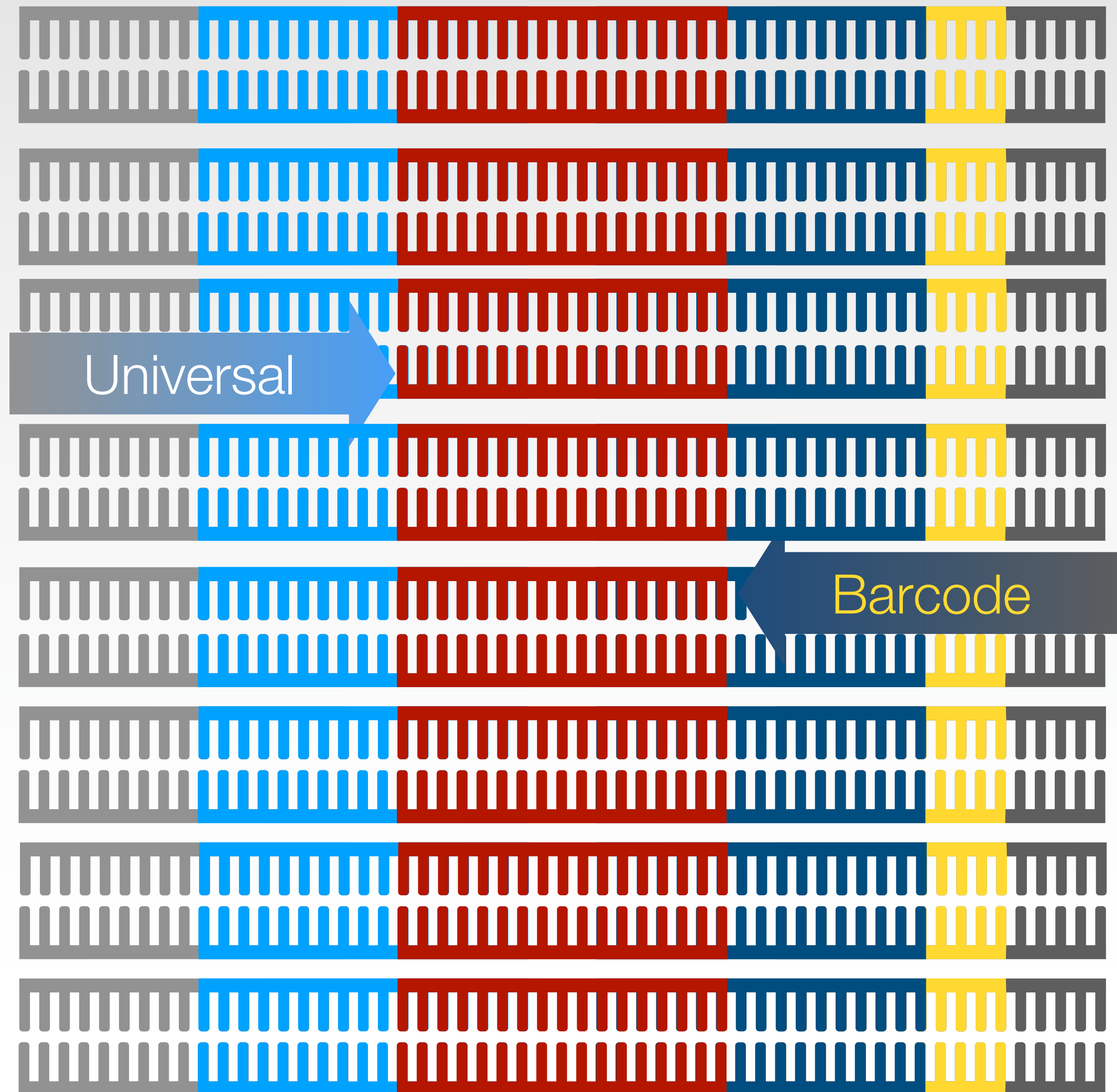
- NEXTflex small RNA

Size selection

- SAGE Pippin Prep
- 140-170 bp

Sequence

- Illumina NextSeq



Bioinformatics: Differential Expression Analysis - miRNAs

DE sequences

- Identify differences in expression between fertility conditions
 - T-test, FDR correction

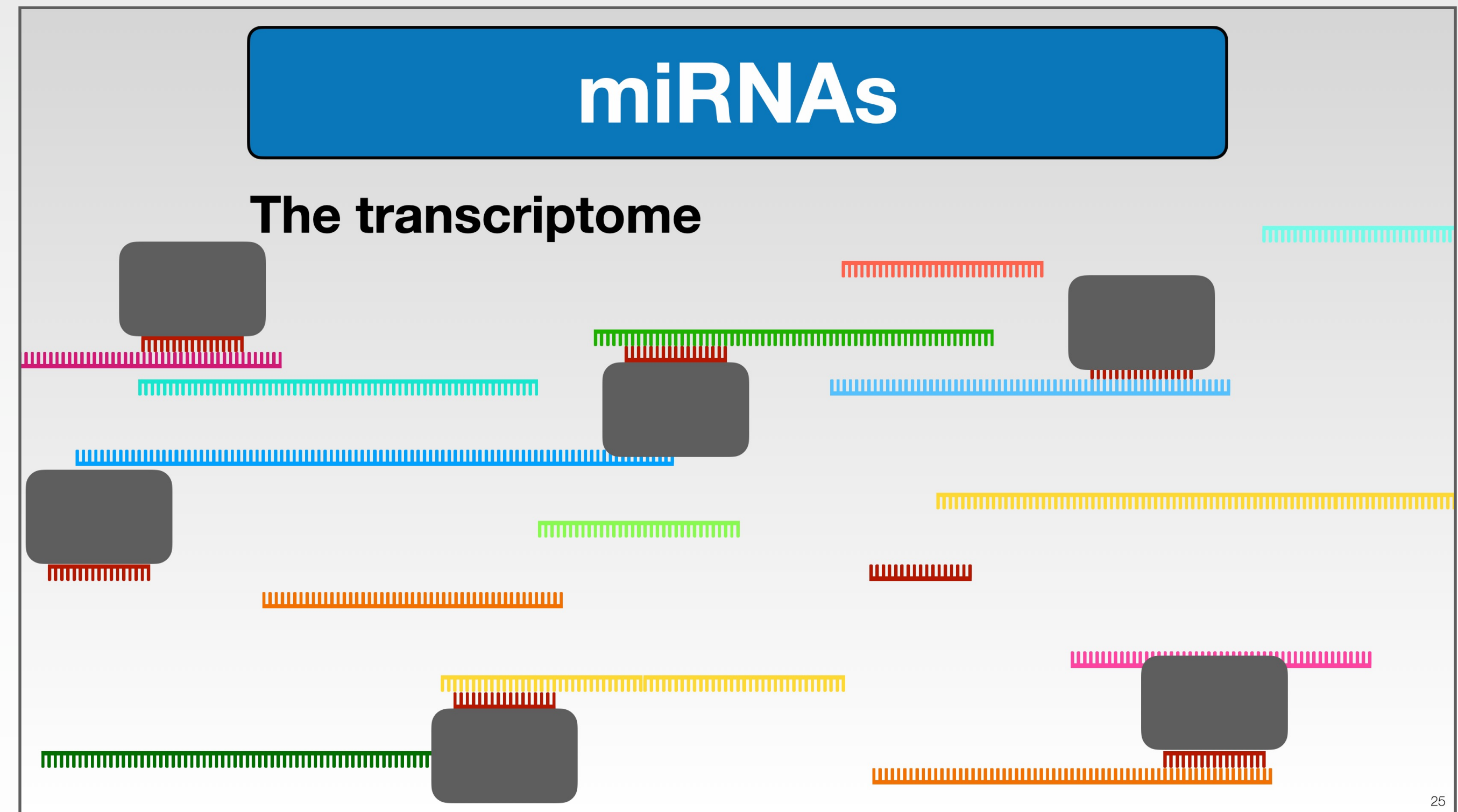
miRNA	Enhanced Mean	Standard Mean	log2FoldChange	pvalue	FDR
miR-2450c-3p	6	0	-0.00	0.000	0.000
miR-2311-5p	4	0	-0.00	0.000	0.000
ppc-mir-2274-5p	2	0	-0.00	0.000	0.000
miR-409a-3p	60	24	-0.02	0.000	0.014
miR-543	136	52	-0.02	0.000	0.018

Correlation Analysis

miRNA:miRNA Relationships

miRNAs may share:

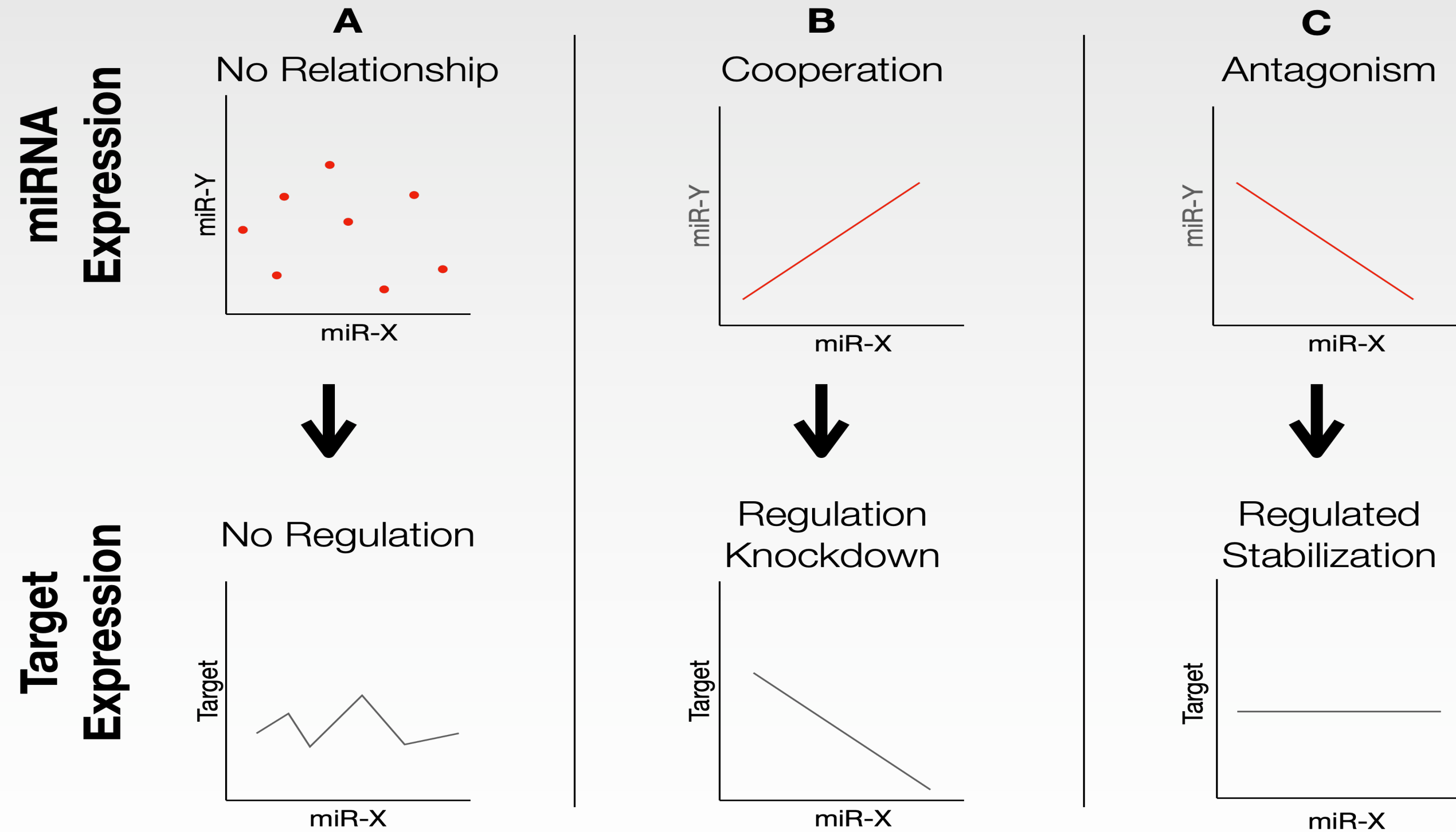
- Gene targets
- Functional pathways
- Transcription regulators



Corral-Vazquez C, Salas-Huetos A, Blanco J, Vidal F, Sarrate Z, Anton E. Sperm microRNA 555 pairs: new perspectives in the search for male fertility biomarkers. *Fertil Steril* (2019) 112:831–556 841. doi: 10.1016

Correlation Analysis

- May represent functional differences that affect fertility which are then reflected in the sperm miRNAs.

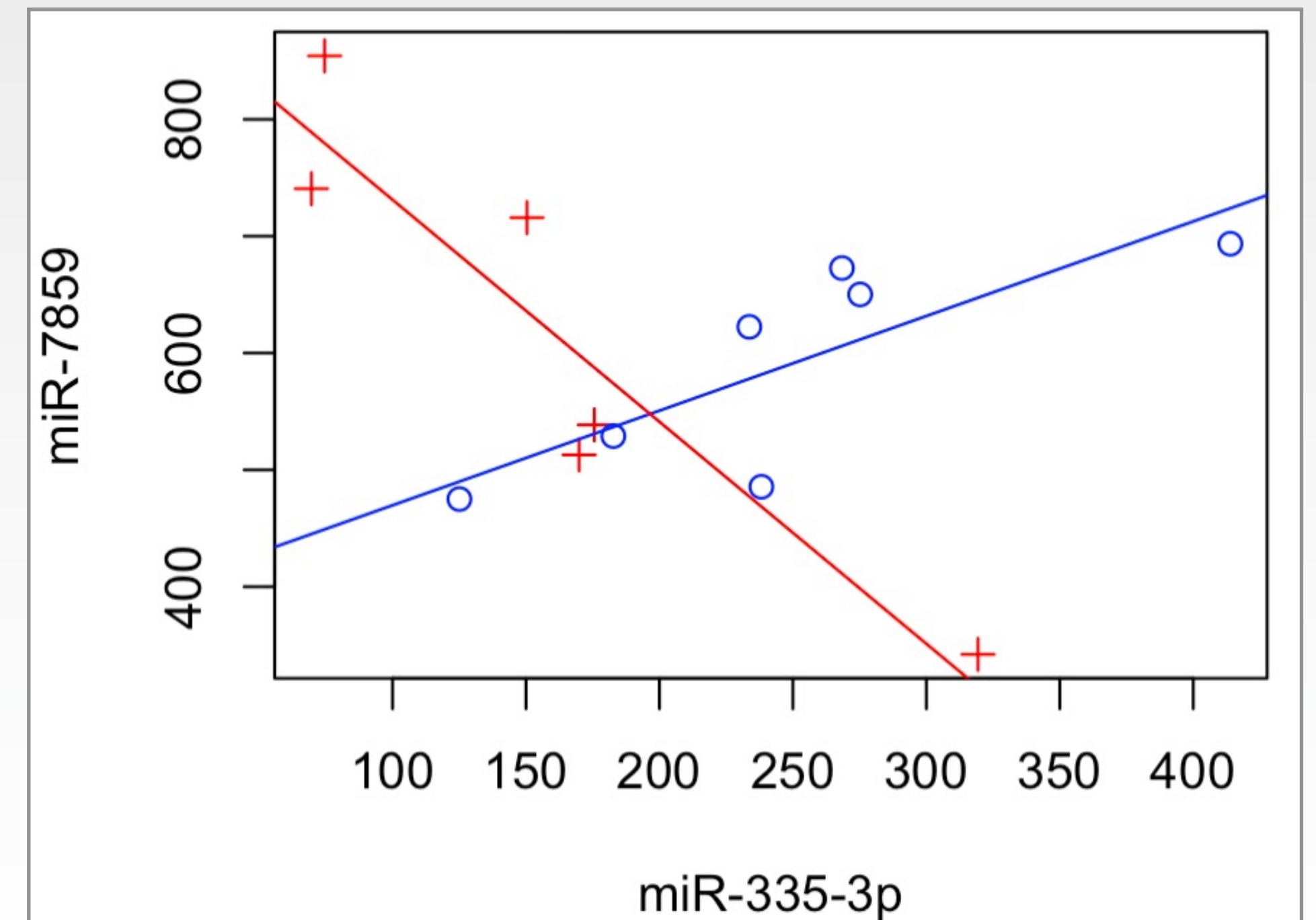


Correlation Analysis

miRNA:miRNA relationships

Analysis requirements

- ♂ Significant Spearman correlation in high and low fertility samples
- ♂ Inverse trends in the different fertility conditions

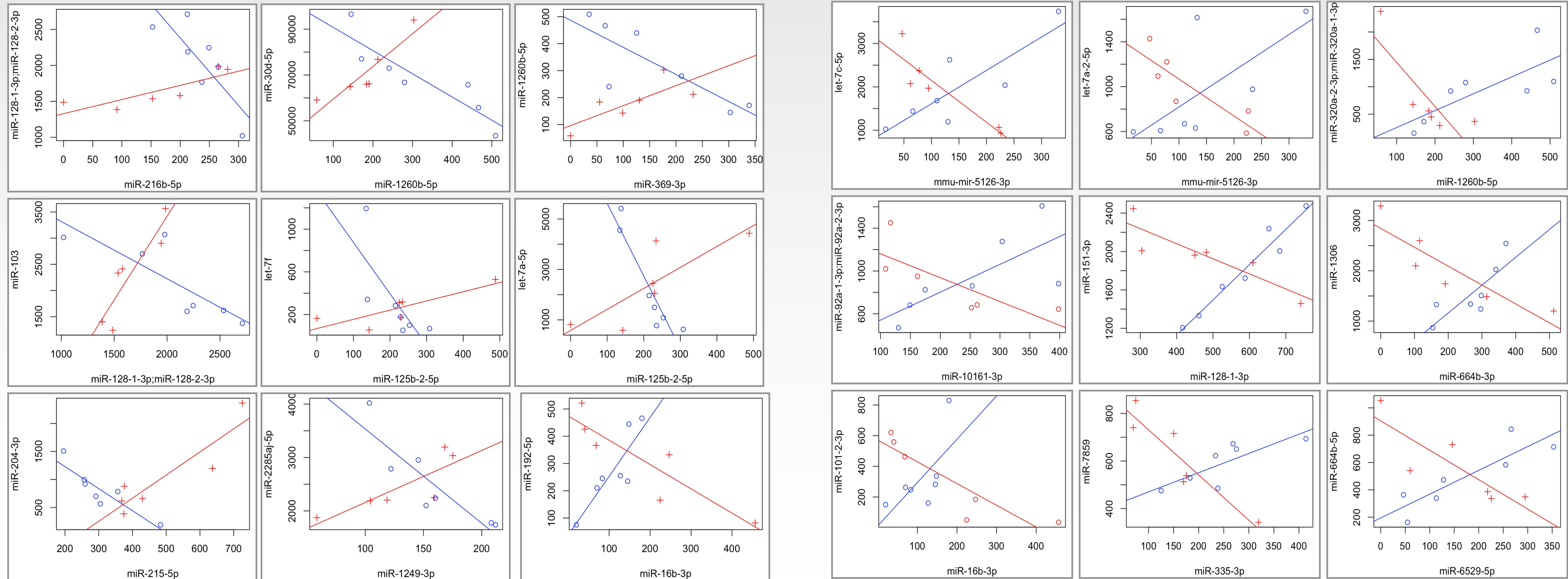


○ Enhanced Fertility

+ Standard Fertility

Correlation Analysis

Multiple significant relationships identified



○ High Fertility + Low Fertility

Summary

Sperm-borne miRNAs

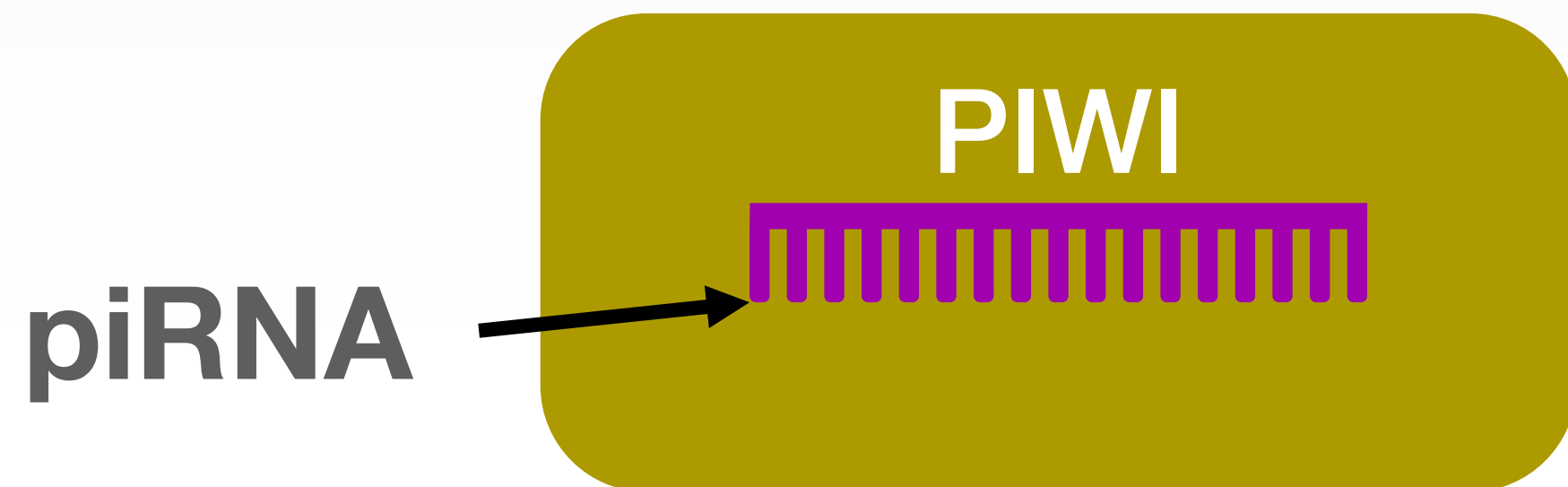
Similar RNAs are Most Abundant

No differences in the rank order or overall placing of the top 5 miRNAs identified

PIWI-Interacting RNAs (piRNAs)

PIWI Proteins

- ~ **P**-element Induced **W**impy testis (Drosophila Phenotype)
- ~ Associate with piRNA to effect RNAi



Transposable elements

- ~ Self-replicating genomic sequences
- ~ Expressed during reprogramming
 - ~ Suppressed to protect genome



BIOLOGY OF REPRODUCTION (2016) 94(4):75, 1–11
Published online before print 24 February 2016.
DOI 10.1095/biolreprod.115.136721

Identification of *PIWIL1* Isoforms and Their Expression in Bovine Testes, Oocytes, and Early Embryos¹

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³Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

⁴Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

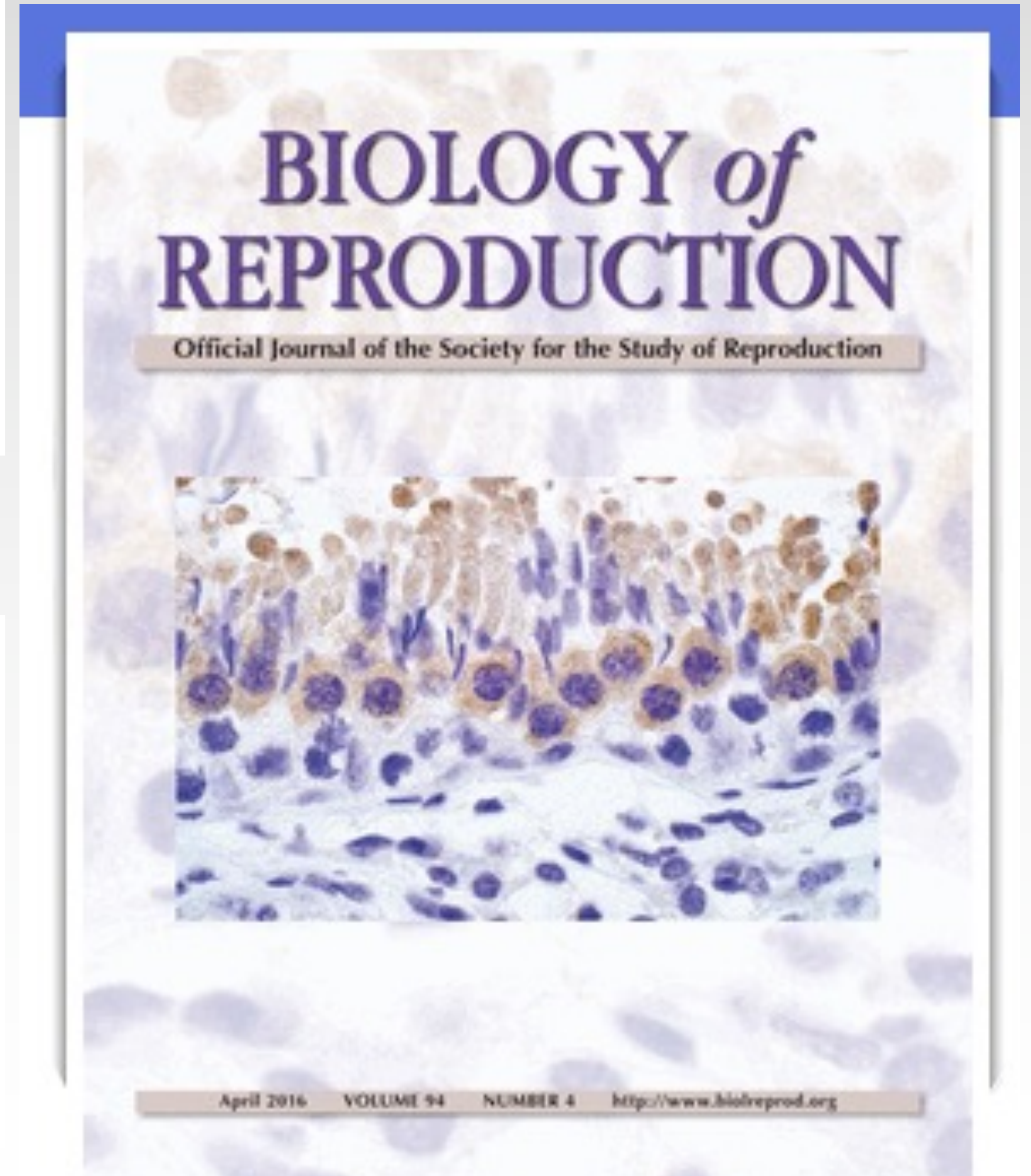
REPRODUCTION
RESEARCH

Bovine piRNA-like RNAs are associated with both transposable elements and mRNAs

Stewart Russell¹, Mehool Patel¹, Graham Gilchrist¹, Leanne Stalker¹, Daniel Gillis¹, David Rosenkranz² and Jonathan LaMarre¹

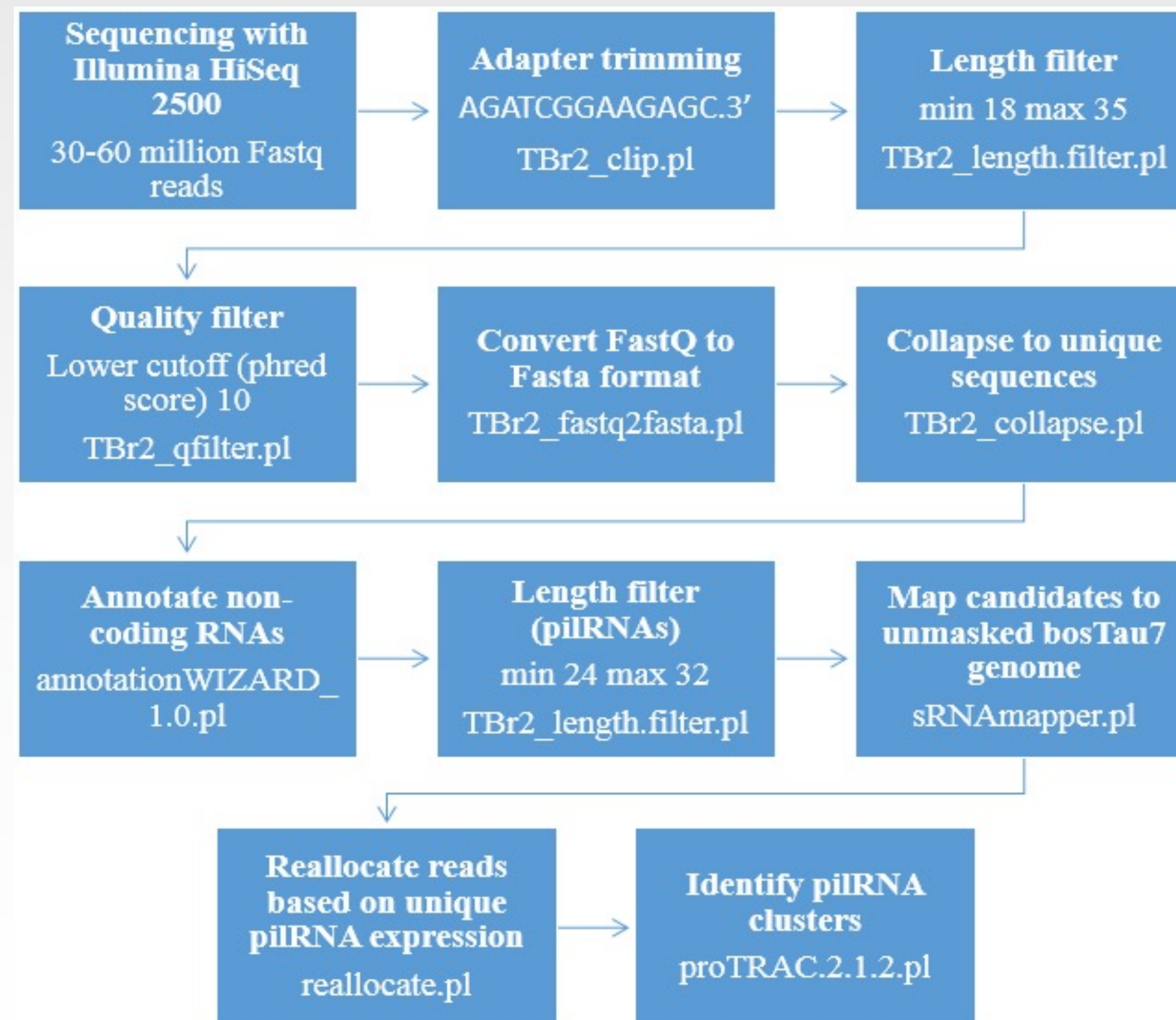
¹Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada and ²Anthropologie, Johannes Gutenberg-Universität Mainz, Mainz, Germany

Correspondence should be addressed to J LaMarre; Email: jlamarre@uoguelph.ca



piRNA Identification “Pipeline”

Collaboration with D. Rozenkranz and colleagues, University of Mainz

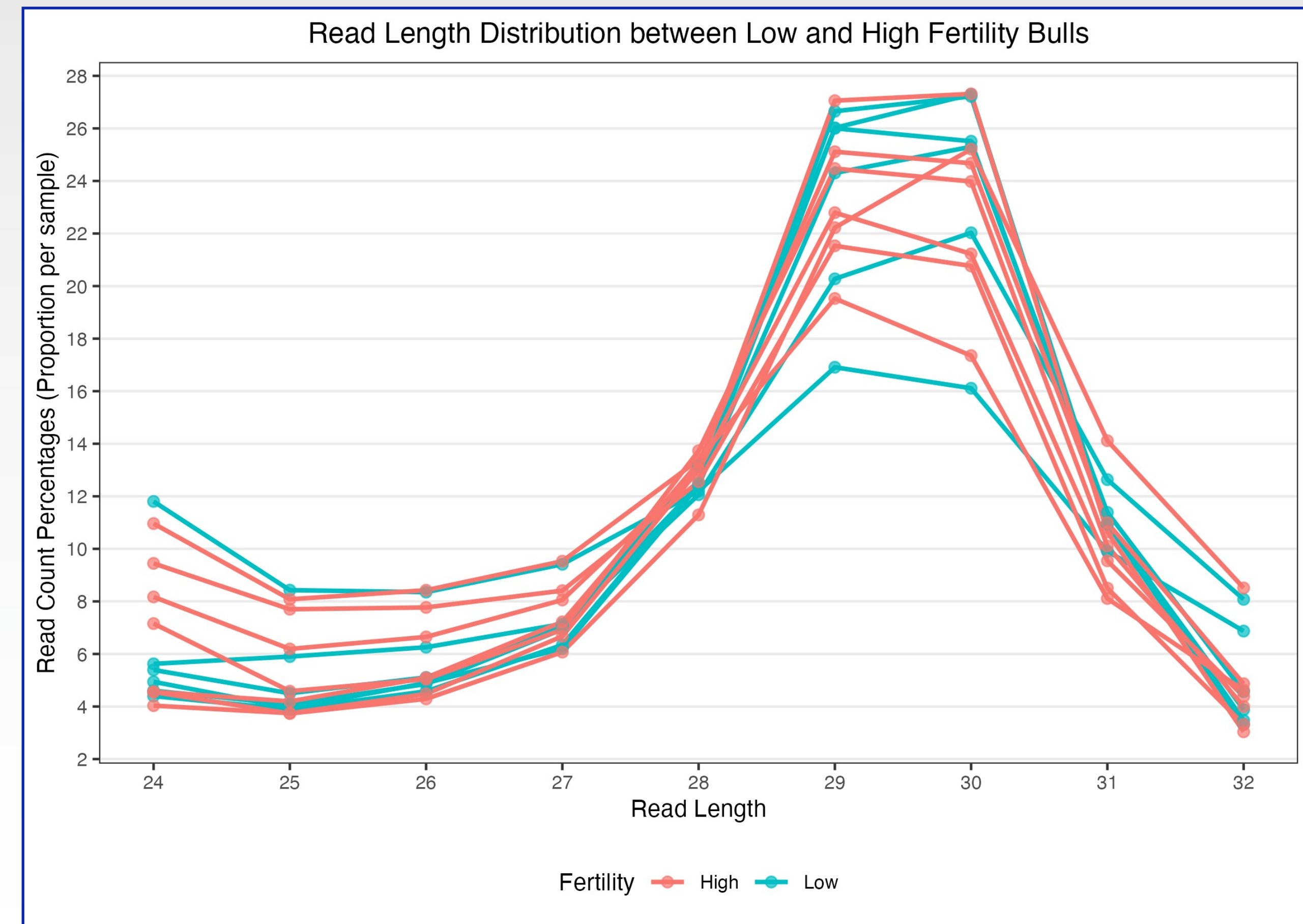


Sperm piRNA Characteristics

Transcript Length

Read length distribution

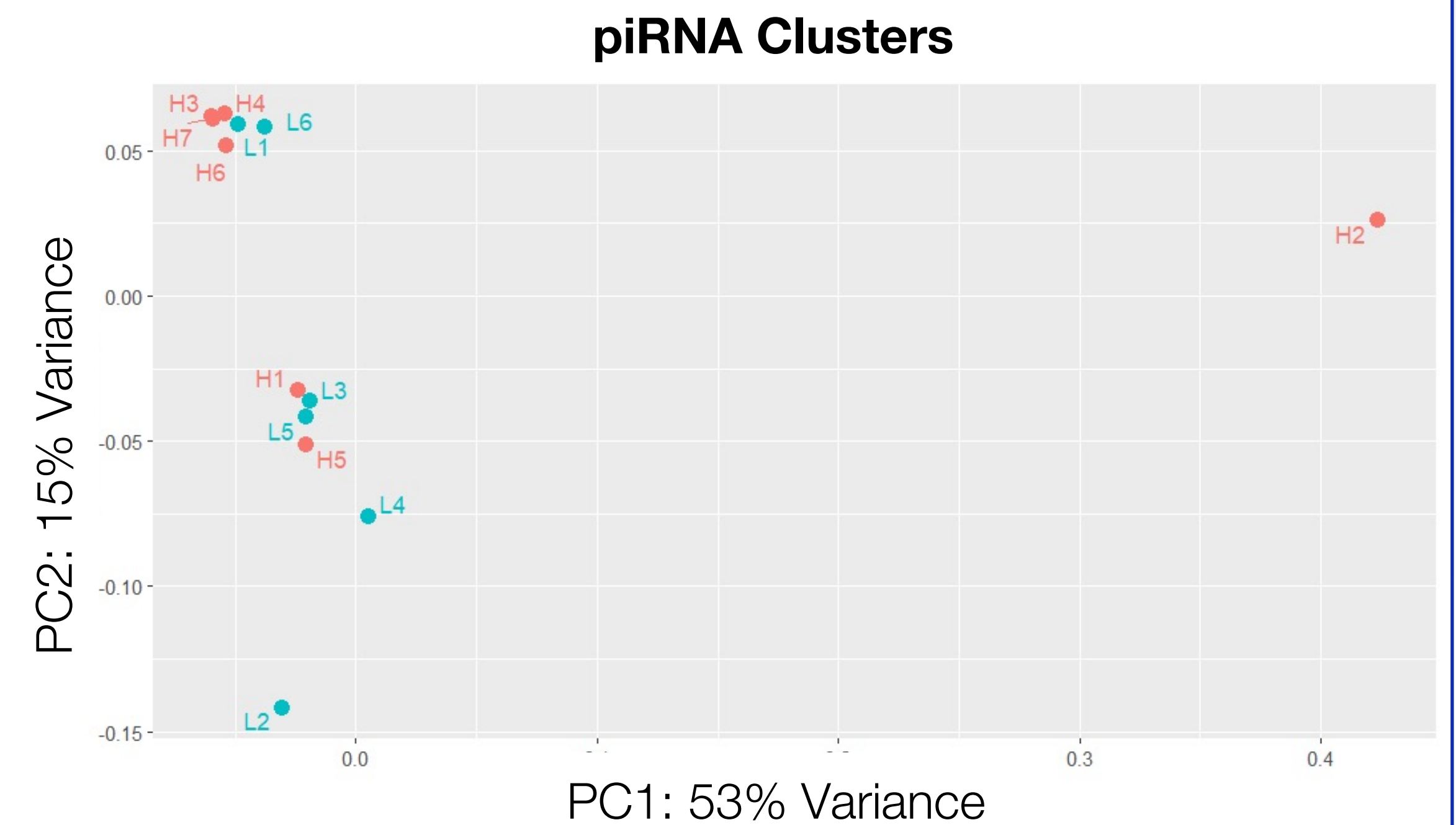
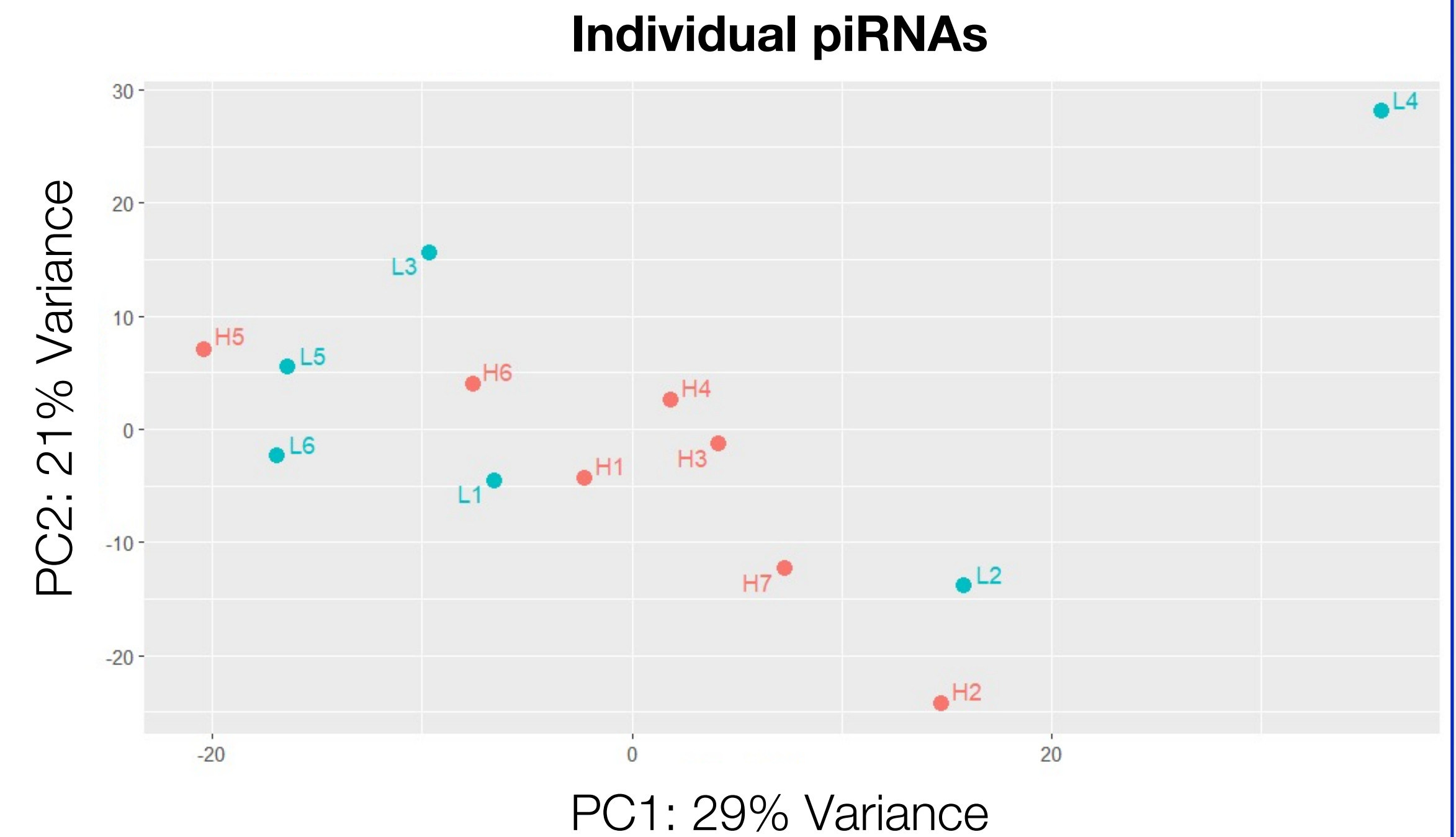
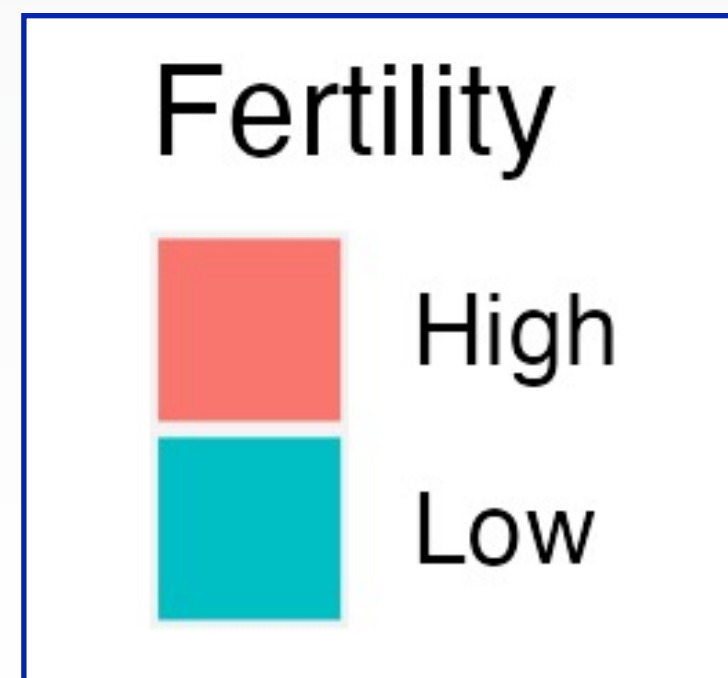
- Consistent across fertility groups
- 29-30 nts most common read length
- Consistent with expected piRNAs



PiRNA Expression

Principal component analysis

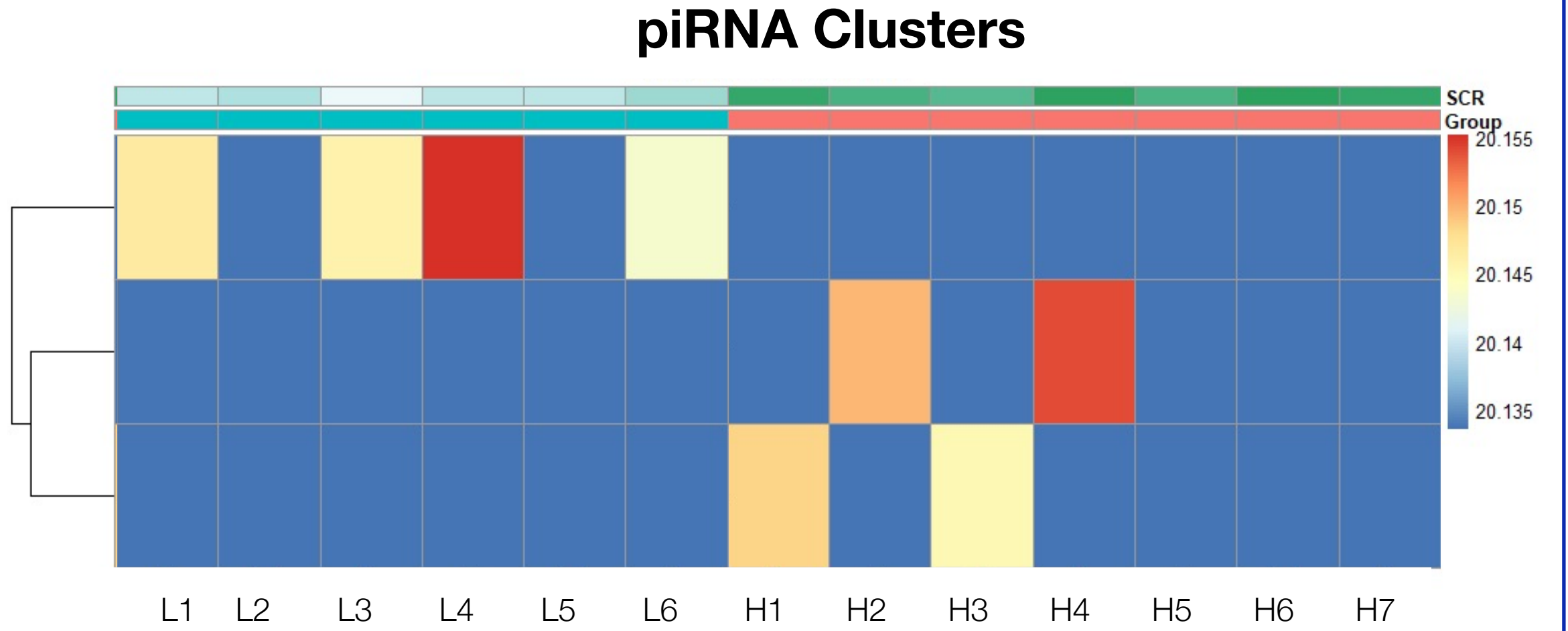
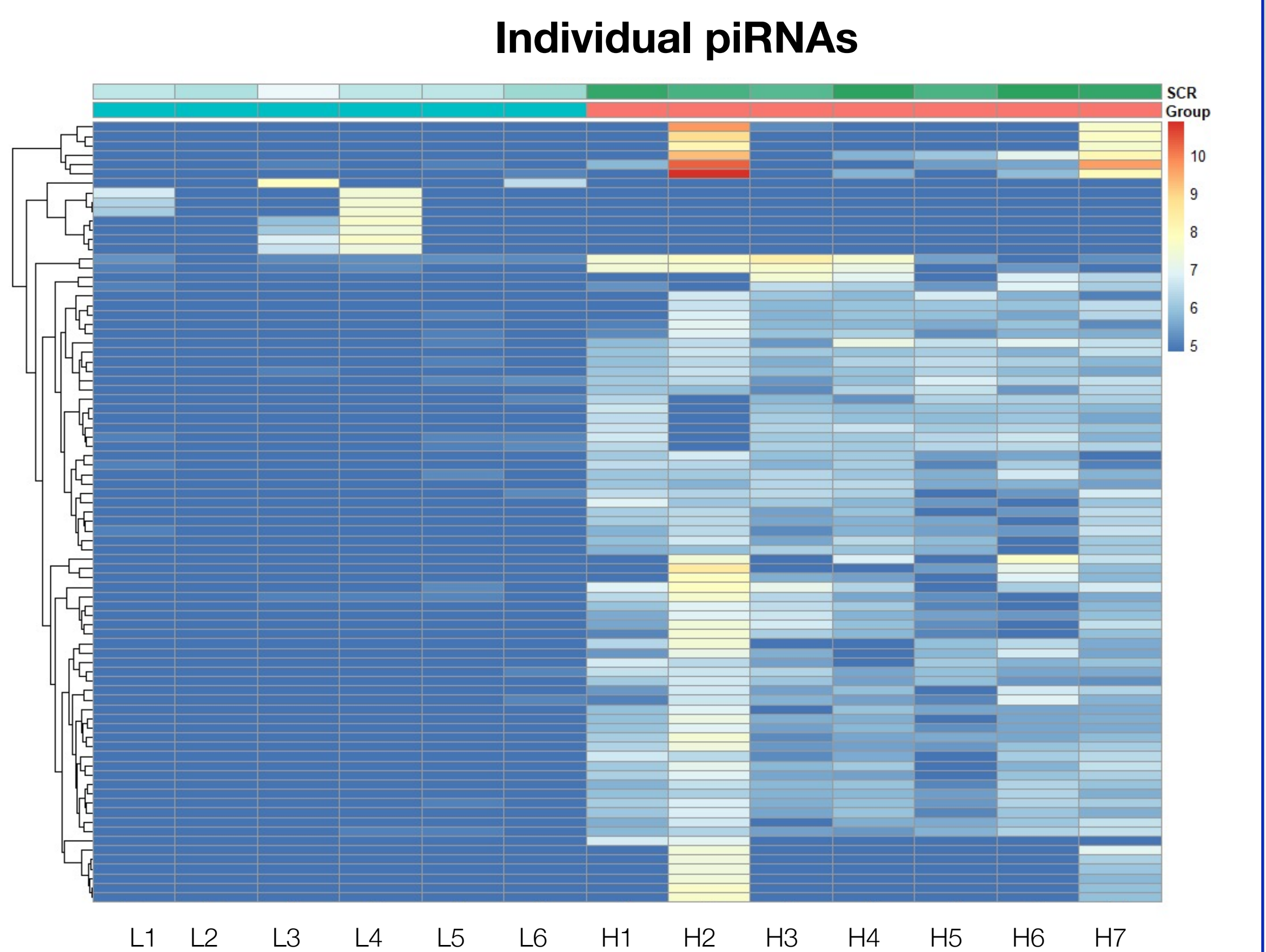
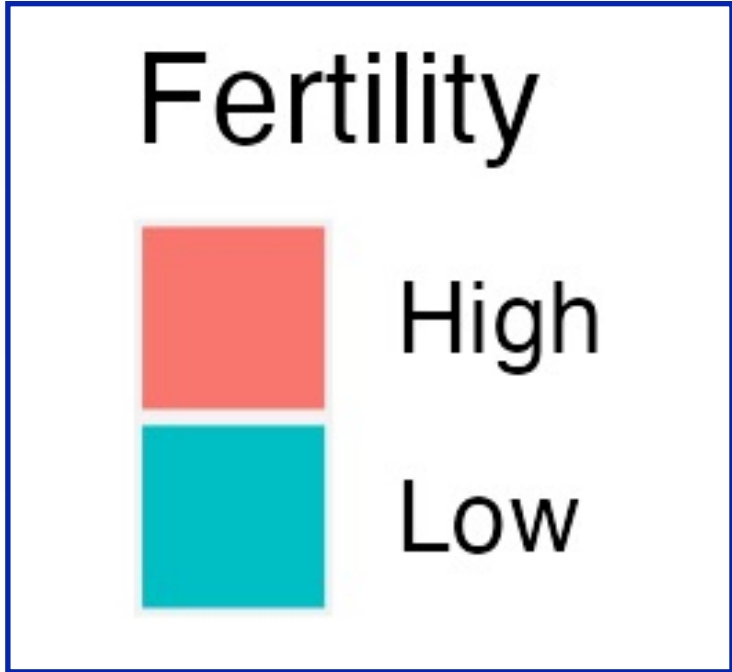
- No overall differences in piRNA expression by individual or cluster



piRNA Expression

Differential Expression

- 83 DE piRNAs
 - Mostly over-expressed in high fertility
 - Very low relative expression
- 3 DE clusters
 - Expressed in few bulls

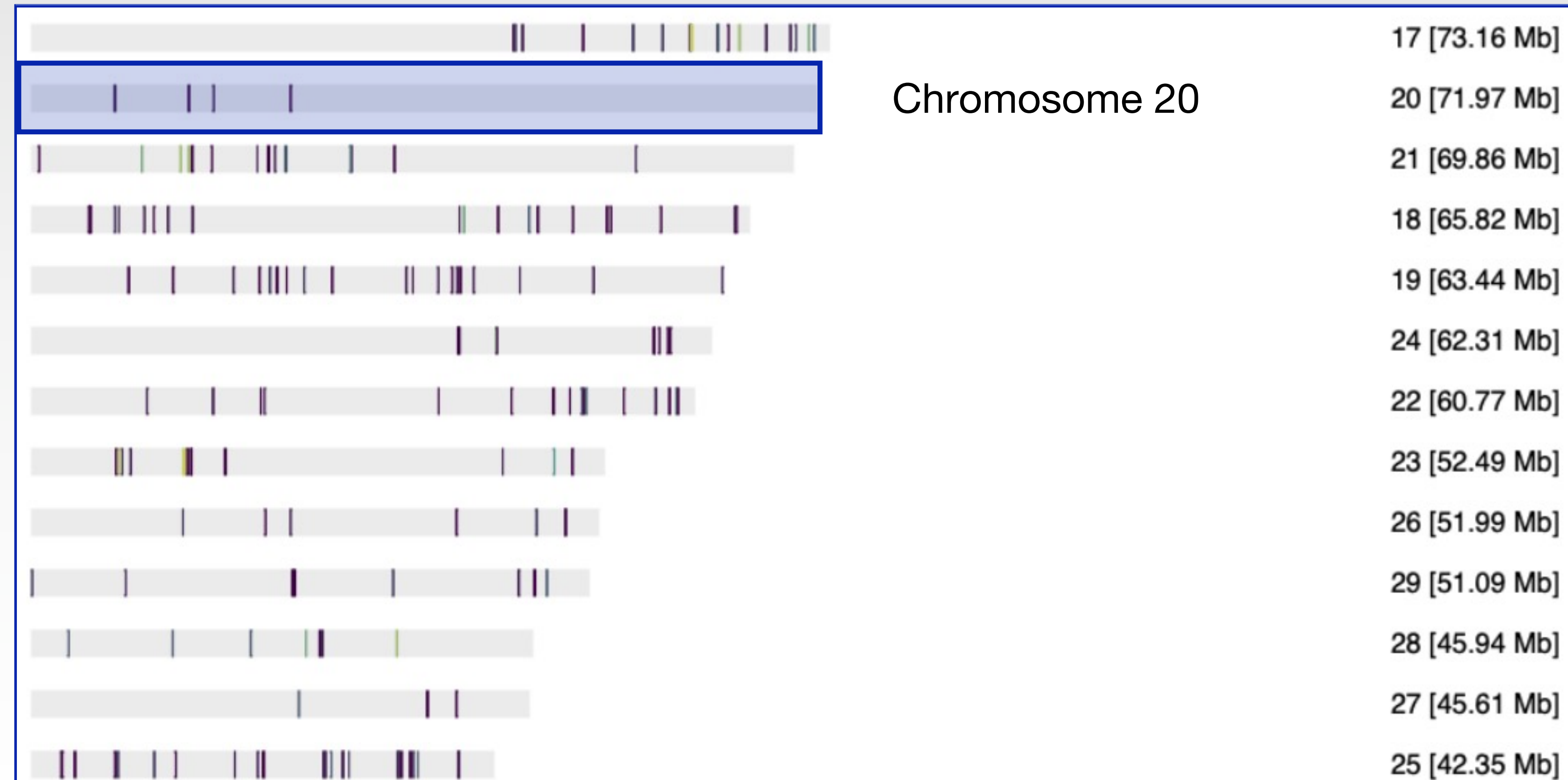


Sperm piRNA Characteristics

Cluster Expression

By chromosome

- Consistent across fertility groups
 - Expected
- No piRNAs from Chr 20
 - Contains 4 clusters



Transposable Element Targeting

piRNAs complementary to expressed TE Transcripts

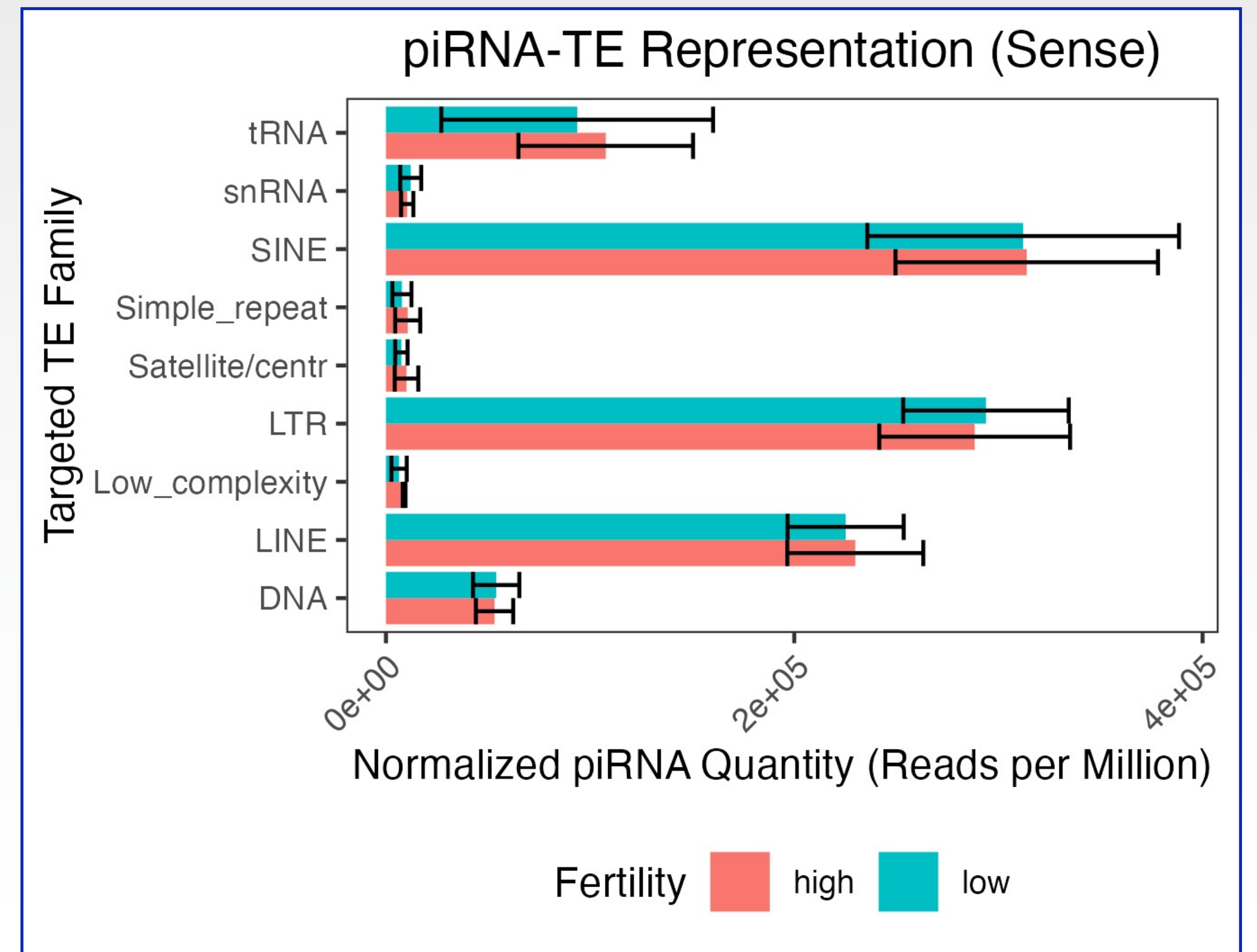
~ Abundant targeting of TEs

~ SINEs

~ LTRs

~ LINEs

No fertility-associated differences

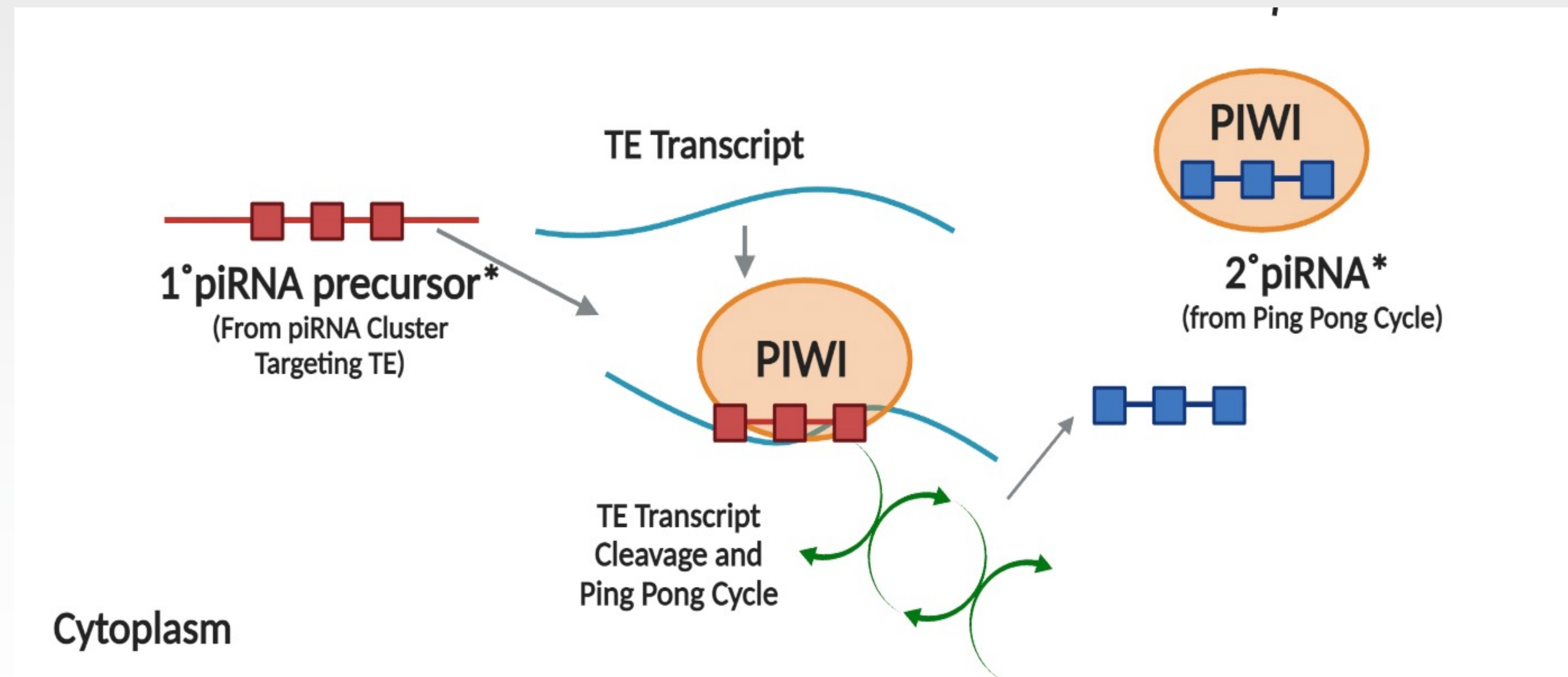


Ping-Pong Pathway

Secondary piRNA biogenesis

Amplification loop

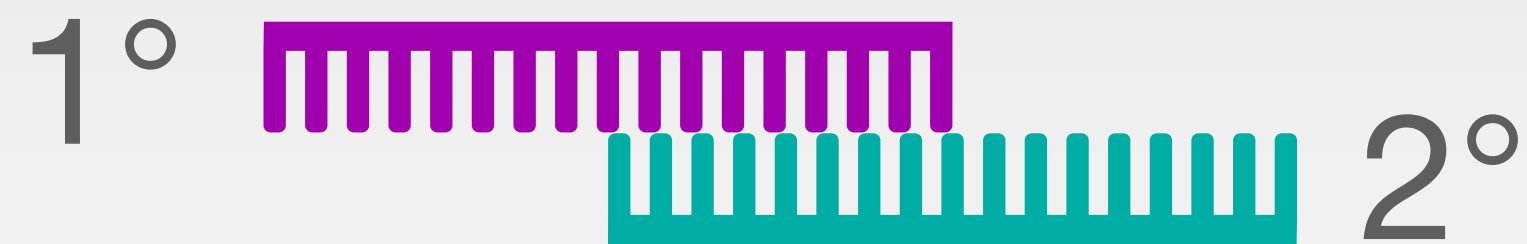
1. Primary biogenesis
 - ~ Yields mature piRNA
2. PIWI association
 - ~ Forms piRISC
3. TE cleavage
 - ~ Yields secondary piRNA
4. PIWI association
 - ~ Forms piRISC
5. Transcript cleavage
 - ~ Ping-Pong repeats



Presence of 2° piRNAs reveals active targeting – Sequences are actually *derived from* the RNA target.

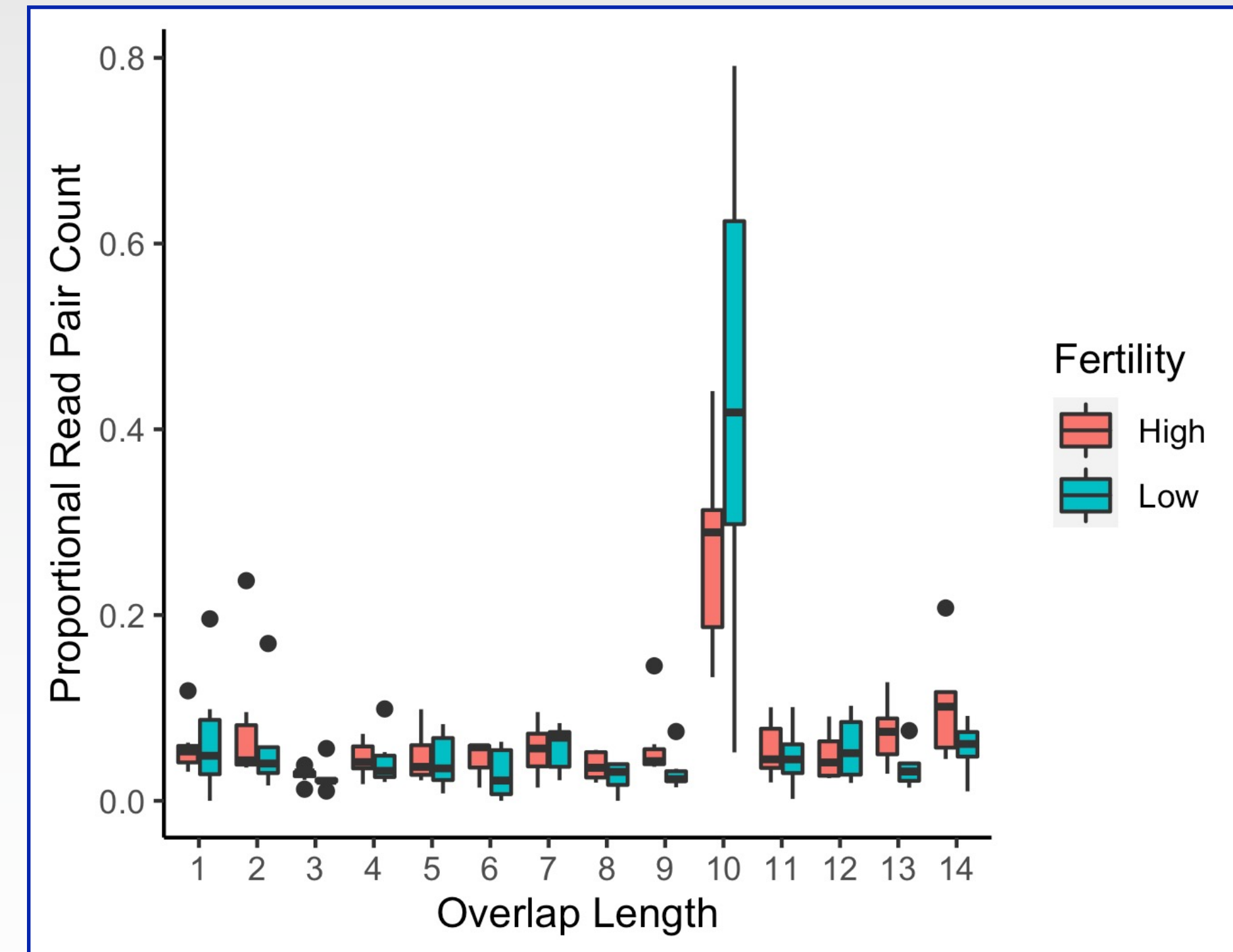
Ping-Pong "Signature" in Sperm RNA

10 nucleotide overlap



Active secondary biogenesis

- Similar overlap profile in both high and low fertility

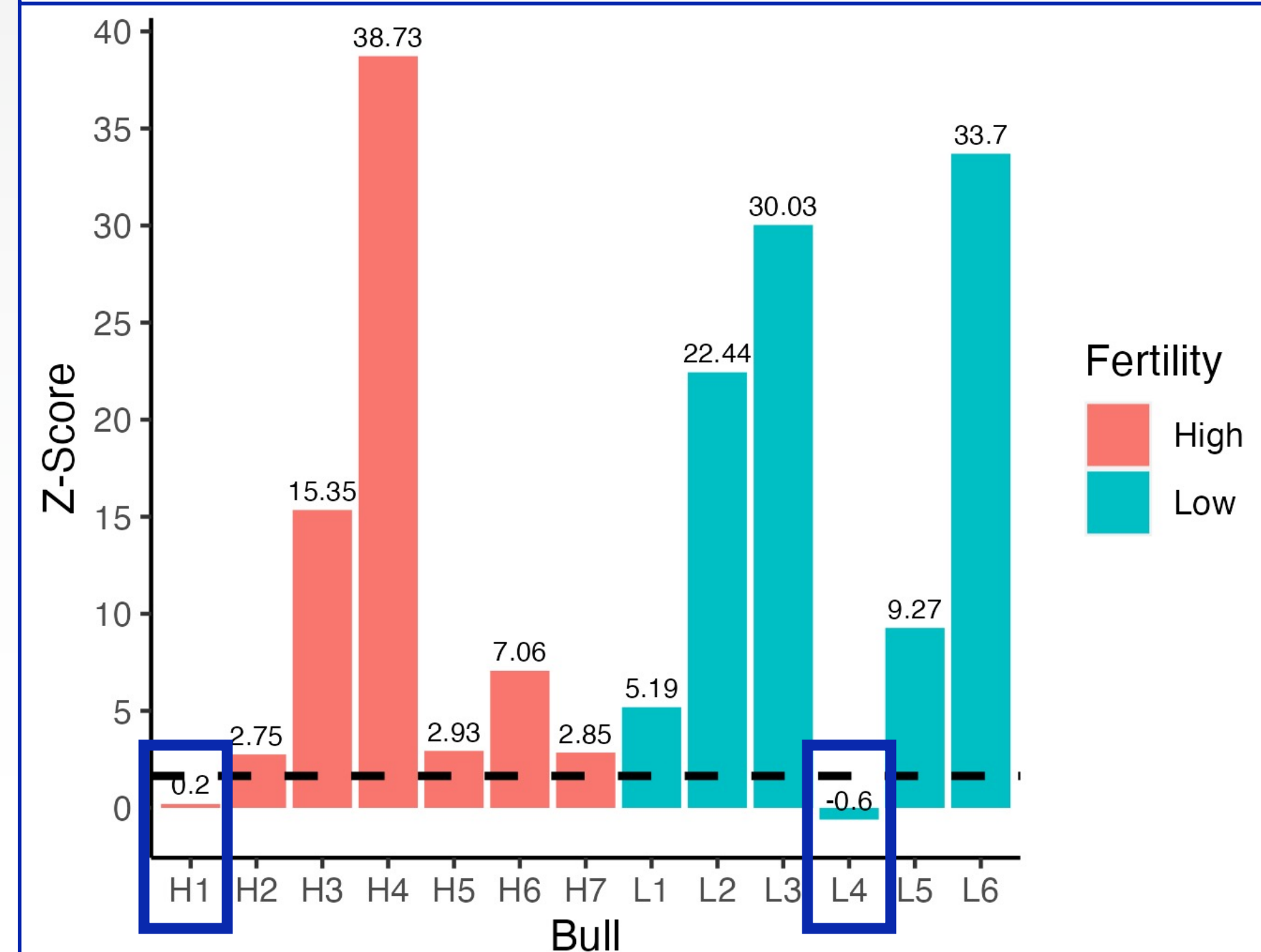
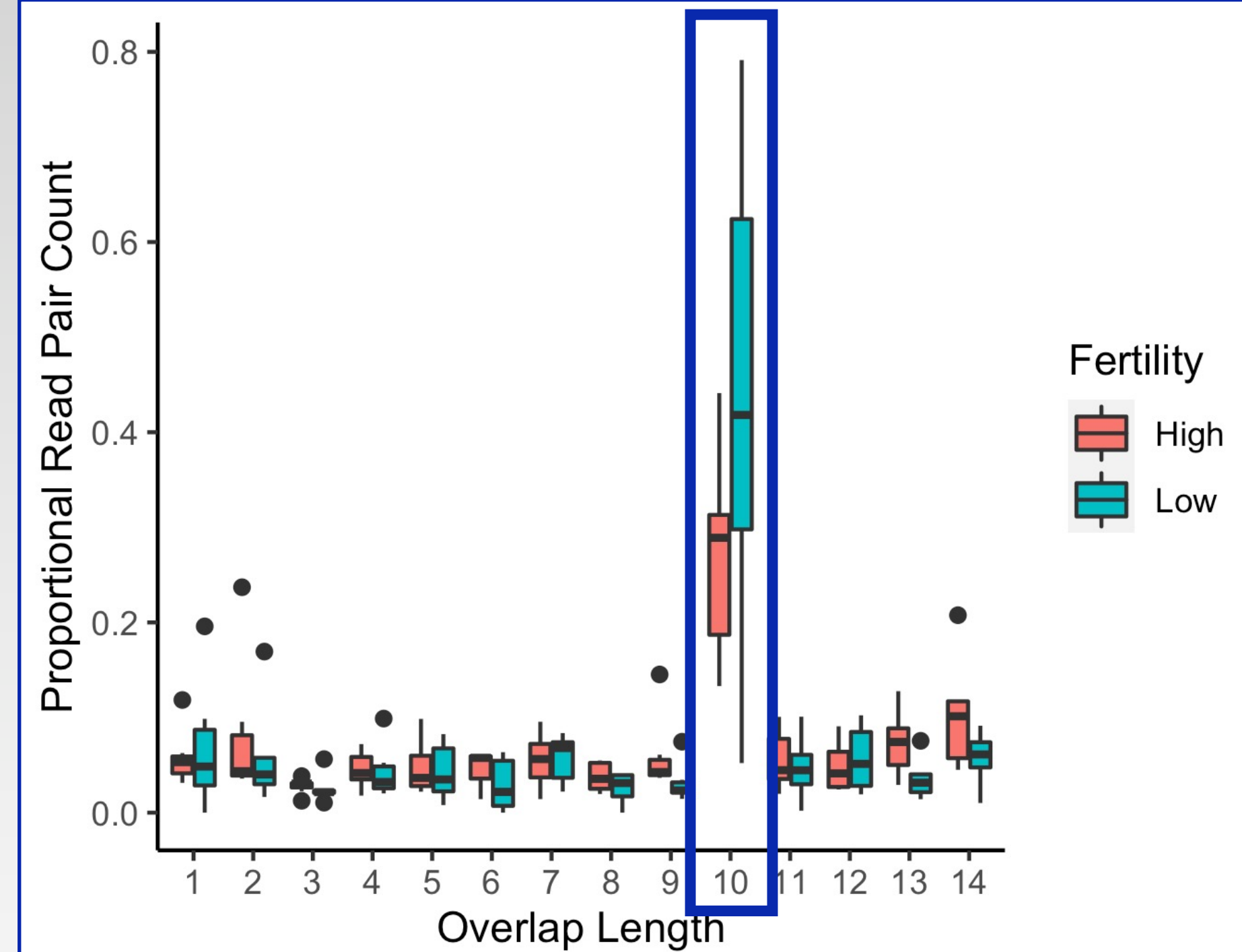


Ping-Pong Signature

10 nucleotide overlap in sequence data

Active secondary biogenesis

- Similar in high and low fertility
- Indicates functional activity of piRNAs
 - Target suppression required for Ping-Pong
- Active Ping-Pong in majority of bulls
 - Some exceptions in both fertility groups



Summary

Sperm-borne piRNAs

TE Targeting

SINEs, LTRs, LINEs
are abundantly
targeted by piRNA in
bovine sperm

Overall Conclusions

Small RNAs represent useful potential biomarkers of bull fertility.

However.....

“Disentangling genetic and environmental effects (on small RNA populations) will require a large number of bulls from several breeds to be raised altogether in the same semen production center to evaluate the breed effect, and to duplicate this design in several semen production center to measure the environmental effect.”

Schibler Group: Sellem *et al.* Epigenetics Chromatin. 2020; 13: 19.

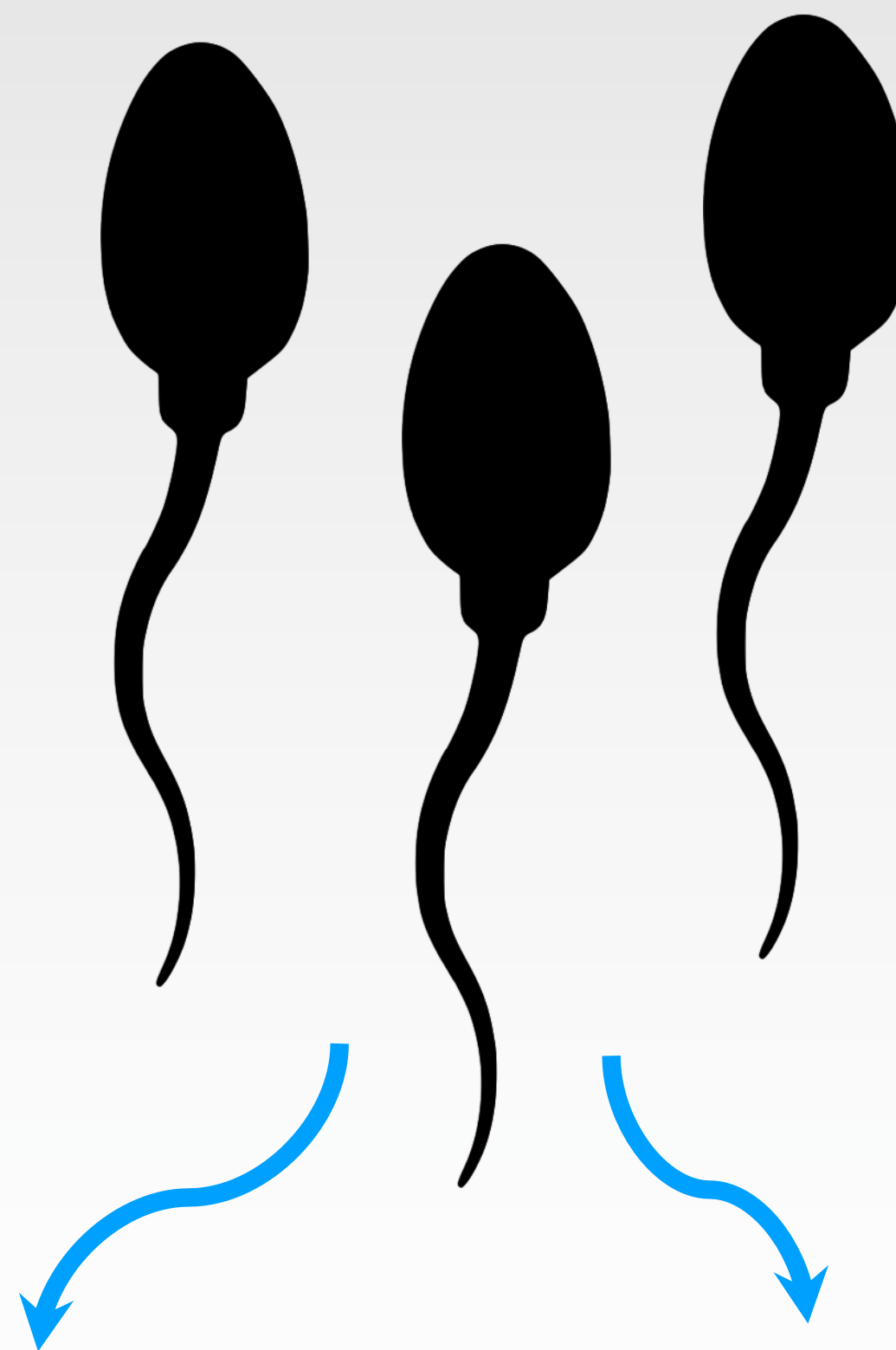
Small RNAs as “Epigenomic Metadata”

“METADATA”



“Good guy” vs. “Bad guy”

“EPIGENOMIC METADATA”



Small RNAs, DNA Methylation, Proteome

“Optimally Fertile” vs. “Subfertile”

Acknowledgements

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Marangaby Mahamat

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Dr. Cliff Librach

Dr. Dan Gillis

Alumni:

Leanne Stalker

Graham Gilchrist

Thomas Parmentier

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Gonzalo Molledo

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