A newly-engineered fly to simplify recombination mapping

Frequency of recombination between our mutation and each P-element insertion.

Table 1: Phenotypes of flies assayed for recombination between the mutation and each P-element insertion. Numbers of flies of each phenotype observed were used to determine the predicted distance between the mutation and each P-element insertion.

**Figure 1: Biogenesis of microRNA and miRNA.** (A) miRNA genes are transcribed and processed to yield short double-stranded RNAs. After further processing, a single miRNA strand is incorporated into the miRNA-induced Silencing Complex (miRISC), which binds to complementary target mRNAs and silences gene expression by means of mRNA degradation, translation block, or both. (B) Several miRNA-based therapeutics designed to regulate target gene expression are currently in clinical trials.

**Figure 2: Isolation of mutants and detection of miRNA activity.** The eye-specific GFP reporter is regulated by miRNA binding to the target 3' untranslated region (3'UTR) (A). EMS mutagenesis was performed and mitotic recombination was used to generate clones of cells homozygous for induced mutations (B). Predicted GFP expression levels in the adult eye if a gene required for silencing is disrupted; corresponding chromosomes shown in B (C). The I1-5 mutant demonstrates a defect in silencing (D).

**Figure 3: Molecularly defined P-element insertions and observations of recombination.** Locations of P-element insertions used for recombination mapping (A). Parental cross between a fly line with a specific P-element insertion and flies carrying the mutation of interest (P), F1 cross between flies heterozygous for both the mutation and a specific P-element and flies carrying the mutation of interest (F1), and flies observed for evidence of a recombination event (F2). (Zhai '04)

**Figure 4: Projected position of the I1-5 mutation.** The Xs indicate possible locations of the mutation based on recombination frequencies from Table 1.

**Figure 5: A new fly line for streamlined data collection for recombination mapping.** Further recombination mapping will be aided by generation of a fly line that allows for removal of uninformative flies and scorings of informative recombinant flies in the F2 generation.

**Figure 6: Heat shock protocol and resulting progeny.** Four days after a mating has been set, larva are heat shocked at 37°C for one hour to kill unwanted flies containing the heat-shock hid transgene. The overall number of flies to be sorted is reduced, simplifying scoring of informative recombinant flies in the F2 generation.