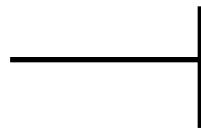


Characterizing mammalian piRNA precursors

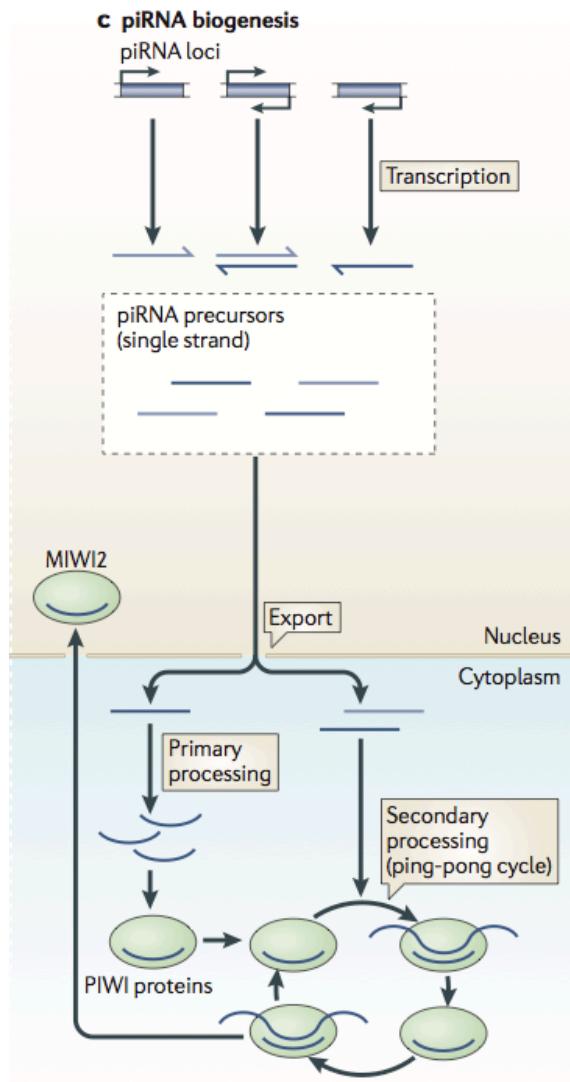
Christian Roy
Biochemistry Chalk talk
Moore/Zamore Labs
11/04/2011

The importance of piRNAs

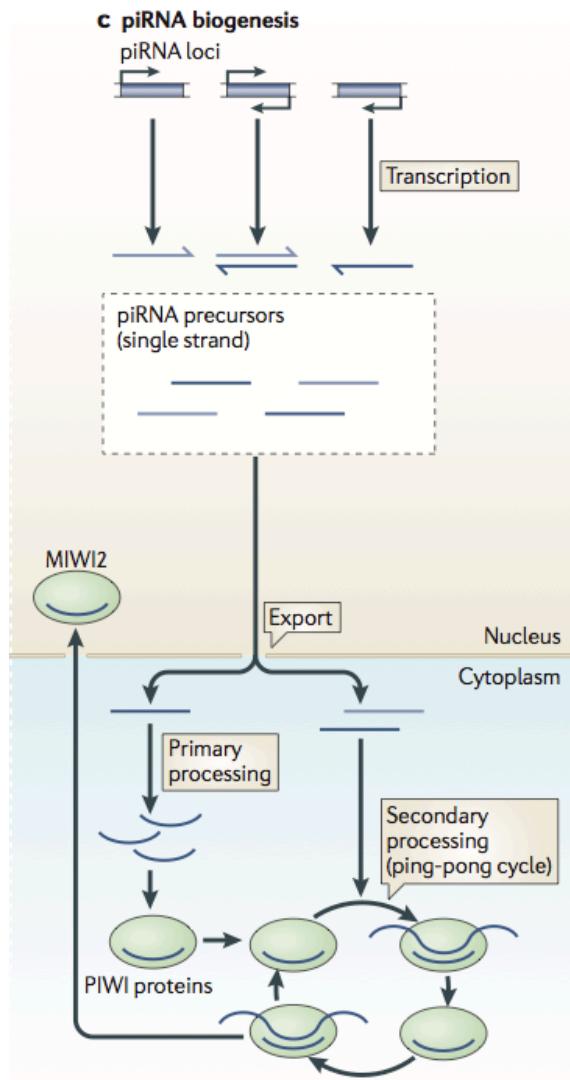
- Sterility is common phenotype to all known pathway mutants
- Human SNPs in Piwi proteins have been associated with male infertility
- Especially in mammals, piRNA function in maintaining fertility largely unknown



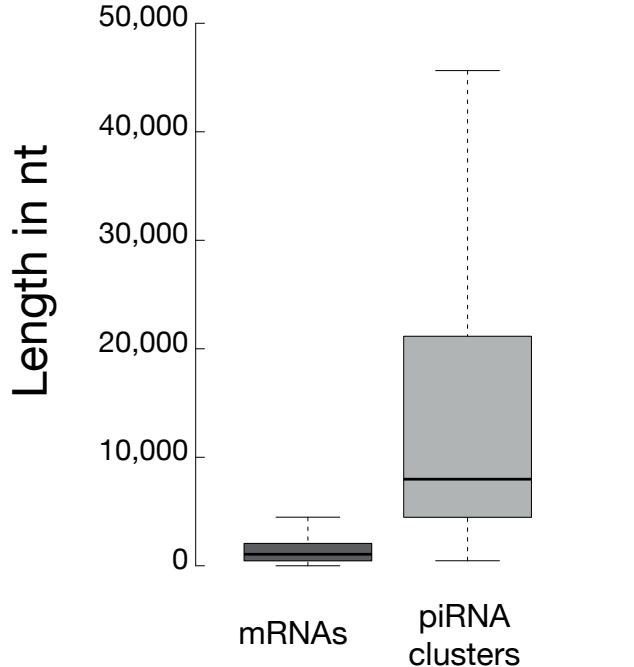
Features of mammalian piRNAs



Features of mammalian piRNAs



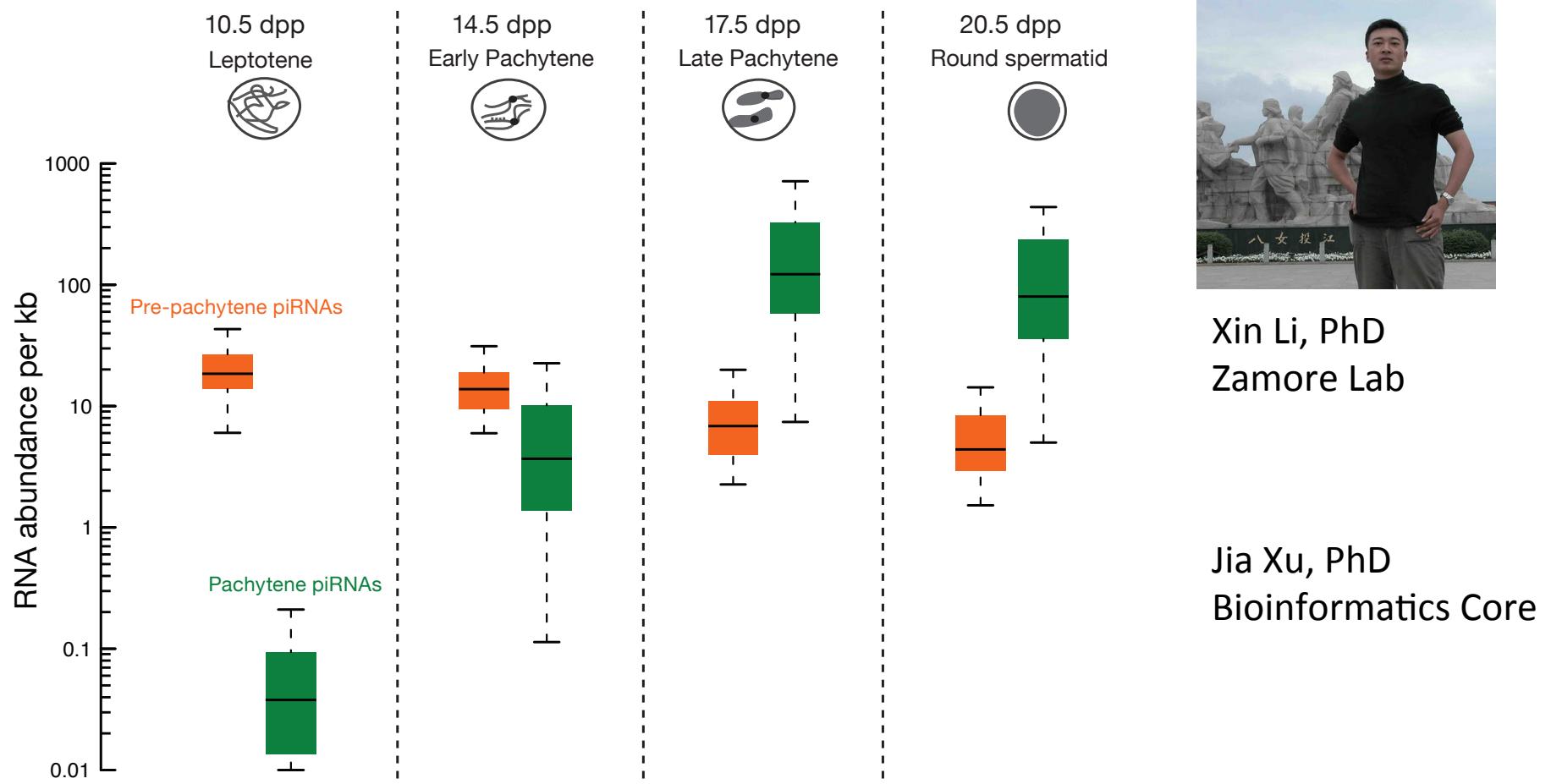
piRNA clusters are very long



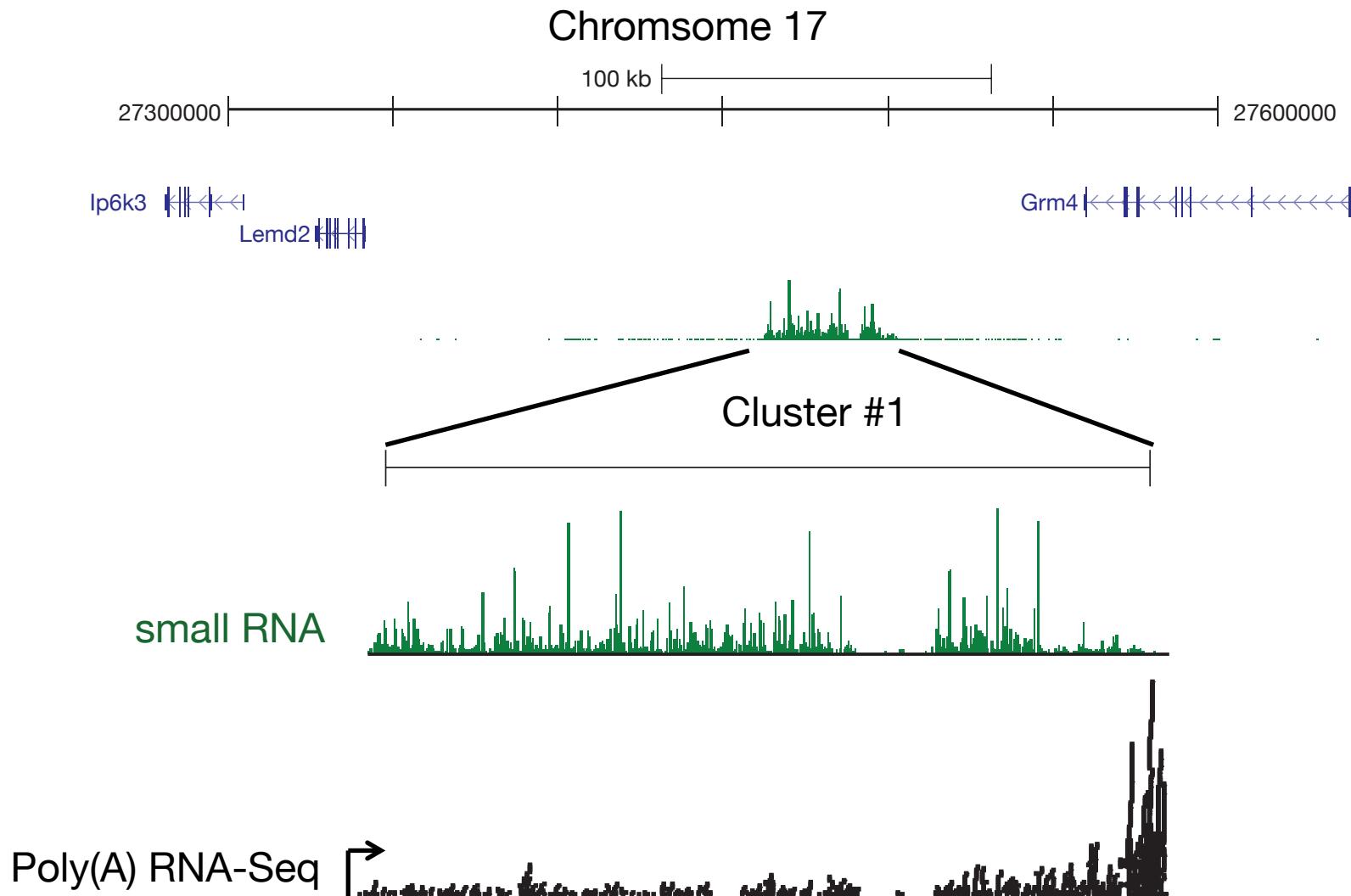
Features of a piRNA

- 23-30 nt small RNA
- 5' Uridine
- 2' O-methylation on 3' end
- Dicer independent
- Binds a Piwi protein
- *Map to transposons*

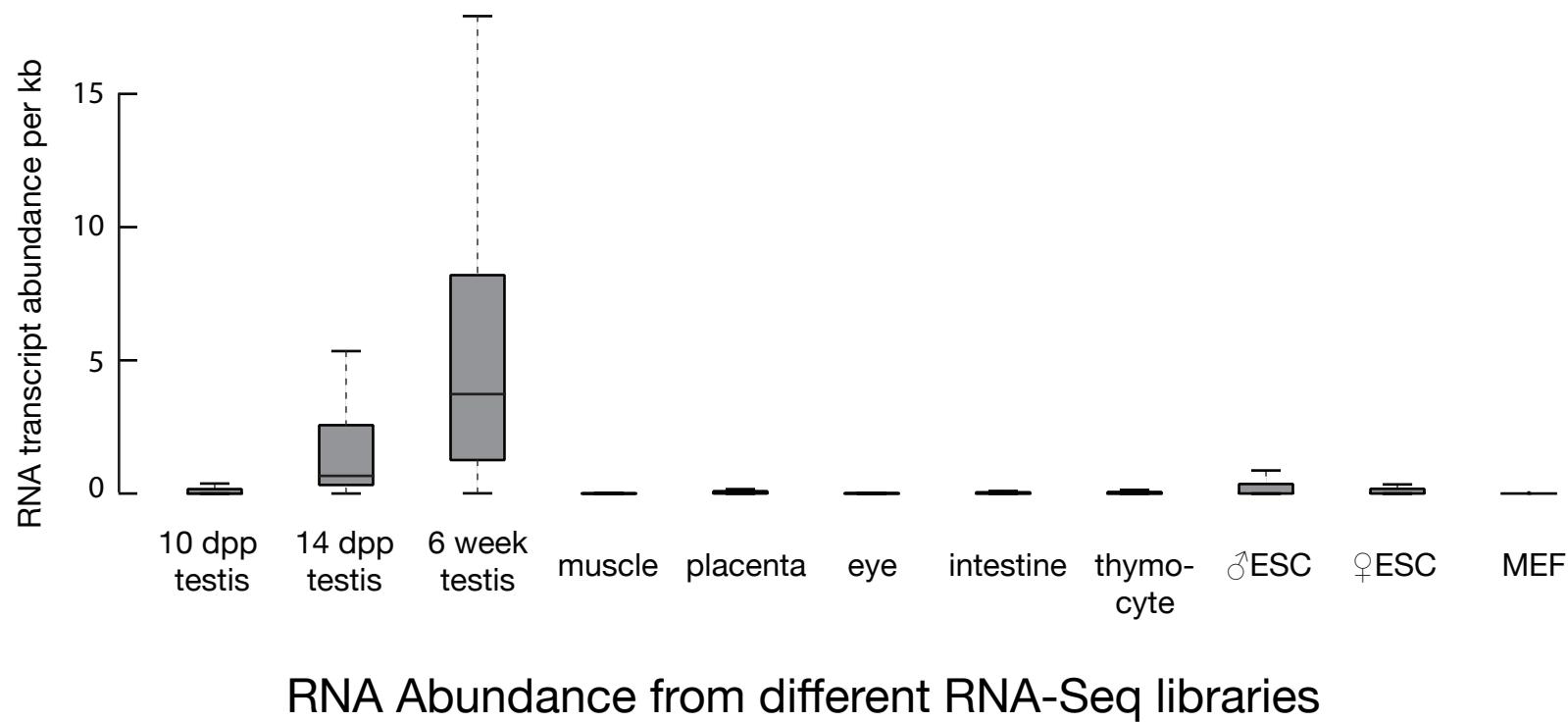
Cluster expression is dynamic



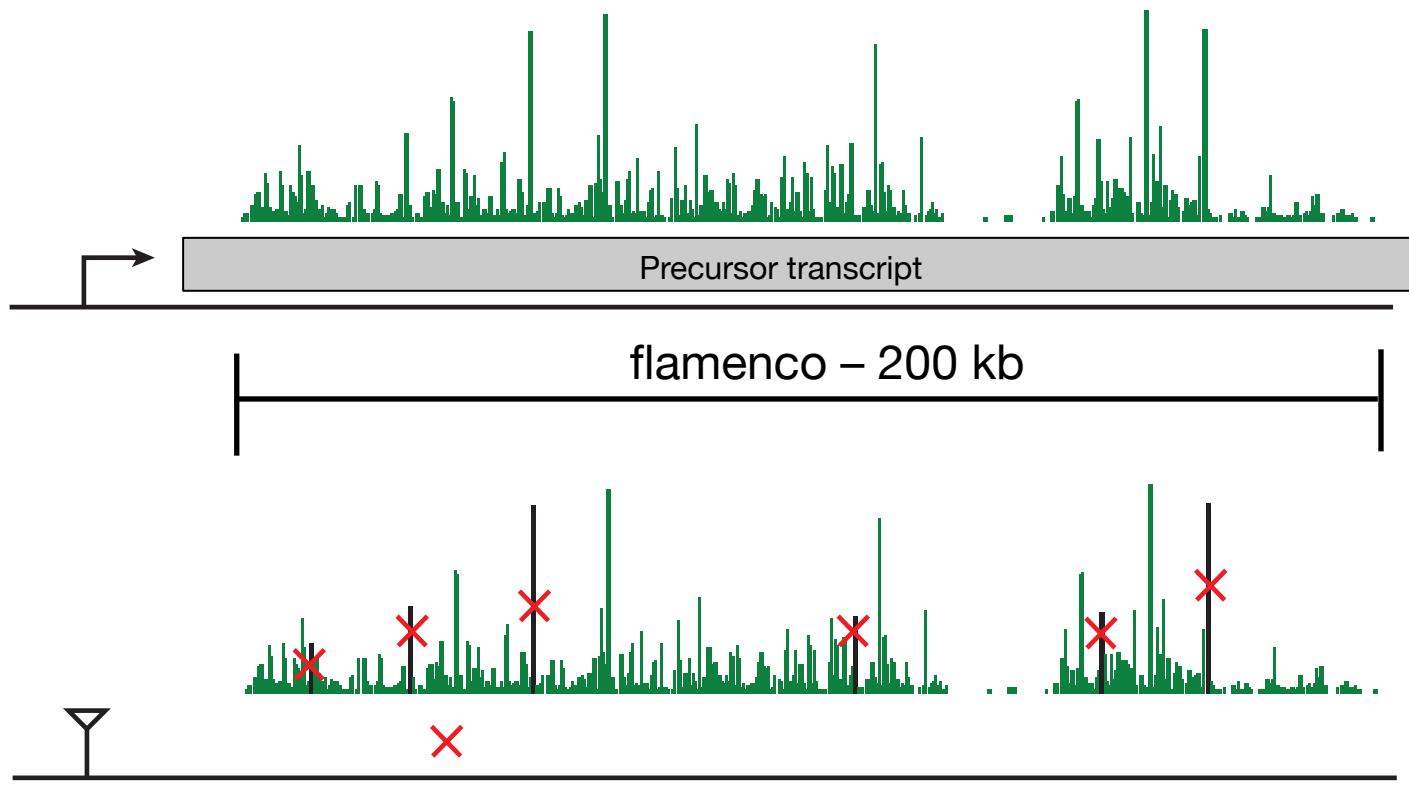
An example piRNA cluster



Precursor transcripts are germline specific



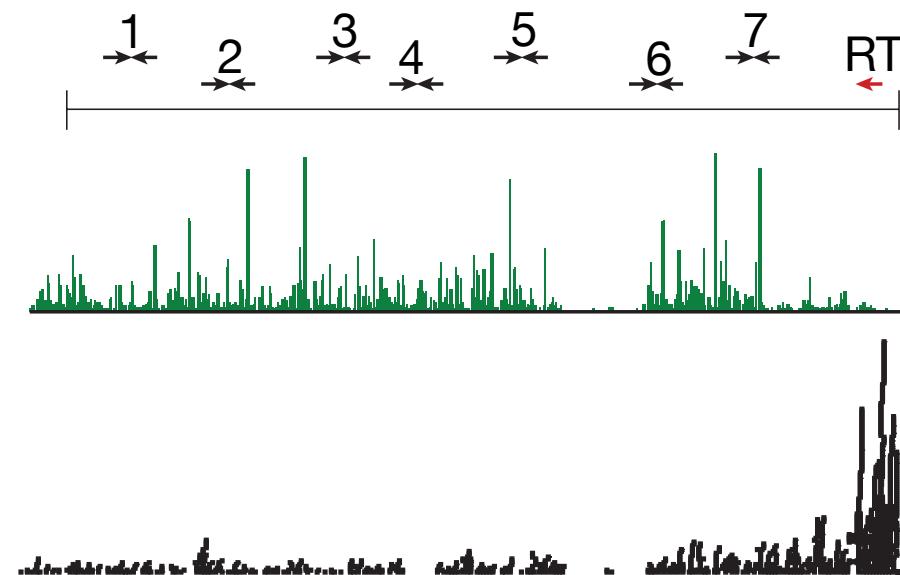
Evidence of contiguous precursor transcripts



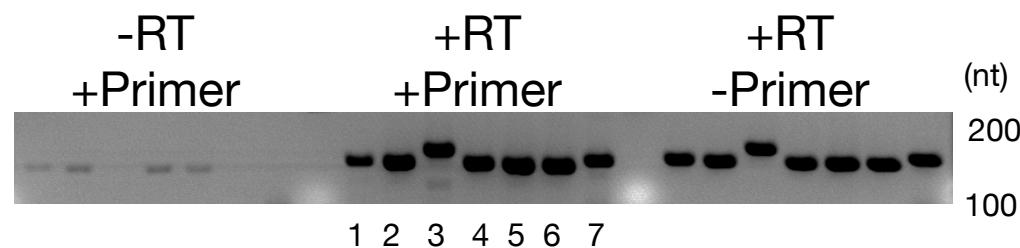
Do precursor transcripts exist as a contiguous piece of RNA?

Adapted from: Brennecke J, Aravin AA, Stark A, et al. Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. *Cell*. 2007;128(6):1089-1103.

RT-PCR analysis of precursor transcripts

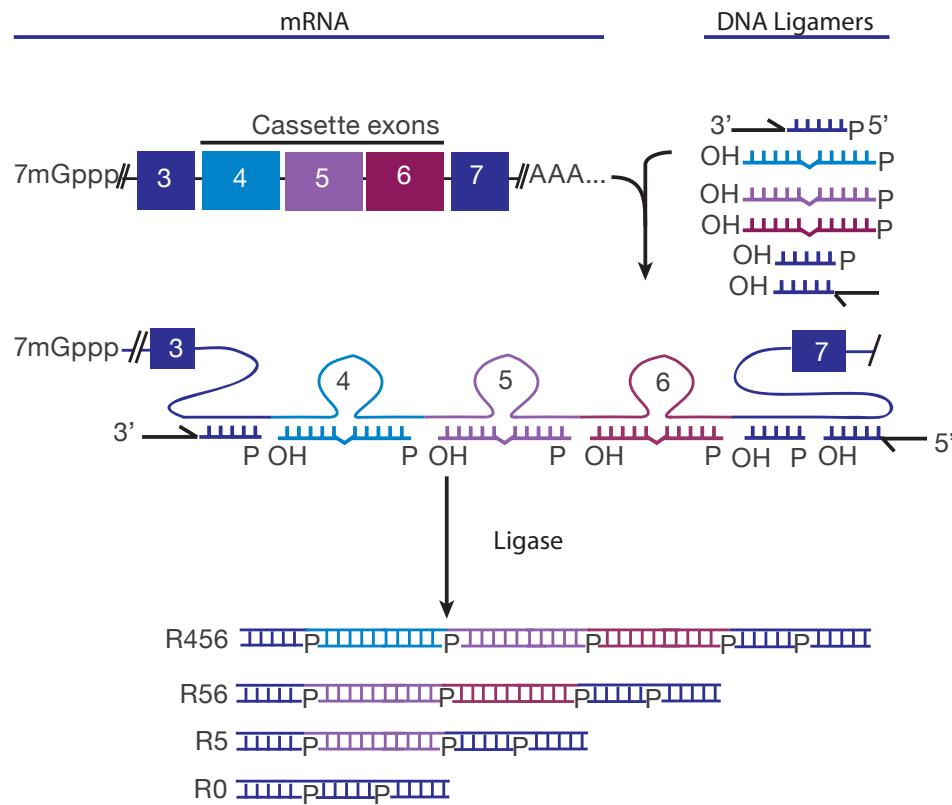


Mouse Testes RNA after DNase treatment

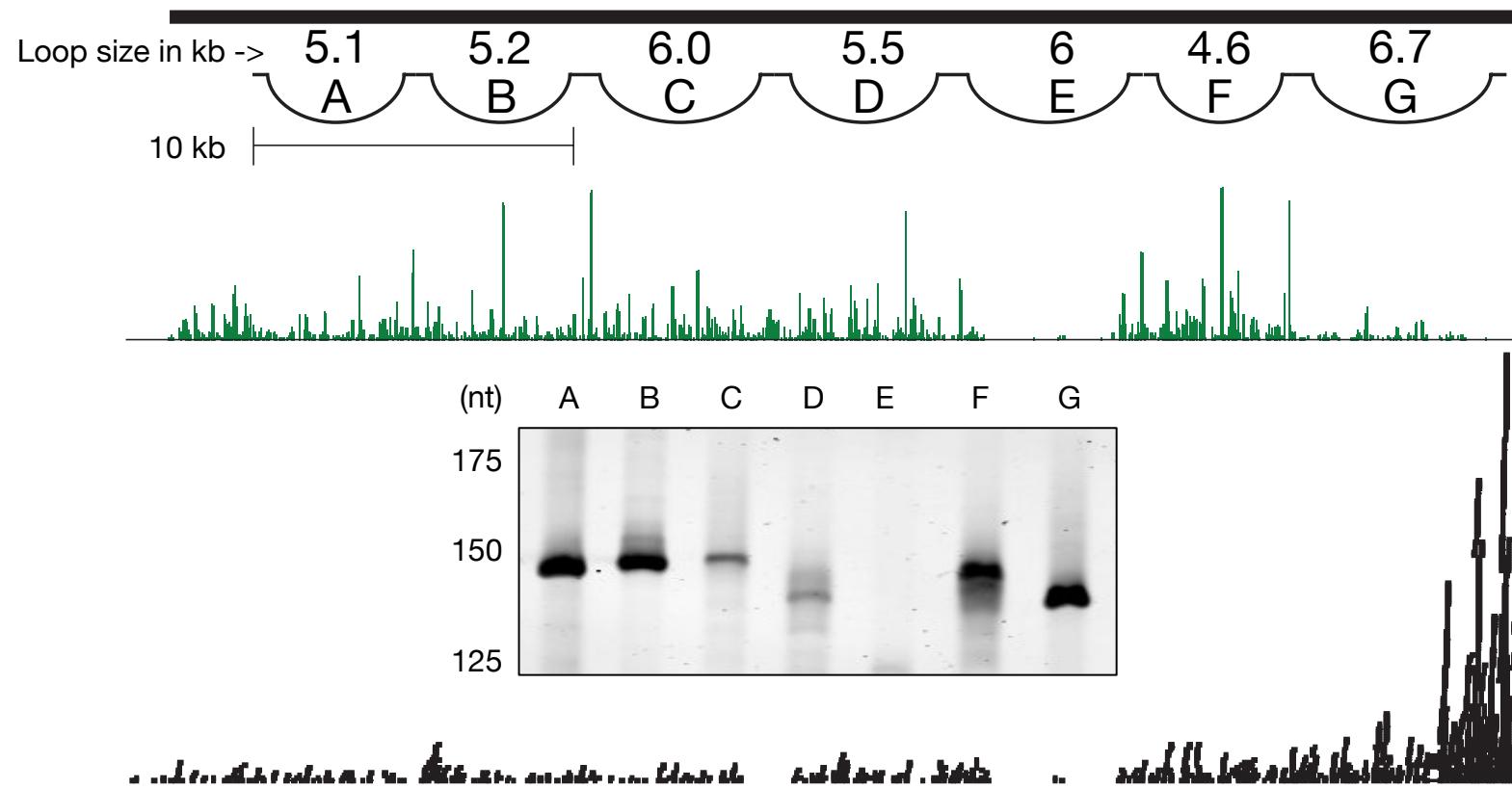


SeqZip

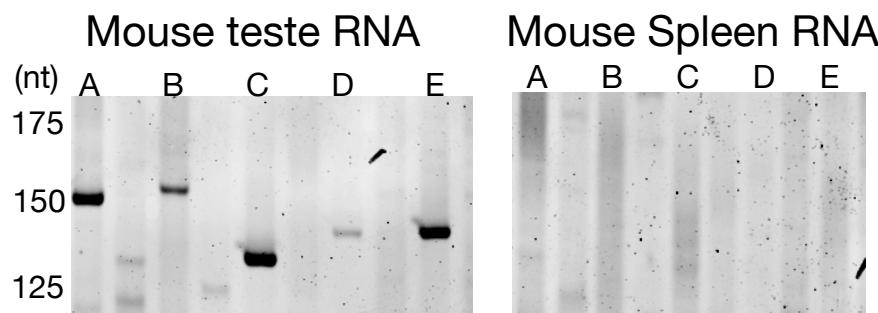
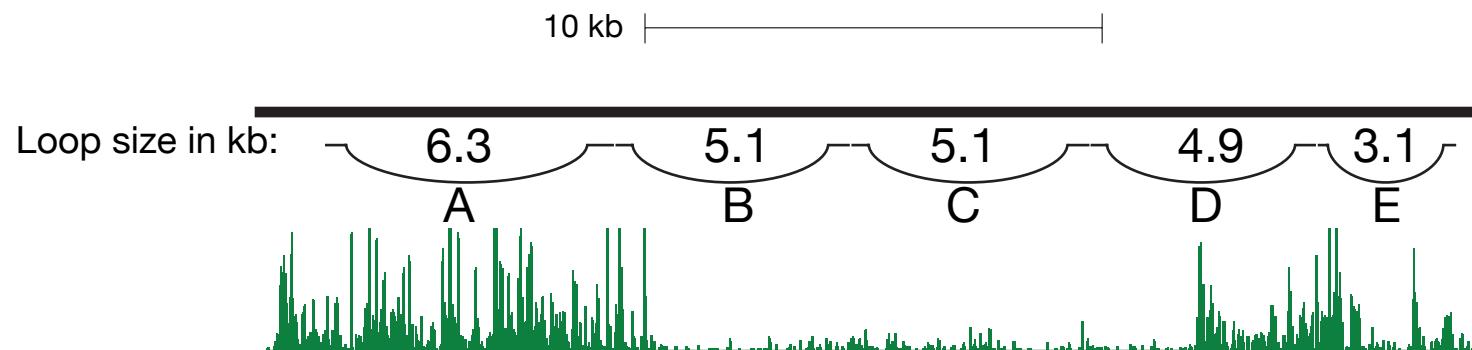
An RT-free methodology to examine RNA sequence content and connectivity



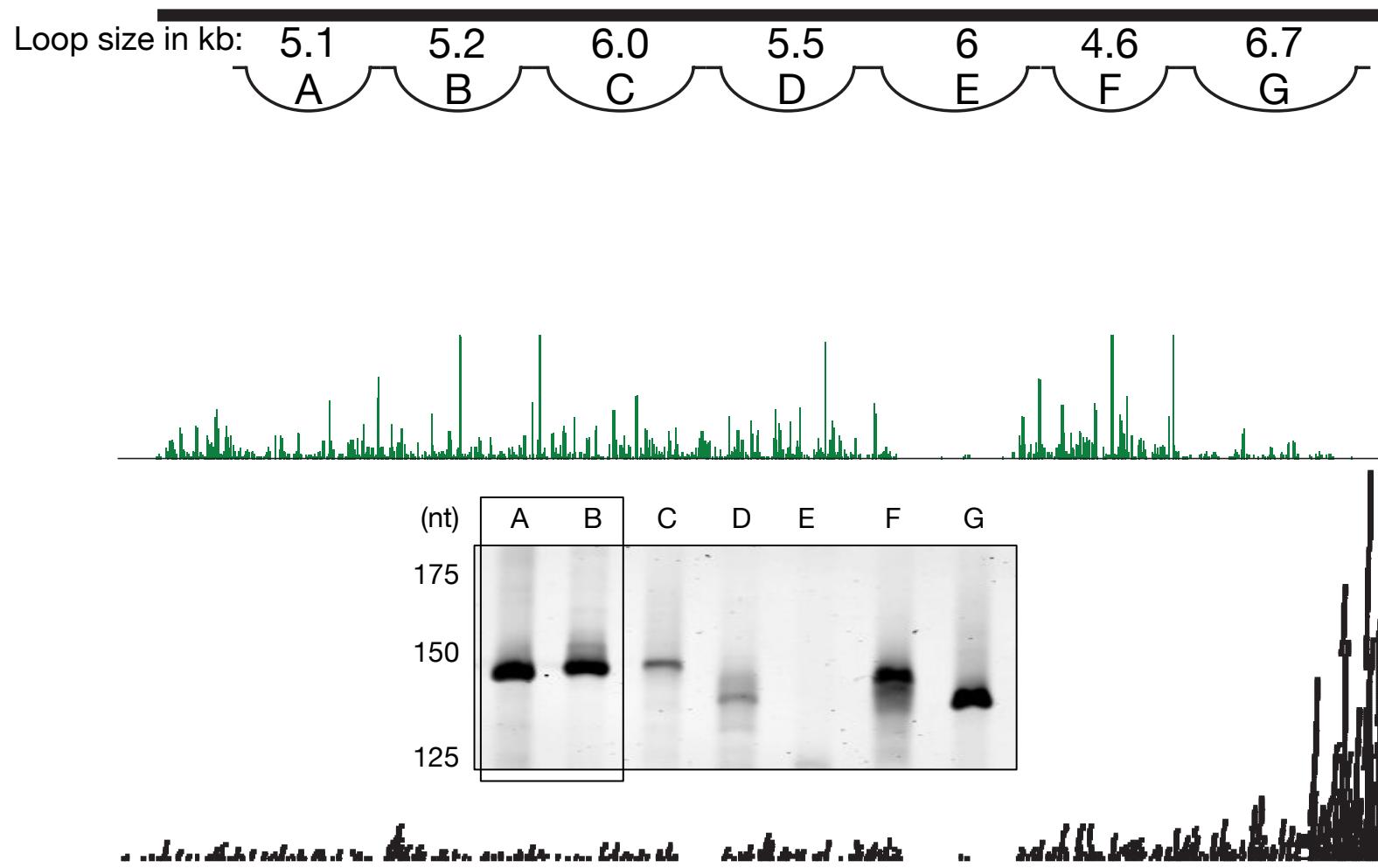
SeqZip analysis of piRNA precursor 1



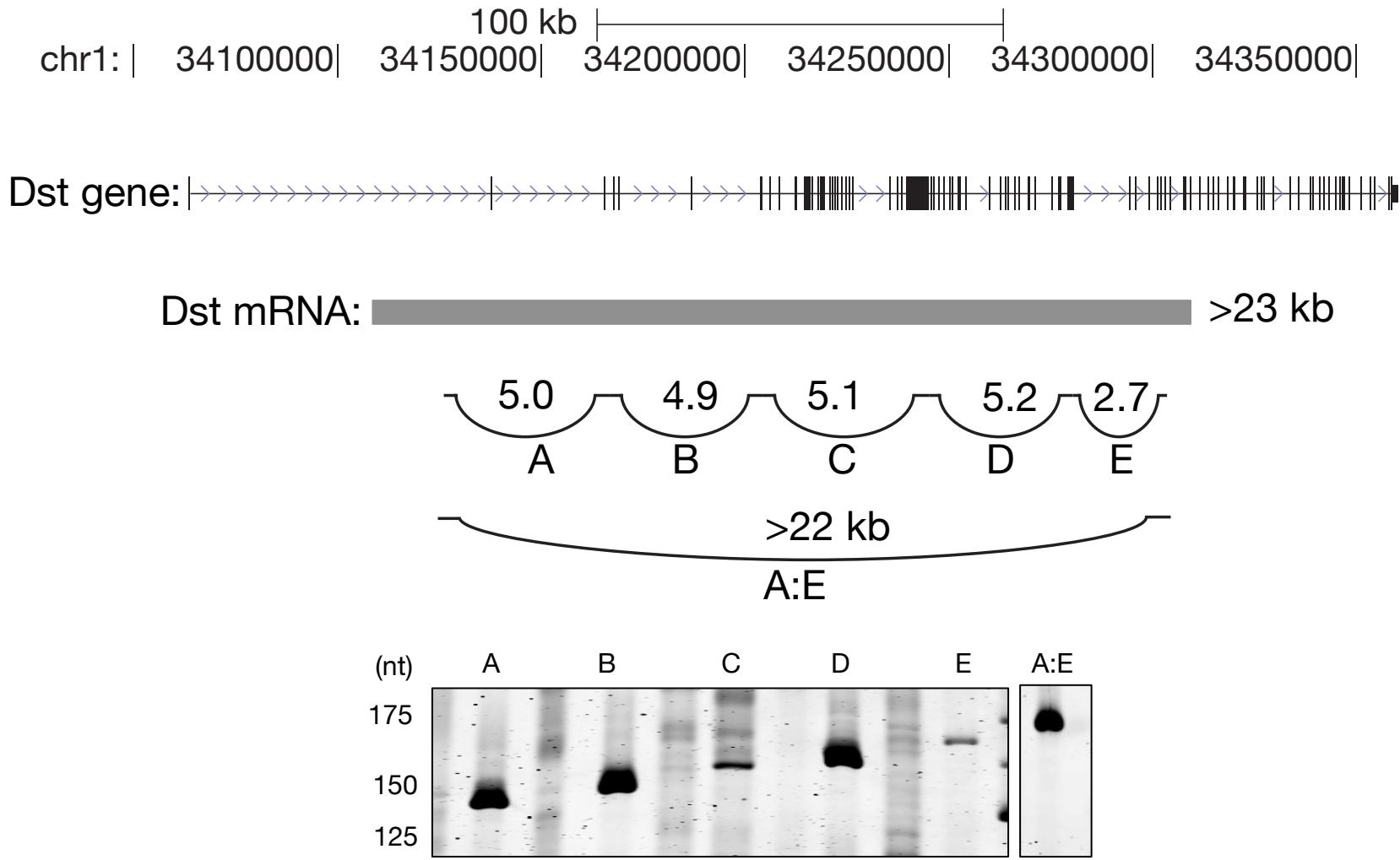
SeqZip analysis of a different precursor



Product abundance decreases with loop length



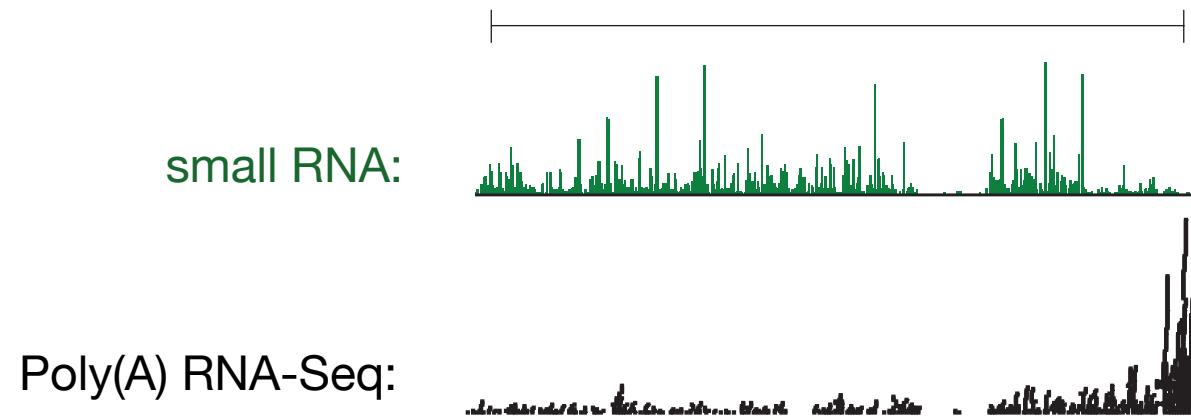
SeqZip connects ends of a long mRNA



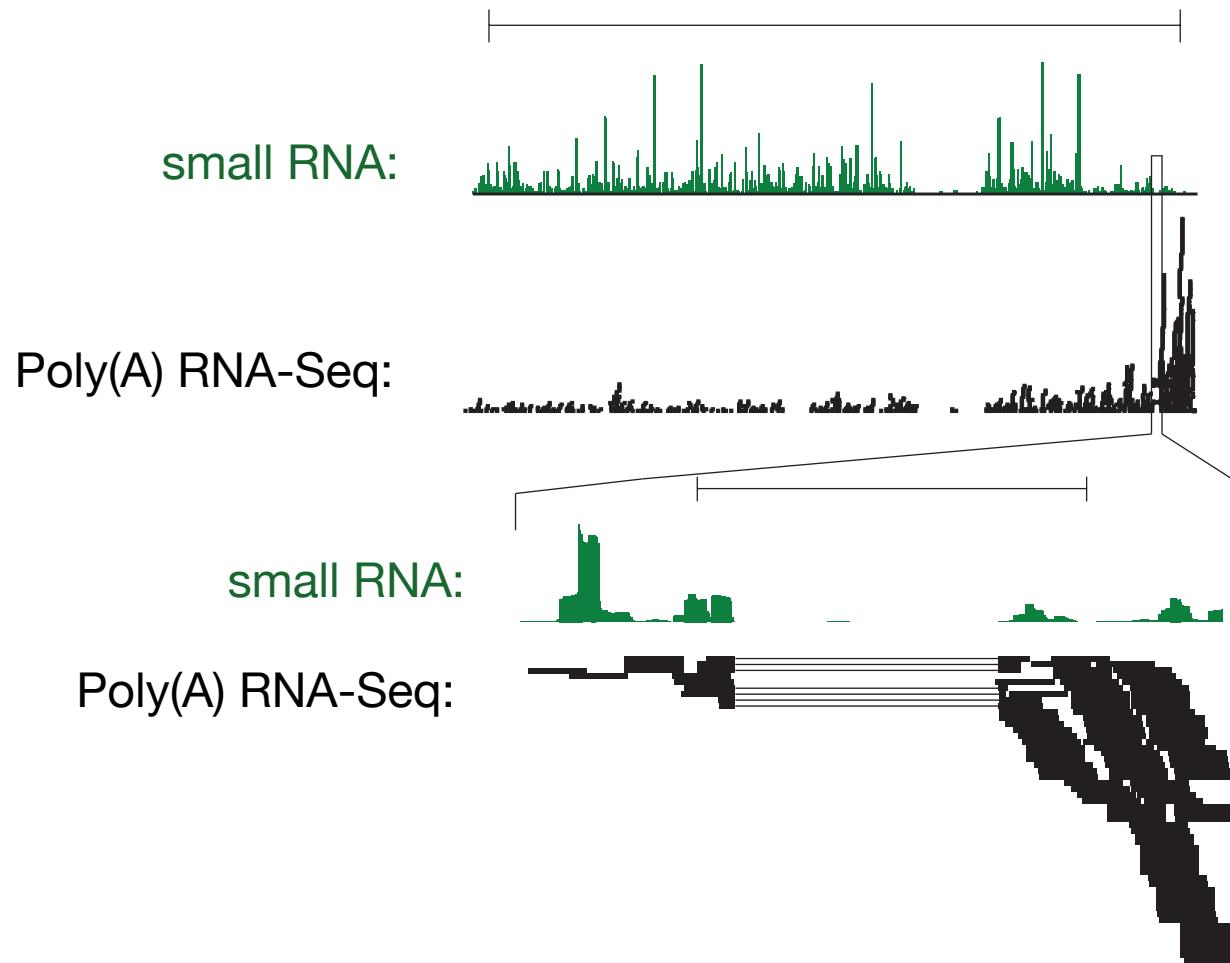
Status of SeqZip precursor analysis

- RT-PCR can not demonstrate continuity of precursor transcripts
- SeqZip indicates continuity of at least 5 kb segments and up to a 10 kb segment
- Have yet to demonstrate continuity between the suspected 5' and 3' of a precursor
- Methodology does demonstrate connectivity >23 kb for a known mRNA

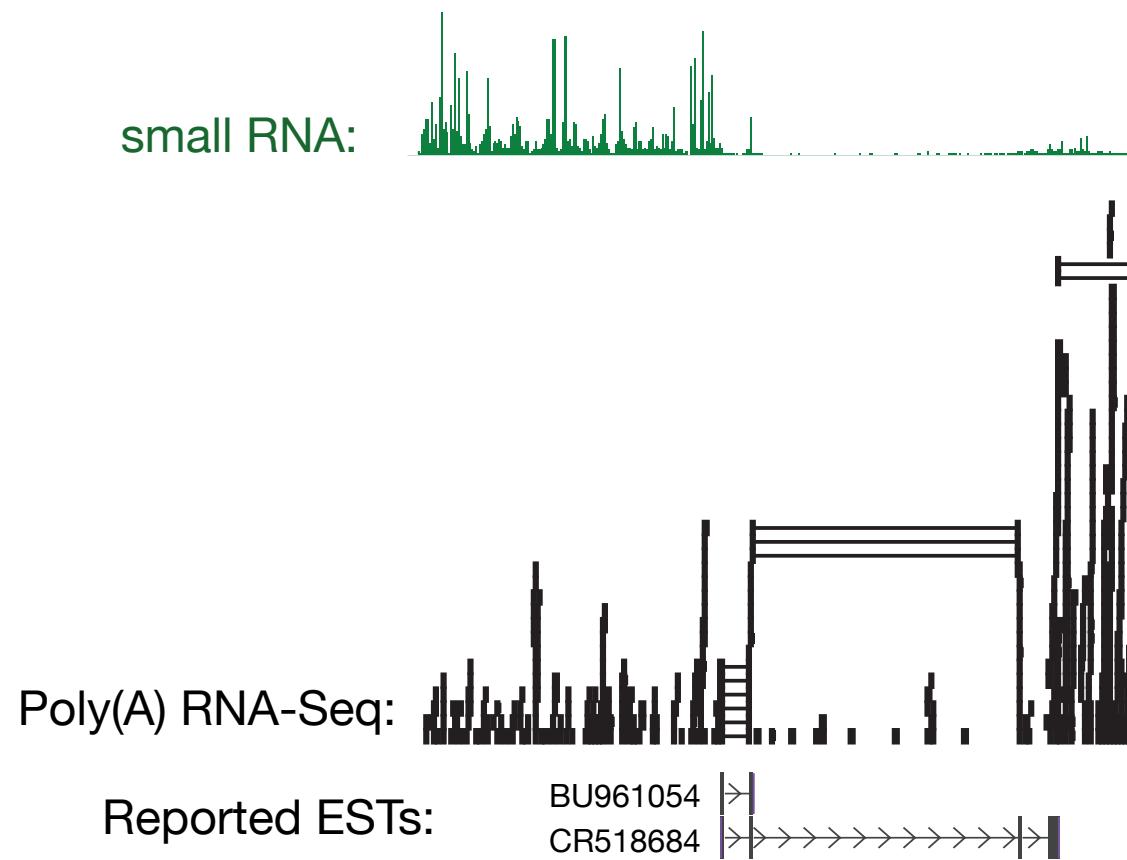
Precursors are spliced



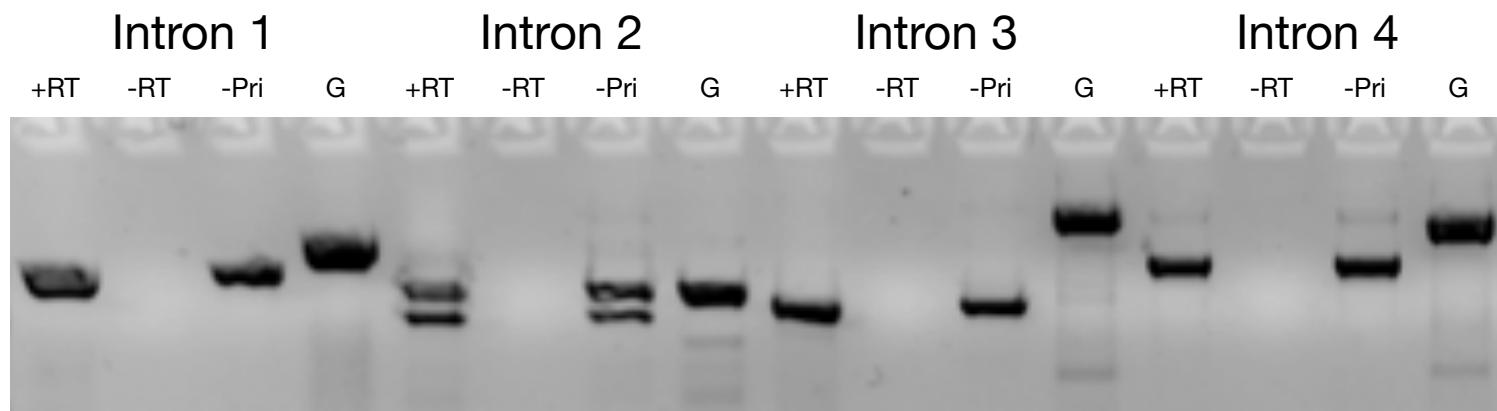
Precursors are spliced



EST evidence for splicing



RT-PCR confirms spliced precursors

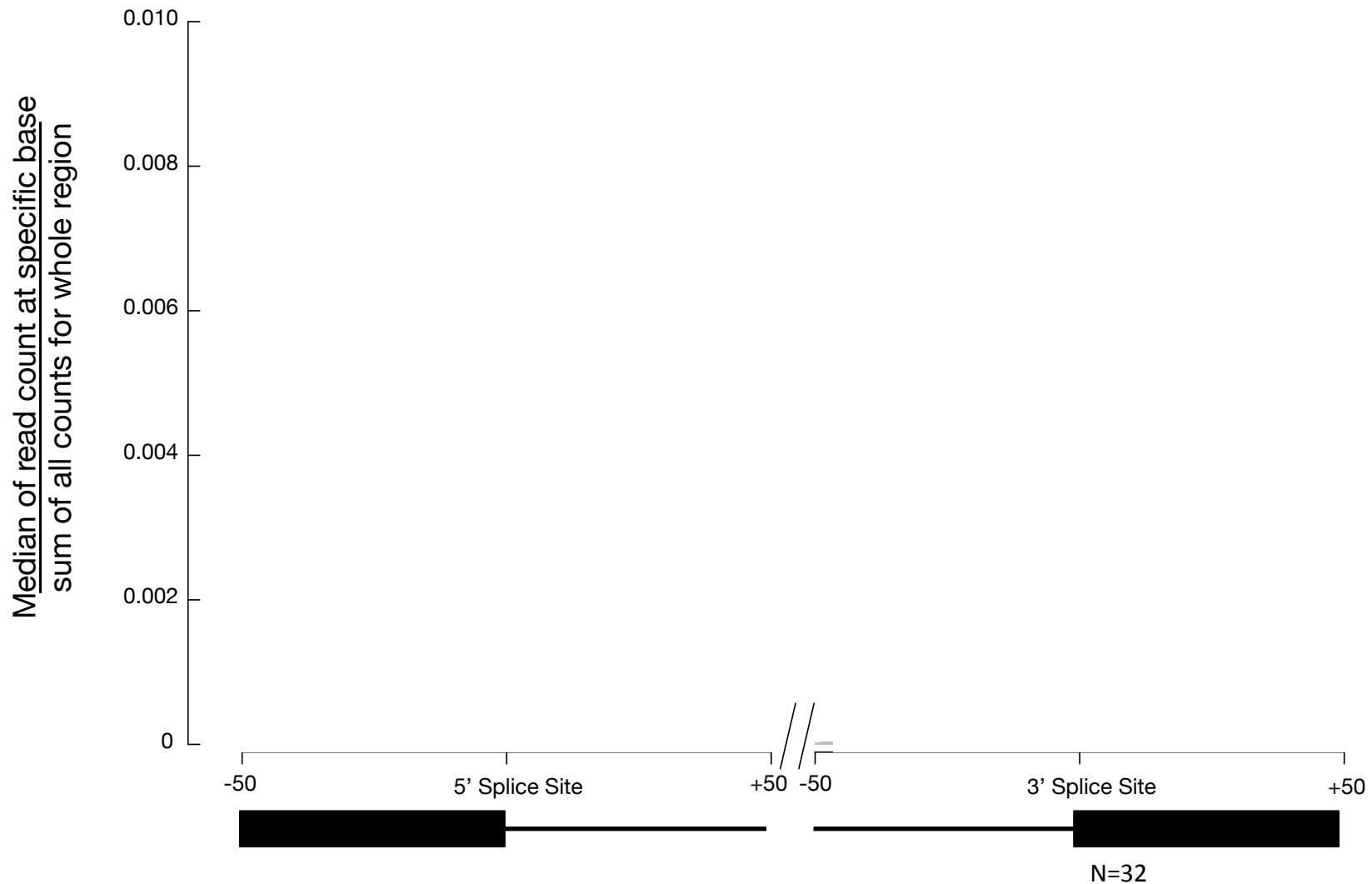


+RT = + Reverse transcriptase
-RT = - Reverse transcriptase
-Pri = No RT primer added
G = Genomic DNA template

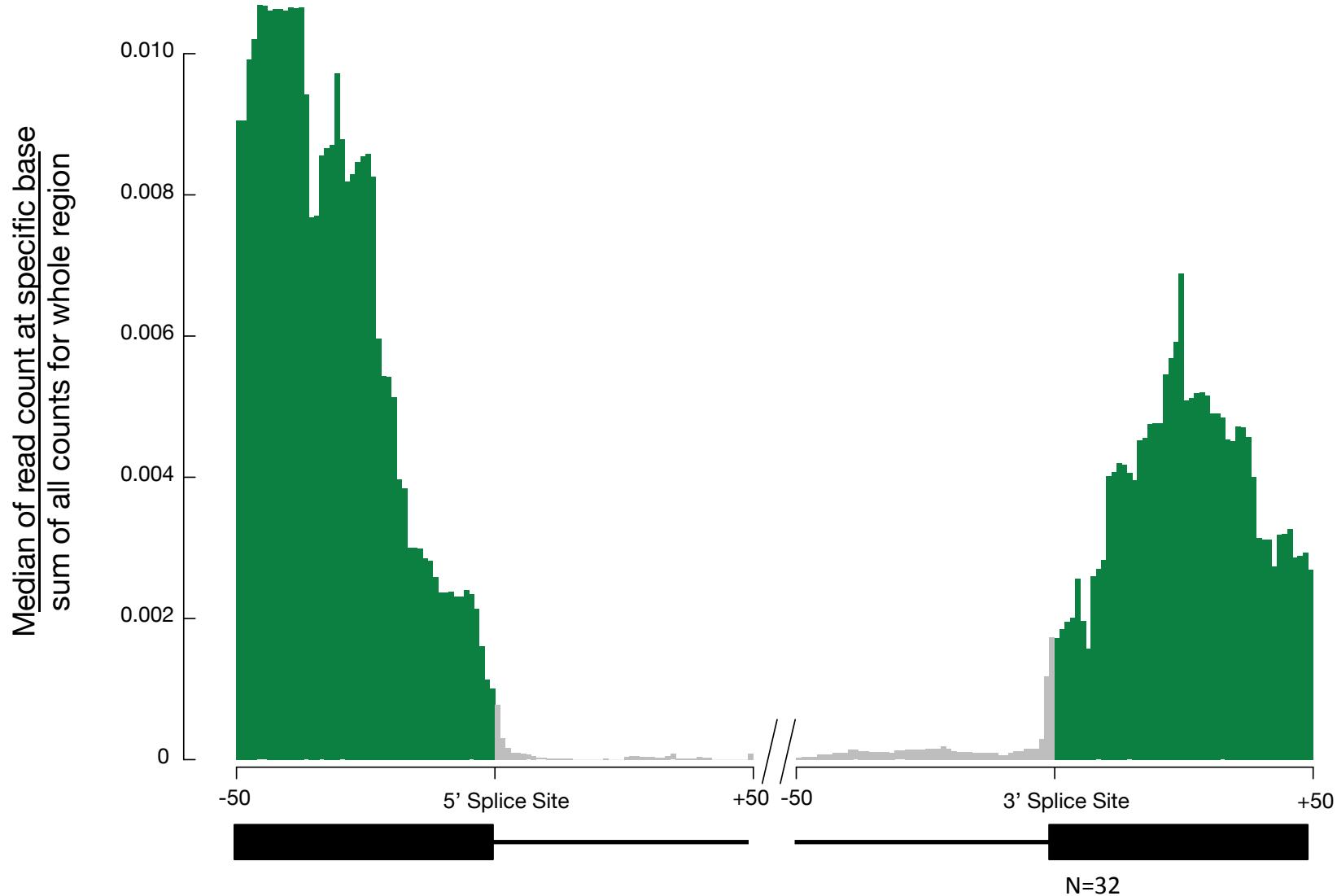
Non-genome mapping piRNAs map to junctions



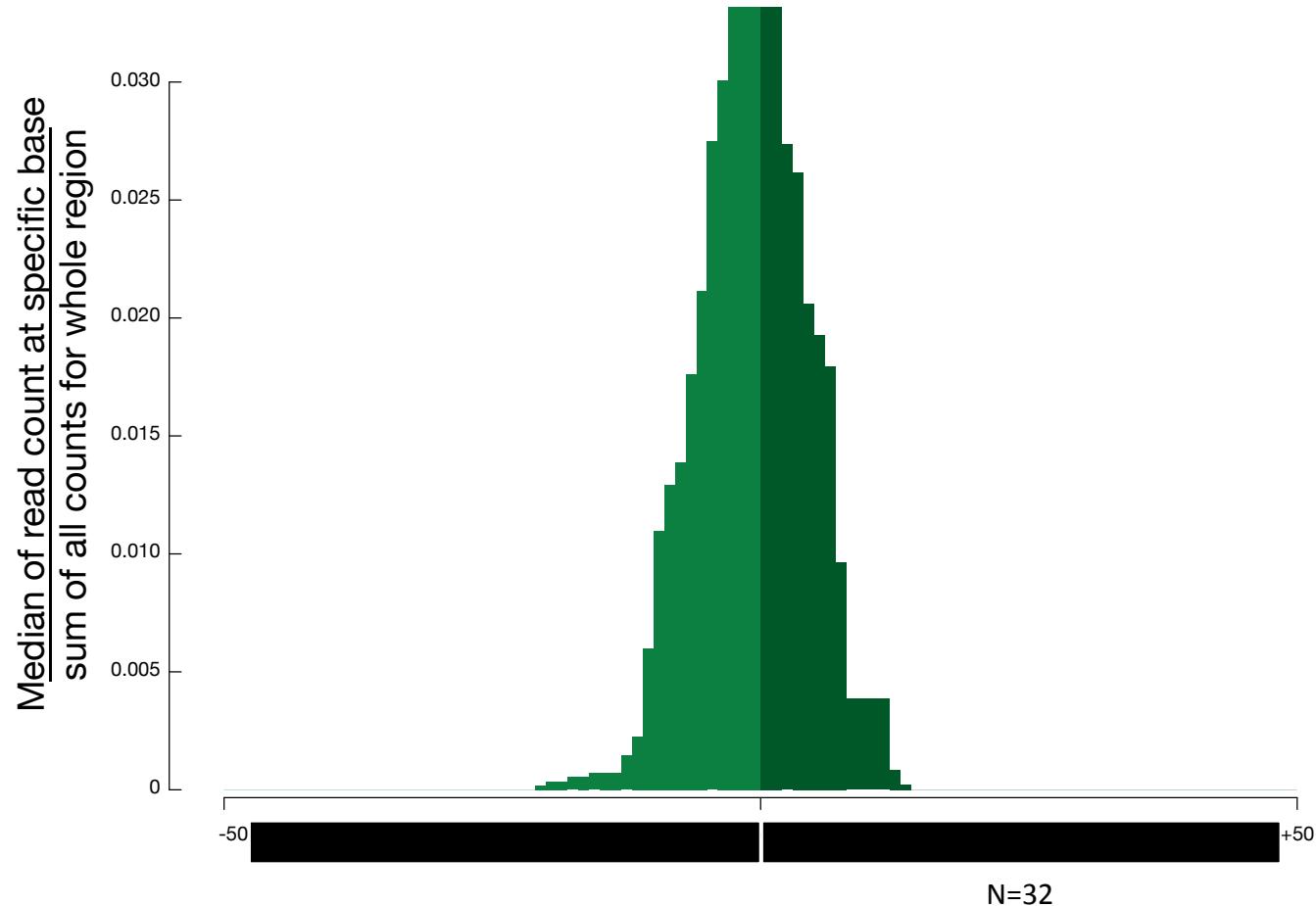
Precursor introns have lower piRNA density



Precursor introns have lower piRNA density



Non-genome mapping piRNAs map to junctions



Not so in pathway mutants that block transcription of precursors!

Summary

- SeqZip indicates precursor transcripts exist as at least 5 kb segments
- SeqZip can loop out >23 kb of an mRNA
- Precursor transcripts undergo splicing
- piRNA are derived from spliced precursors

Future goals

- Modifications to SeqZip protocol to increase loop limit
- Deep sequence the 5' end of precursors
- Identify more introns within cluster using deeper or more even RNA-Seq coverage

Thanks!

Moore Lab

Melissa J Moore

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Rina Paladino

Jing Yan

Weijun Chen

Inna Shcherbakova

Victor Serebrov

Alper Kucukural

Akiko Noma

Alicia Bicknell

Lingtao Peng

Guramrit Singh

Eric Anderson

Erin Heyer

Kelly Limoncelli

Danny Crawford

Tiffany Lopes

Luis Gustavo Morello

Can Cenik

Ami Ashar

Emiliano Ricci

Blandine Mercier

Zamore Lab

Phillip D Zamore

Xin Li

Gwen Farley

Cindy Tipping

Alicia Boucher

Tiffanie Covello

Zhao Zhang

Stefan Ameres

Timothy Chang

Bo Han

Carlos Fabian Flores

Ryuya Fukunaga

Elif Sarinay

Keith Boundy

Desiree Boltz

Jogender Singh

Liang Meng Wee

Chengjian Li

Alicia Boucher

Tracey Lincoln

Jennifer Broderick

Wes Solomon

Wei Wang

TRAC

Zhiping Weng

Scot Wolfe

Job Dekker

Bioinformatics Core

Jia Xu

General Comments

- Do not start with ‘SO’
- Start with piRNAs (Why I should care)
- Compare length of clusters to something audience knows
- Titles on 1 line – make them a conclusion
 - Always have the title in same place!
- All fonts are Helvetica Neue
- Do not tell what you are to tell – just tell!
- Tags = ‘Sequence Reads’
- Change RPKM to RNA Abundance per KB
- Change all numbers to KB with 1 sig digit