A. small RNA extraction
PAGE purification
(size-selection)

input reads (fastq)

analyze read quality (fastQC)
trim adapters (trimmer)

trimmed reads

Align to rRNA (Bowtie) → rRNA reads
Align to microRNA hairpin (Bowtie) → miRNA reads
non-rRNA reads, non-miRNA reads
size-selection (piPipes) → siRNA reads
non-rRNA, non-miRNA, 23-29 nt reads

Align to transcriptome (Bowtie)
Transcriptome mapped reads at:
genes, piRNA clusters, and transposons (BAM/BED)
count estimation (Express)
antisense/sense counts
(normalize with mRNA hairpin reads)
normalized counts
(DESeq2)
differential expression analysis at:
piRNA clusters, transposons, coding genes

B. RNA extraction
ribo-zero treatment

input reads (fastq)
Align to genome (STAR)
genome mapped reads (BAM)
count estimation (TeTranscript)
abundance counts at:
transposons and genes
(DESeq2)
differential analysis at:
genomes, transposons

ping-pong activity analysis
A  
antisense and sense piRNAs mapping to FB-element transposon

Wild-type  
klp10A\text{RNAi}

B  
antisense and sense piRNAs mapping to BAR/1 transposon

Wild-type  
klp10A\text{RNAi}
A. 

**Replicate 1**

5' to 5' overlap, $Z = 20.5$

**Replicate 2**

5' to 5' overlap, $Z = 22.3$

**Replicate 3**

5' to 5' overlap, $Z = 38.1$

---

B. 

![Box plots showing ping-pong ratio for different conditions](image)

- **Wild-type 1**
- **Wild-type 2**
- **Wild-type 3**
- **Klp10A$^{RNAi}$ 1**
- **Klp10A$^{RNAi}$ 2**
- **Klp10A$^{RNAi}$ 3**
### A.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>klp10A&lt;sup&gt;RNAi&lt;/sup&gt;</th>
<th>Wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### B.

- **PC1**: 77% variance
- **PC2**: 16% variance

**Wild-type replicates**

**Klp10A<sup>RNAi</sup> replicates**

### C.

- **13,665 genes analyzed**
- (>10 read count): adjusted p-value < 0.1

- Log<sub>2</sub> fold change > 0 (up): 477, 3.5%
- Log<sub>2</sub> fold change < 0 (down): 1264, 9.2%
A

control

nos>|klp10A^RNAi|

B

control

nos>|klp10A^RNAi|

Number of filmed cells

Time after metaphase (min)

Piwi at nuage

Piwi nuclear only

S10 Fig
### Table C

<table>
<thead>
<tr>
<th></th>
<th>GSC and SG</th>
<th>SC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>nos&gt;klp10A&lt;sup&gt;RNAi&lt;/sup&gt;</td>
</tr>
<tr>
<td>Interphase cells</td>
<td>0.36±0.95</td>
<td>4.36±4.44</td>
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<tr>
<td>with cytoplasmic</td>
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<td></td>
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<tr>
<td>Piwi foci</td>
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</table>

**S11 Fig**