

Supplemental 1 Zili cDNA sequence. The 5' and 3' UTR are indicated in lower case.

Supplemental 2 piRNAs binding to Ziwi and Zili. (A) RNA isolated from immuno precipitations with Ziwi or Zili antibodies was ³²P end-labelled and analyzed by electrophoresis. Ziwi and Zili antibodies both pull down a complex containing a distinct class of small RNAs of about 27nt long. (B) Size distribution of cloned piRNAs, showing a distribution around 26 nucleotides for Zili and 27 for Ziwi. (C) Annotation of identified RNA species. piRNA population can be divided into piRNAs mapping to 'repetitive elements', 'coding regions' or 'not annotated'. 'Other RNAs' consists of tRNA, rRNA, snRNAs and snoRNAs. Percentages are given for each group. Four libraries; Ziwi and Zili immunoprecipitations from ovary and testis, all show distinct mapping to repeat associated regions and a smaller proportion to coding regions. (D) Coding piRNAs usually map once, while piRNAs mapping to repetitive regions more often map to several locations in the genome. piRNAs classified as 'not annotated' show a distribution very similar to that of piRNAs from repetitive elements, indicating that 'not annotated' piRNAs may very well hold many piRNAs that map to repetitive sequences.

Supplemental 3 Ziwi and Zili bind piRNAs of opposite polarity. (A) Part of chromosome 14 in UCSC genome browser showing piRNA clusters with distinct strand switching corresponding to blocks of repeats that switch orientation (arrows). Blue bars are sense piRNAs and red bars are antisense piRNAs. (B) Graphic representation showing Ziwi and Zili piRNAs mapping to the same loci on chromosome 8. The x-axis shows complete chromosome 8, while the y-axis shows plotted unique reads (red) and weighted reads (blue), clustered in loci on the basis of a maximum distance of 100 kilobases (kb) between two consecutive loci. Ziwi piRNA loci are depicted above the midline, while Zili piRNA loci are depicted below the midline.

Supplemental 4 Evidence for piRNA ping-pong. (A) Left graph showing a 10-nucleotide overlap between Ziwi piRNAs on opposite strands, while the right graph shows nucleotide distributions at the 5' and 3' ends of these overlaps. (B) Left graph showing a 10-nucleotide overlap between Zili piRNAs on opposite strands, while the right graph shows nucleotide distributions at the 5' and 3' ends of these overlaps. (C) Graph showing a 19-28 nucleotide overlap between Ziwi piRNAs on the same strand, suggesting a preference for certain sequences in the primary processing or amplification. (D) Graph showing a 19-28 nucleotide overlap between Zili piRNAs on the same strand, suggesting a preference for certain sequences in the primary processing or amplification.

Supplemental 5 Transposon-derived piRNAs show a ping-pong signature. Nucleotide distribution of piRNAs mapping to several transposon species, showing a clear preference for 5' uracil, most prominent in Ziwi antisense (minus) piRNAs. While Zili sense (plus) piRNAs have a distinct preference for adenosine at position 10. This holds true for LTR transposons (*BEL*, *Gypsy*), as well as non-LTR (*CRI*, *SINE*) and DNA transposons (*EnSpm*, *Helitron*).

Supplemental 6 Exonic piRNAs show a ping-pong signature. (A) piRNA population mapping to genes, either antisense or sense to the gene. (B) Graphical representation of piRNA population mapping to genes in different libraries. Ziwi piRNAs are mostly antisense to coding regions of genes, while Zili piRNAs are mostly sense. Left panel represents piRNAs that are antisense to genes and the right panel represents piRNAs that are sense to genes. Left and right panel together are 100%. (C) Graph shows a 10-nucleotide overlap between Ziwi and Zili piRNAs mapping to exons of genes (C) or to a set of exonic piRNAs selected for unique mapping-positions and excluding genes with alternative splicing (C'). (D) Graphs showing nucleotide distributions at the 5' and 3' ends of these overlaps, showing a 5' preference for uracil and a 3' preference for adenosine in overlapping piRNA sequences in these exons. This suggests that piRNA amplification, as seen in transposon classes, also takes place with exonic piRNAs.

Supplemental 7 *zili*^{-/-} gonads. (A) Zili (brown) staining is lost in *zili*^{-/-} gonads (6 wpf), indicating that Zili antibody staining is specific. Scale bars are 100µM. (B) At 5 wpf, *gypsyDRI* and *ngaro* transcripts (LTR elements)(purple) are found in the nucleus of germ cells of female and male gonads.

Supplemental 8 Transposon transcripts in *zili*^{-/-} and *zili*^{L590P/-} gonads. Elevated levels of transposon transcripts in *zili*^{-/-} gonads at 5 wpf compared to female and male gonads at 5 wpf. Since *zili*^{-/-} gonads are phenotypically more similar to male gonads they may be compared with those. However in quantitative PCR analyzes both male and female *zili*^{+/+} gonads were pooled. Transposon levels in *zili*^{L590P/-} ovary are not increased compared to *zili*^{+/+} ovary. *GypsyDRI* (LTR),

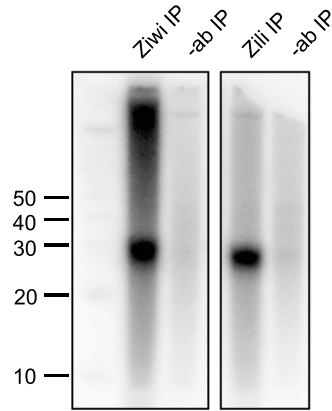
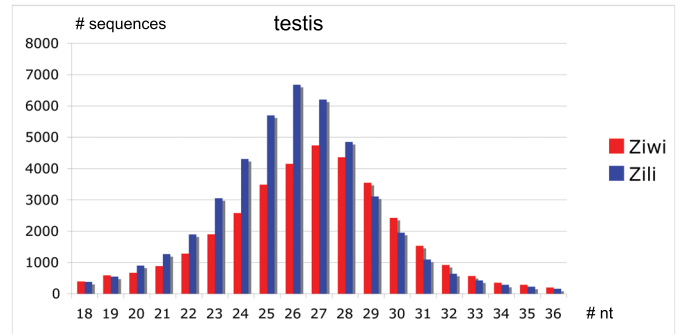
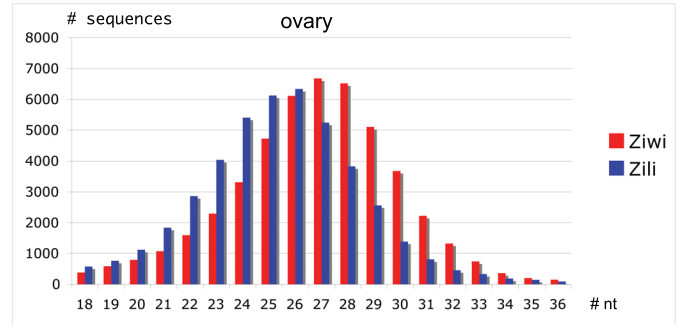
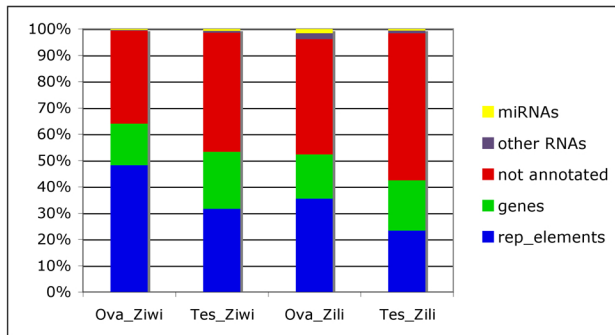
LTR2 (LTR), *DIRS1a* (LTR), *ngaro* (non-LTR) were tested (purple). Scale bars are 50µM.

Supplemental 9 Characteristics of *zili*^{-/-} germ cells.

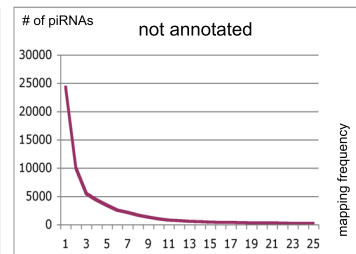
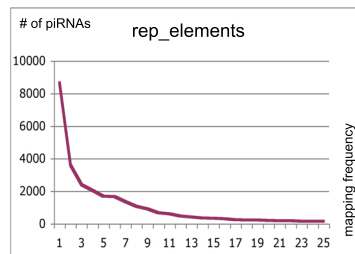
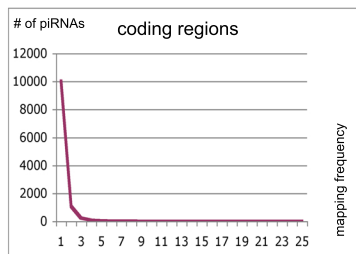
(A) At 5 wpf, *nanos* (purple) is present in oocytes but cannot be detected in developing testis. *zili*^{-/-} gonads are negative for *nanos*, indicating that female development does not occur. Scale bars are 100µM. (B) At 5 wpf, *proliferating cell nuclear antigen (PCNA)* (purple) is present in female and male germ cells, as well as in *zili*^{-/-} germ cells, indicating that *zili*^{-/-} germ cells are still proliferating. Scale bars are 100µM. (C) At 5 wpf, cleaved Caspase-3 is rarely seen in *zili*^{+/+} gonads. *zili*^{-/-} gonads are negative for cleaved Caspase-3, indicating that these mutant germ cells are not undergoing apoptosis. A mouse intestine serves as a positive control and has several apoptotic cells. Scale bars are 100µM.

Supplemental 10 Model for piRNA amplification pathway. (a) Initiation of the cycle begins with a piRNA cluster, often annotated as transposons sequences, which generate primary piRNAs. These piRNAs are antisense to expressed transcripts and bind to Ziwi. (b) These Ziwi-piRNA complexes can identify and cleave mRNA, between position 10 and 11 of the 5' end of the piRNA. This generates sense piRNAs, which bind to Zili. (c) These Zili-piRNA complexes mediate cleavage of the piRNA cluster transcript, resulting in new antisense piRNAs capable of binding to Ziwi. As long as these secondary antisense piRNAs are able to bind and silence their targets, more sense piRNAs will be generated thereby amplifying this silencing loop. (d) Zili-piRNA complexes may target complementary DNA sequences, possibly resulting in epigenetic modifications. (e) Ziwi is maternally deposited in the embryo, providing the offspring with a pool of piRNAs, capable of passing on the epigenetic profile of the germ cells and providing an early defense against transposons. (f) If Zili protein is lost, a primary pool of piRNAs will be present however amplification of the cycle is less effective with only Ziwi left to cleave transcripts. (g) This leaves the developing germ cells with a reduced ability to fight transposon threats, resulting in increased levels of transposon transcripts. This is accompanied by a defect in germ cell differentiation, ultimately followed by germ cell loss. (h) With an altered Zili protein, Zili function may be reduced or changed. Meiotic defects in the female germ line are observed (i).

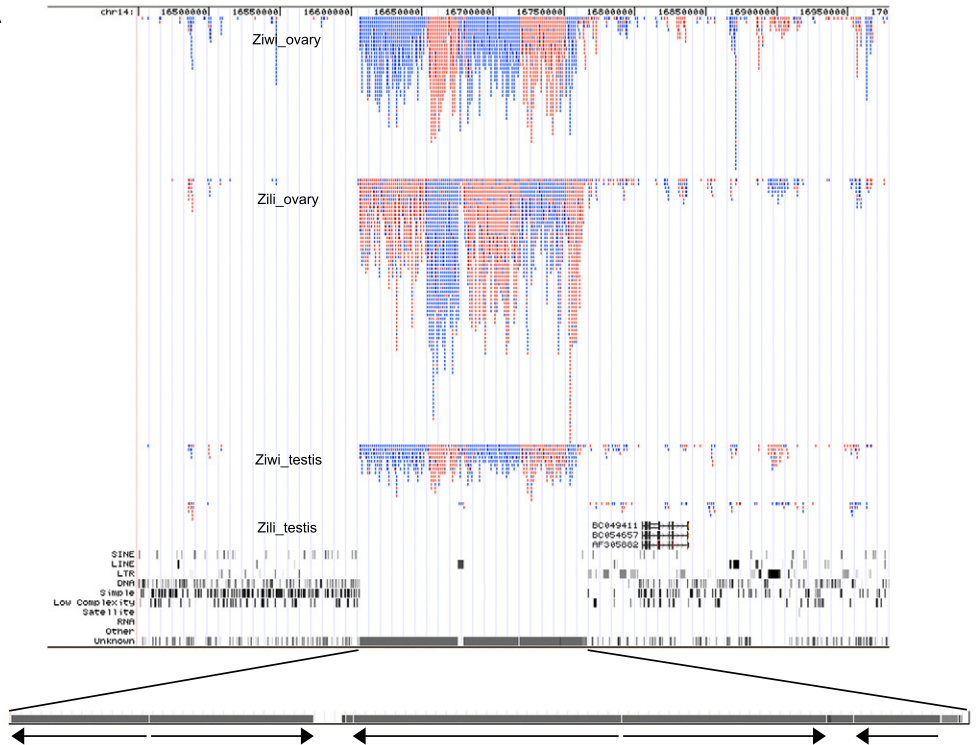
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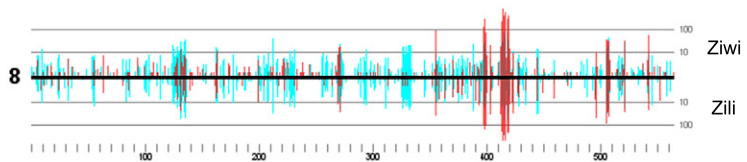
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genes	7318	15.8%	6267	21.6%	6553	16.8%	7354	19.1%
not annotated	16393	35.3%	13169	45.3%	17116	43.9%	21516	55.9%
other RNAs	151	0.3%	207	0.7%	846	2.2%	364	0.9%
miRNAs	181	0.4%	210	0.7%	660	1.7%	262	0.7%
total	46398		29044		39022		38472	

D


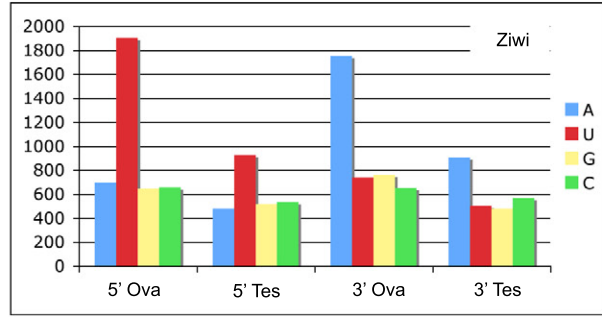
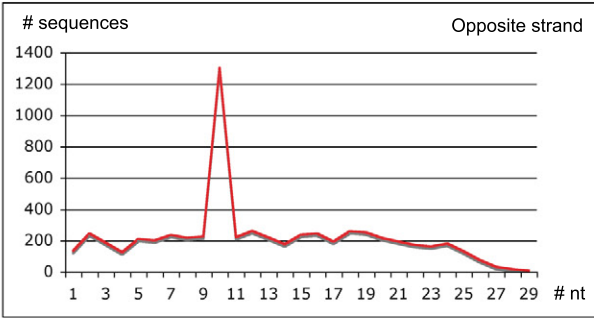
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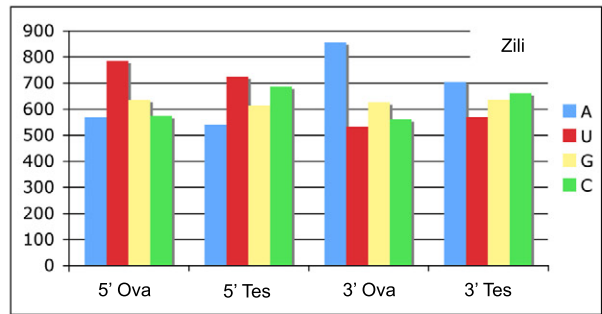
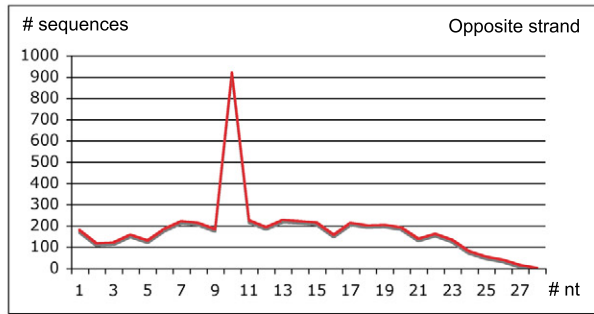
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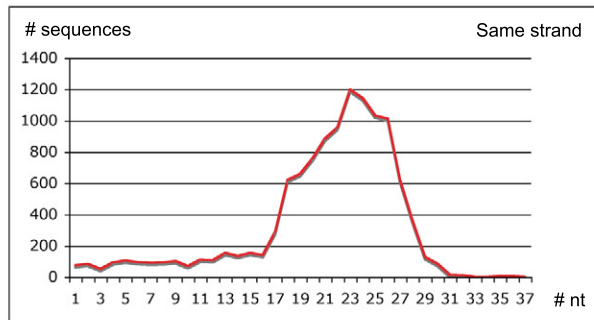
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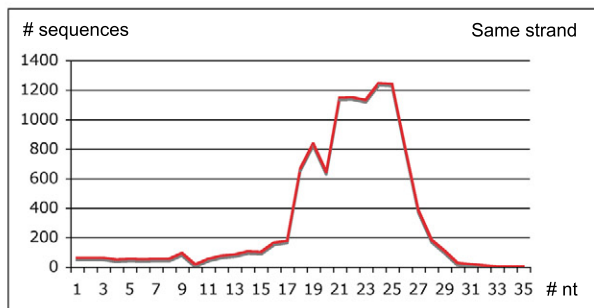
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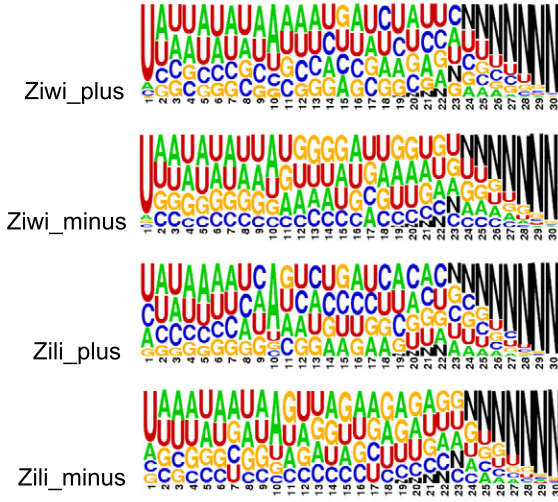
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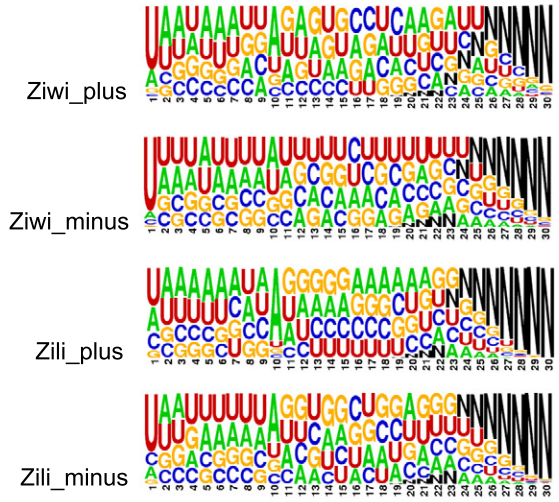
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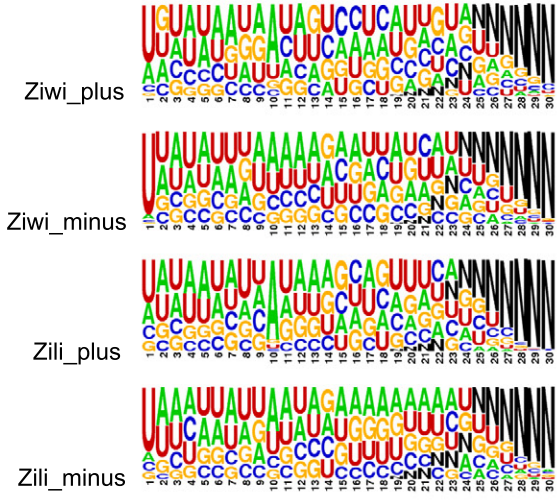
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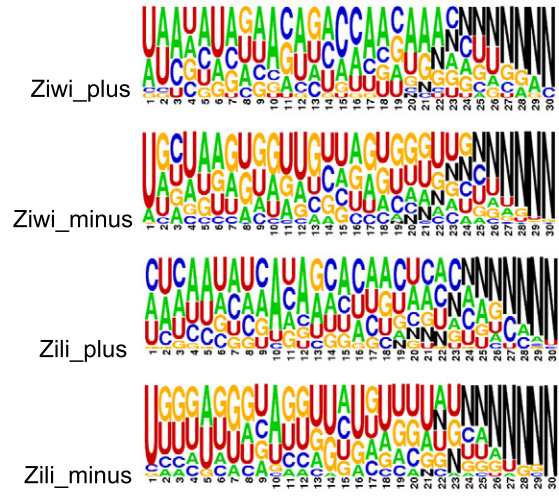
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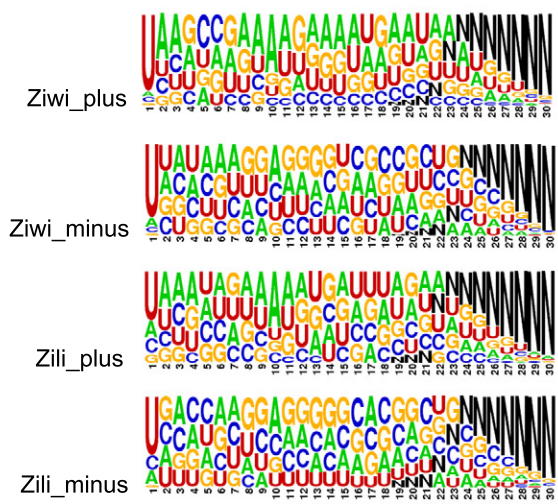
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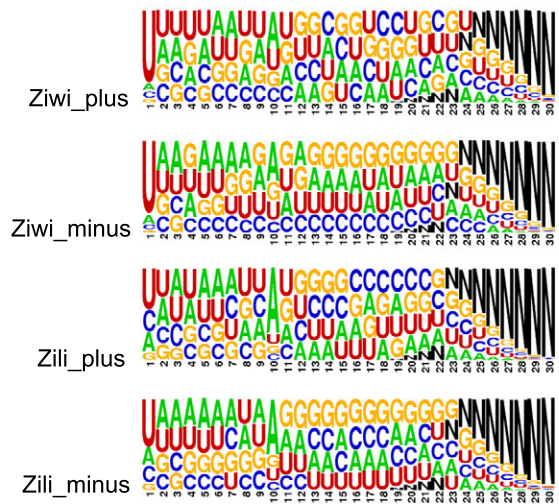
Helitron (DNA)



SINE (non-LTR)

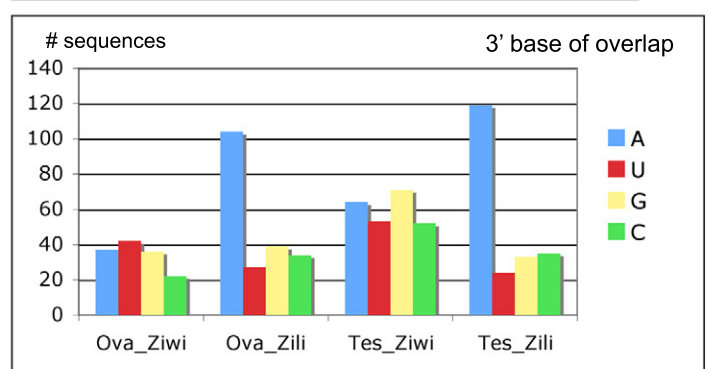
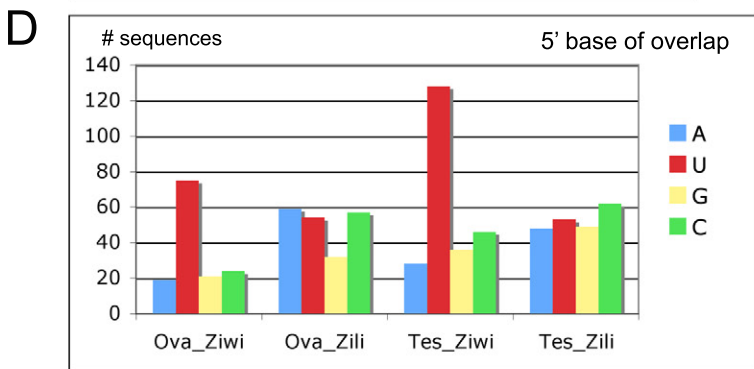
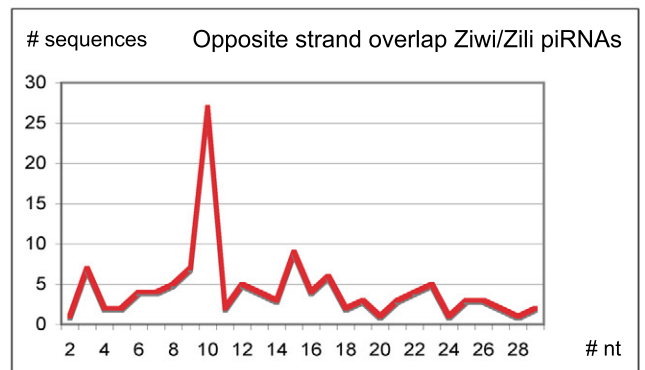
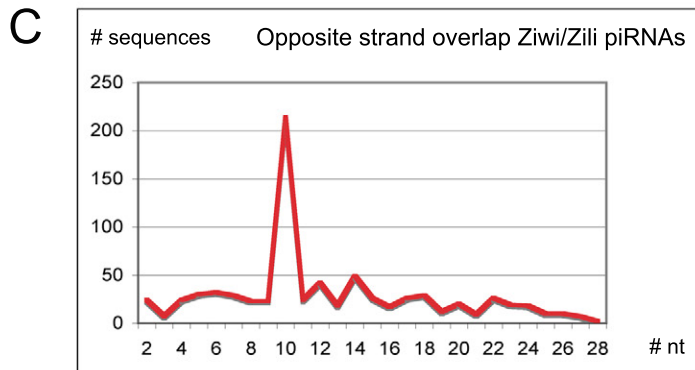
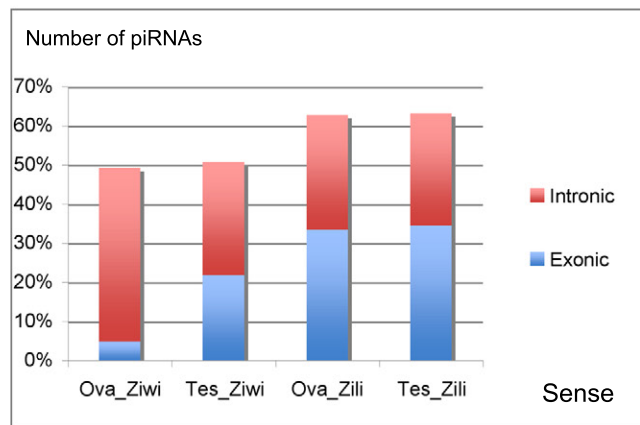
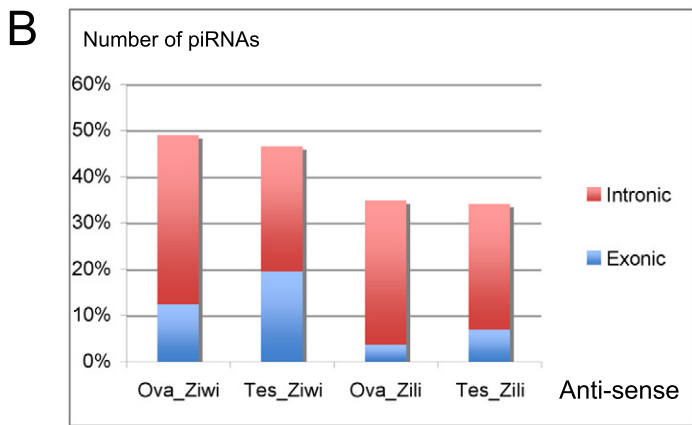


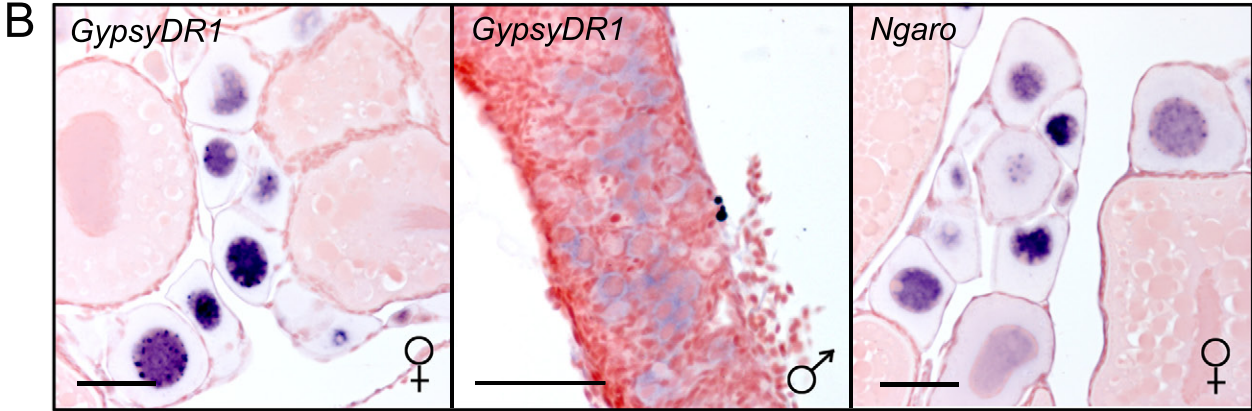
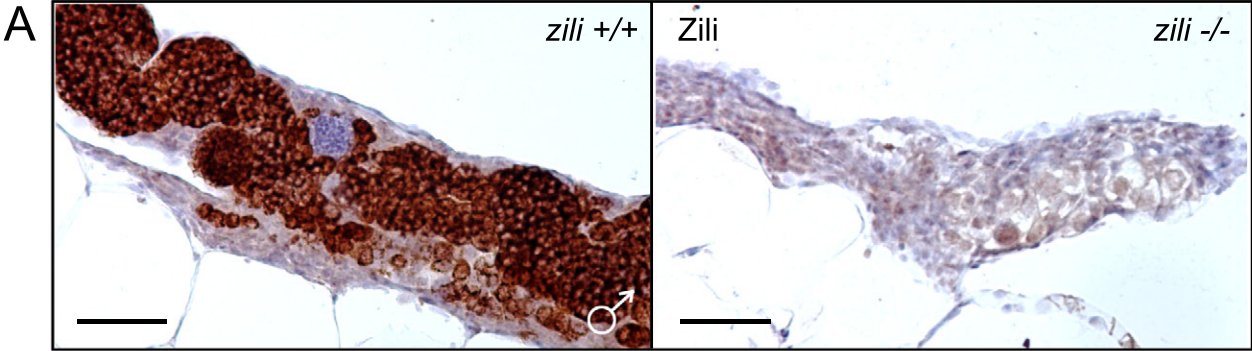
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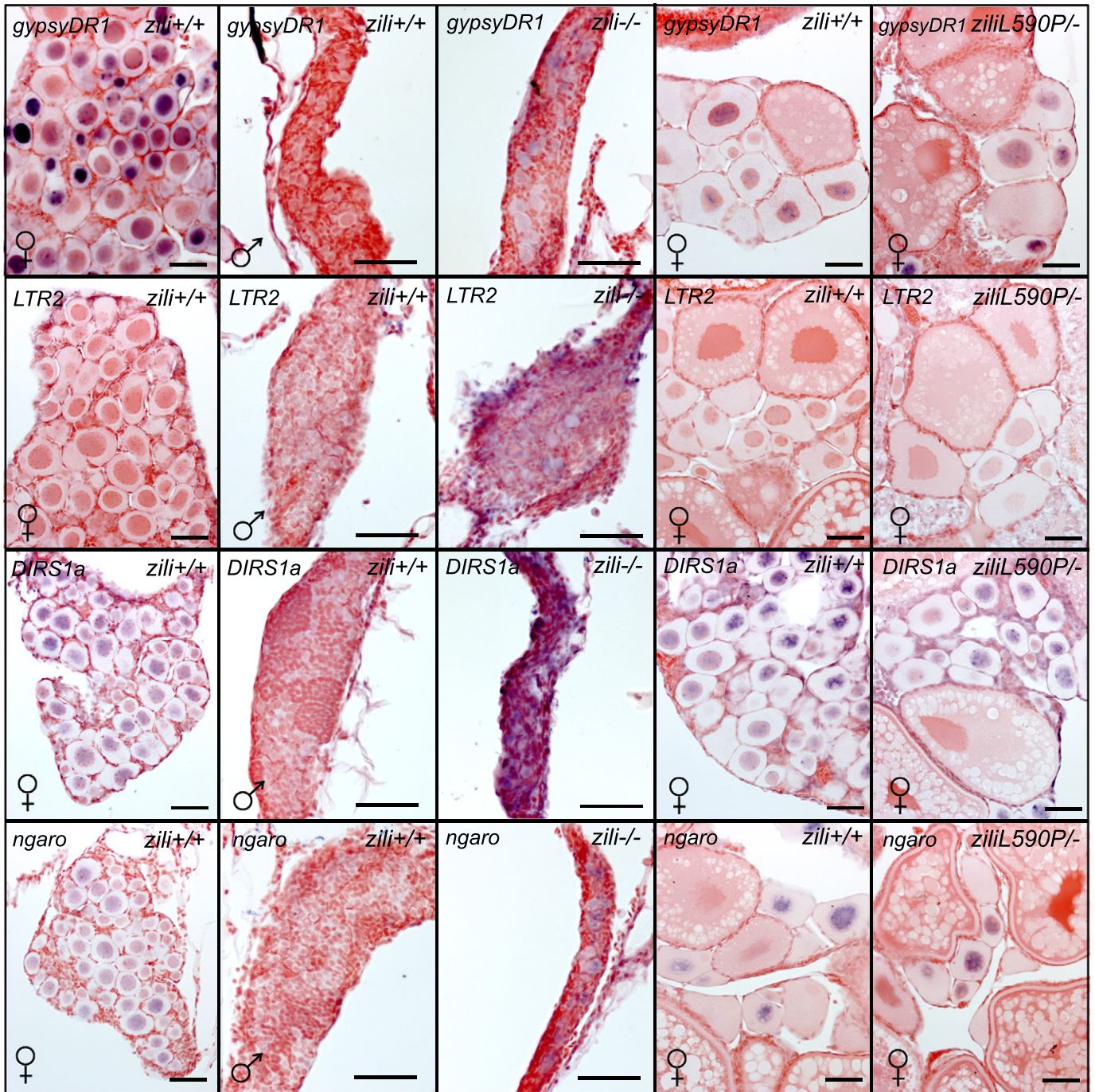


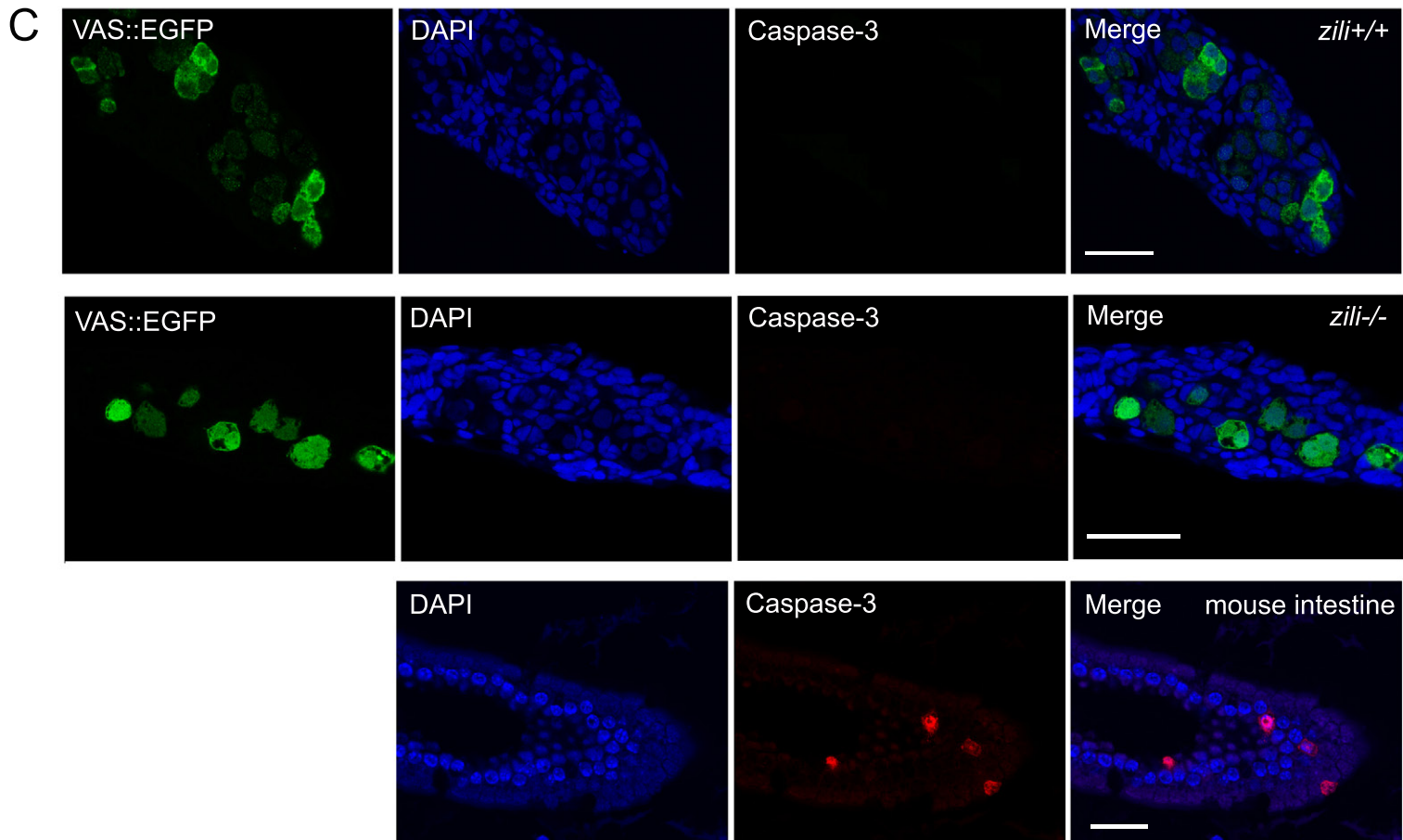
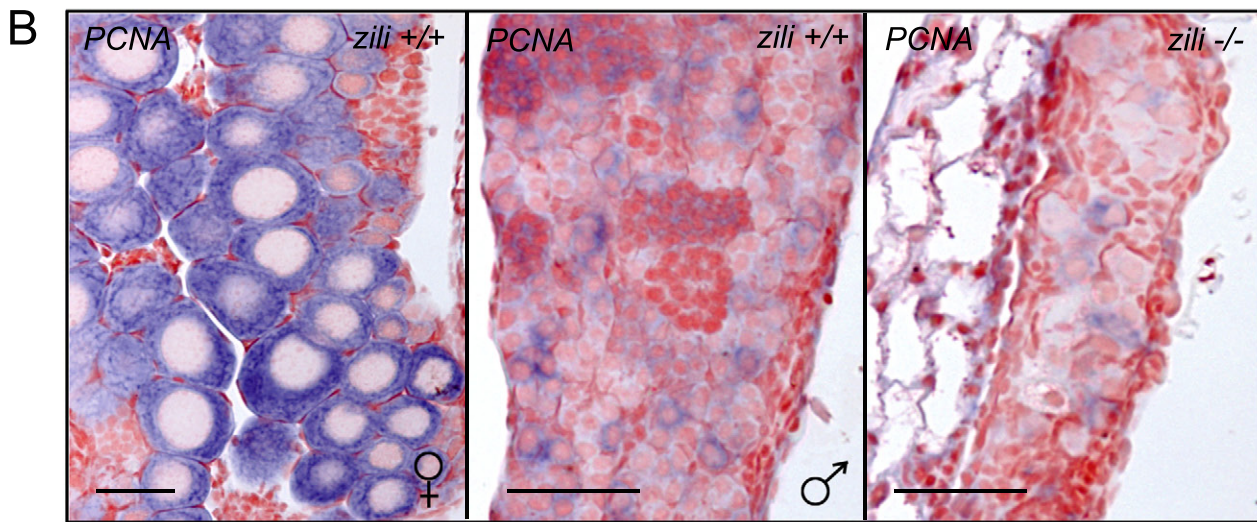
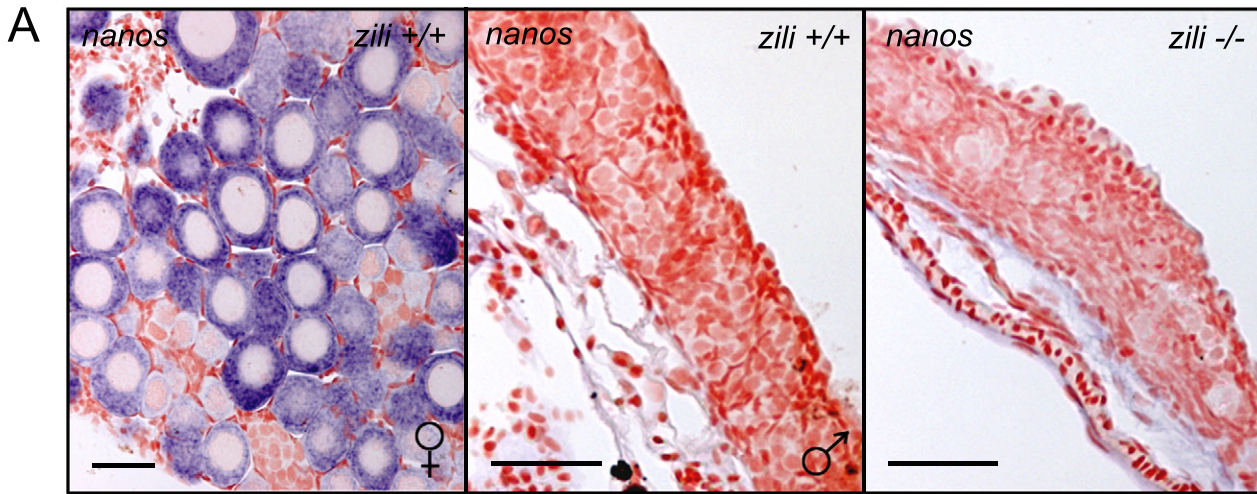
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Antisense_Boundary	94	80	22	46
Antisense_Exonic	902	1226	239	505
Antisense_Intronic	2686	1693	2046	2009
Sense_Boundary	35	87	132	144
Sense_Exonic	353	1370	2186	2537
Sense_Intronic	3248	1811	1928	2113
total	40380	29170	34616	34604

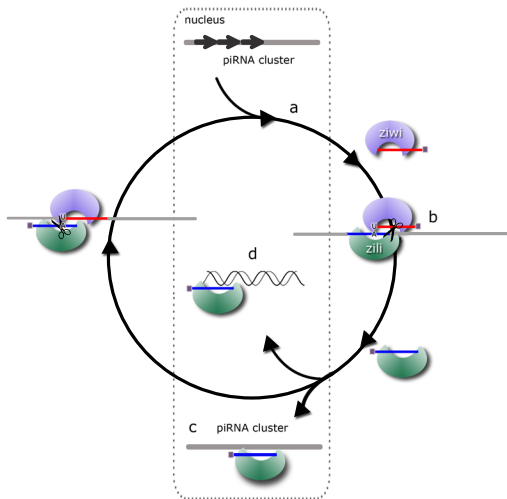




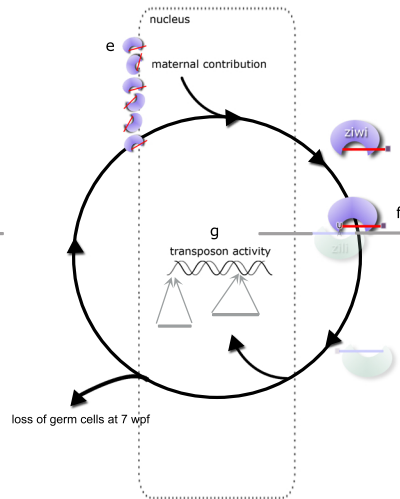




zili^{+/+}



zili^{-/-}



zili L590P/-

