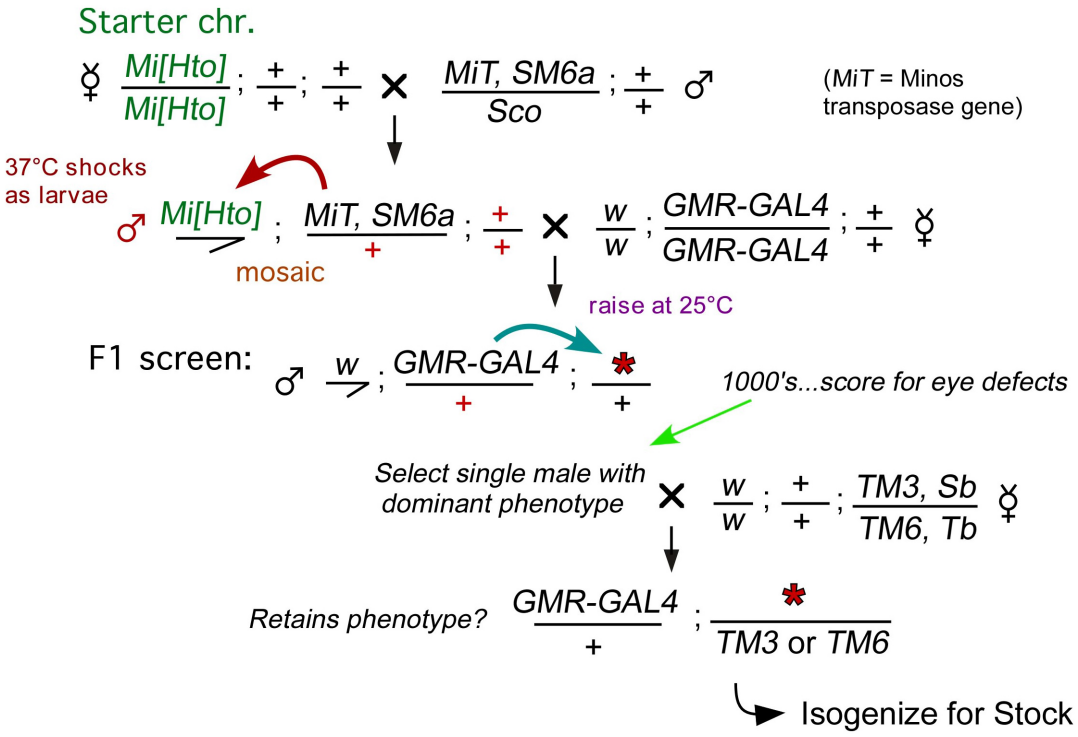
Protocol for performing a *Hostile takeover* (*Hto*) screen for inducible, disruptive protein traps

-Obtain a *Starter* chromosome carrying one or two inserts of *Minos[Hto]*.

--The current recommended *Starter* is *RENX05,X20* with two inserts of *Hto* version 2 on the X. Both inserts can make RFP but neither causes a phenotype (*REN* stands for RFP+, eyes normal with *GMR*); both can hop and make new protein fusions. This *Starter* carries no *w+*.

--Genetic background: If you wish, put a clean chromosome 2 and/or 3 into the *Starter* line to receive new hops; the current *Starter* lines have random, unselected backgrounds. We often recombine the new hops against w^{1118} after they are recovered, so we don't try to clean the chromosomes before the screen.



1. Cross the *Starter* chromosome to the heat inducible *MiT* line {http://flybase.org/reports/FBtp0021508.html}. For example, for the *Starter* X chromosome *RENX05,X20*, use homozygous *Starter* virgins X *MiT, SM6a* / *Sco* males.

2. Brood this cross frequently to keep offspring fairly synchronized.

3. To induce transposase, the progeny are heat shocked (1 hr @ 37°C) on ~day 3 after hatching, when they are 2nd or small 3rd instar larvae; thereafter the germline is a mosaic of new *Hto* hops.

--Use a single HS to get fewer double inserts, or HS twice on successive days to increase the occurrence of new hops.

4. Isolate the mosaic males, *Starter* / Y; *MiT, SM6a* / +.

5. Cross 2-3 of these mosaic males to several virgins bearing a GAL4 driver, in this example, *GMR-GAL4* stock BL-1104. Raise at 25°C.

Caution: For *GMR-GAL4* BL-1104, stock flies raised at 25°C will have a severe rough eye due only to the driver, that is not seen at room temp or in heterozygotes. So, be certain your *GMR* flies are virgins at this step.

6. Each set of males in one vial is assigned an identifier (Set A, Set B, etc), in order to detect potential clusters of mutants that all carry the same insertion.

7. Each set is brooded 2-3 times for ~2-3 days each, and the progeny larvae (F1) are split into new vials as they grow, to prevent overcrowding and loss of potentially weak mutants. Be sure to mark all broods with the identifier; you may recover copies of the same event in, for example, Set A, brood 1 and Set A, brood 2. Each set should give ~1000 F1 offspring and ~2 positives (rough-eyed, in a *GMR* screen).

8. As they eclose, the F1 adults are scored *en masse* under a dissecting scope for defects. For *GMR*: We keep any "hits" in which the fly that has a uniform disruption across the eye, even if mild (misaligned ommatidial bristles or worse). We do not keep flies with a "scar", broken row of ommatidia, or misaligned bristles, only in a small part of one eye; these almost never breed true.

> --If the F1 fly carries the *MiT* chromosome, then you will usually see mosaicism for the *Hto* phenotype; these hits can be recovered as long as the phenotype appears in clones in BOTH eyes.

9. Give each mutant F1 a unique 3-letter code. We use letters instead of numbers since this is more memorable, searchable, and makes for easier record keeping. We try to avoid 3-letter codes similar to known gene or protein names.

10. Cross the F1 mutant to balancer-carrying flies (we use *TM3*/*TM6*) in order to isolate the affected chromosome. The genetics becomes very diverse depending on whether the hit is a male or female, and which chromosome carries the new insert. The optimal case is that *Hto* landed on chromosome 3 in a male (red asterisk on page 1):

(single mutant F1) ***w-*** from *GMR* / ***Y*** ; ***+*** / ***GMR*** ; ***Hto*** / ***+*** X ***TM3*** / ***TM6*** virgins

(single mutant F2) ***w-*** from *TM* stock / ***Y*** ; ***+*** from *TM* stock / ***GMR*** ; ***Hto*** / ***TM6***

X ***TM3*** / ***TM6*** virgins

a few males & females from this cross will start a clean stock: ***w-*** ; ***+*** / ***+*** ; ***Hto*** / ***TM6***

This stock is white-eyed and has no phenotype. Retest next the generation against *GMR* to ensure the desired insert/phenotype is there.

Note that you should get rid of all chromosomes from the original mutant male (red above) that may have extraneous *Hto* inserts. The *GMR* chromosome is useful to have around to see the phenotype, but this chromosome will not be clean if the original hit was a female or carried *MiT*, and so it should be replaced. The X is replaced by passaging through a male, and 3 is replaced by the *TM* balancers.

Also note you must pass the line through a single male in the F2 or later to make sure the *Hto* bearing chromosome is isogenized.

11. The final clean, isogenized stock is ready for testing to ensure it carries a single insert, as is usually the case. This could be assessed at the DNA level by various PCR/cloning/sequencing methods. We like to test this by recombination as well. Cross the *Hto* stock to the *GMR* stock and recover female progeny, in which there is recombination of the *Hto* chromosome. Cross to w^{1118}. Score a large number of *GMR*-carrying progeny for both RFP signal in the eye (by fluorescence dissecting scope), and the presence of the original *GMR>Hto* rough eye phenotype. If there are any progeny that have RFP but lack the phenotype, then you have two functional inserts. In this case you could map both and do a specific recombination expt to remove the extra insert, or perform introgression of a wild type chromosome over several generations (very easy but takes a while.)

--Kevin Edwards, 2013