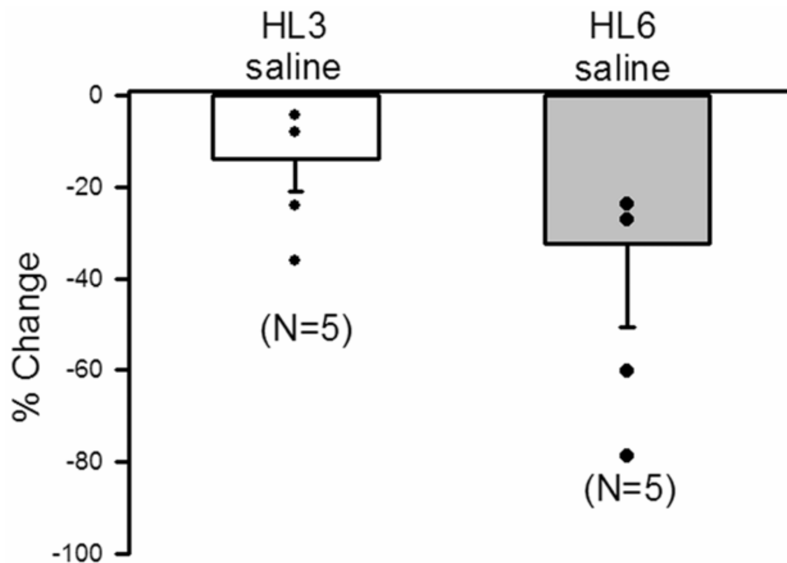

2004). High throughput screening is also possible to assess multitudes of pharmacological agents or mutational screens with these approaches presented.

Figure 5. The average heart rate was taken during the first five-minute period and the period from 15 to 20 minutes and a percent change was determined. The mean change and the ± SEM are shown.

Acknowledgment: We thank Dr. Ruthann Nichols (Univ. of Michigan) for helpful comments regarding the optical means of monitoring heart rate in larvae.

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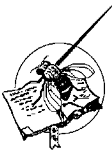

"Marker removal" screen to generate an improved wing disc GAL4 driver.

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Introduction

The GAL4-*UAS* system allows one to test for interactions between gene constructs that would be lethal if expressed constitutively (Brand, *et al*., 1994). The *Drosophila* wing is a particularly

appealing place to study interactions among genes responsible for patterning, signal transduction, growth, and morphogenesis, since the wing is not required for viability and it provides a flat, two-dimensional pattern that is easily quantified. To perform interaction studies in the wing, one can combine a wing disc-specific GAL4 driver with two or more *UAS*-regulated responder transgenes (*e.g.*, Guichard, *et al*., 1999). The phenotypes of flies with the driver and each responder, alone and in combination, are then compared.

To identify which constructs are present in a given fly, one may rely on the *miniwhite* gene that marks most *UAS* transgenes. Since the pigmentation provided by *miniwhite* is additive, flies with one versus two responders can be distinguished by scoring the intensity of eye color (*e.g.*, yellow versus orange). Unfortunately, this approach is nearly impossible for two of the most commonly used wing-specific drivers, *A9-GAL4* and *P[GawB]Bx*^{MS1096} (hereafter, "*MS1096*"; Capdevila and Guerrero, 1994; Marquez, *et al*., 2001). Both of these wing drivers are located on the X chromosome and express *miniwhite* very strongly, resulting in near wild-type red eyes. When *A9-GAL4* or *MS1096* is present, it drowns out the additional *white* activity provided by typical *UAS* responder transgenes, making it difficult to detect the responders. We solved this problem by generating a derivative of *MS1096* that lacks *miniwhite*. The derivative, *MS1096w*, is functionally indistinguishable from the original *MS1096* in terms of strength and specificity of GAL4 expression, but it produces no eye pigment and thus allows for accurate scoring of other *white*-marked constructs.

Results and Discussion

The w^- derivative of *MS1096* was obtained using an F_1 "marker removal" screen. Transposase gene *delta2-3* was used to induce imprecise excisions (actually failed break repairs; Engels, *et al*., 1990) of the *MS1096 P[GawB]* element, and the excisions were screened for those that inactivate *miniwhite* but leave the *GAL4* gene intact. The screen is diagrammed in Figure 1. Mosaic w^{1118}, *MS1096* males bearing *CyO*, *H[Pdelta2-3]* were crossed to *FM7c*/+; *UAS-Pez*, and the female progeny that carried *FM7c* but not *CyO* (*MS1096**/ *FM7c*; *UAS-Pez* or +) were examined. *MS1096*-driven overexpression of *Pez*, which encodes a tyrosine phosphatase, leads to small wings (Edwards, *et al*., 2001, and in preparation). Thus, these progeny primarily consisted of red-eyed flies, in which the *miniwhite* marker in *MS1096* was unaffected, and flies with normal-sized wings, in which *UAS-Pez* was absent or the *GAL4* region of *MS1096* was excised. We screened for rare F_1 females with light eye color that retained the small-wing phenotype indicative of *MS1096* driving *UAS-Pez*. (Note that any *UAS* responder, such as *UAS-Egfr*, could have been used in place of *UAS-Pez*, so long as it produced fertile adults with a scorable phenotype.) 5800 F_1 flies were scored, of which ~1/16 were of the appropriate genotype for the screen, and two balanced females were identified in which *MS1096** was lacking *miniwhite* yet positive for GAL4 activity. The

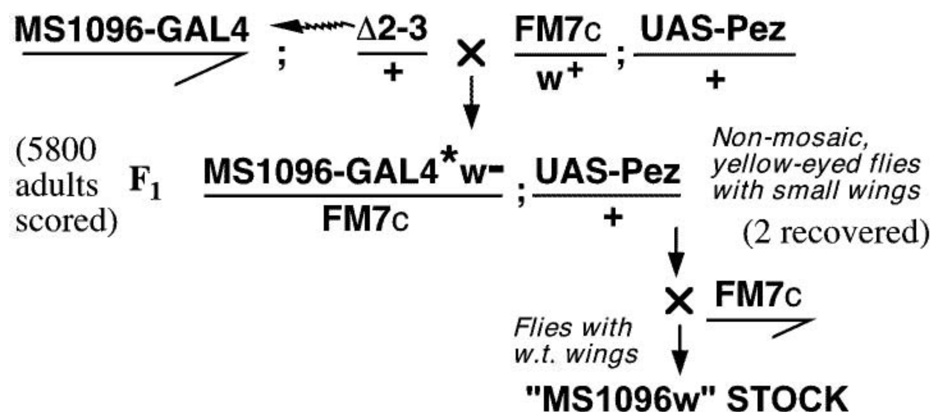

Figure 1. Schematic diagram of the "marker removal" screen (see text for specific genotypes).

positive females were crossed to *FM7c* males, and non-*UAS-Pez* progeny were selected to make stocks. One line, named *MS1096w*, was retained, re-isogenized, and characterized.

MS1096w hemi- and homozygotes have white eyes, indicating that *delta2-3* activity has completely inactivated *miniwhite*. To determine if *MS1096w* still functions as a strong, specific wing driver, we crossed it to several responders with known *MS1096* phenotypes. In all cases *MS1096* and *MS1096w* were indistinguishable. An example is shown in Figure 2. Both *MS1096w*/+ and *MS1096*/+ females, each driving one copy of *UAS-Egfr*, display extra wing vein material and distorted vein pattern characteristic of excess EGF signaling (*e.g*., Guichard, *et al*., 1999).

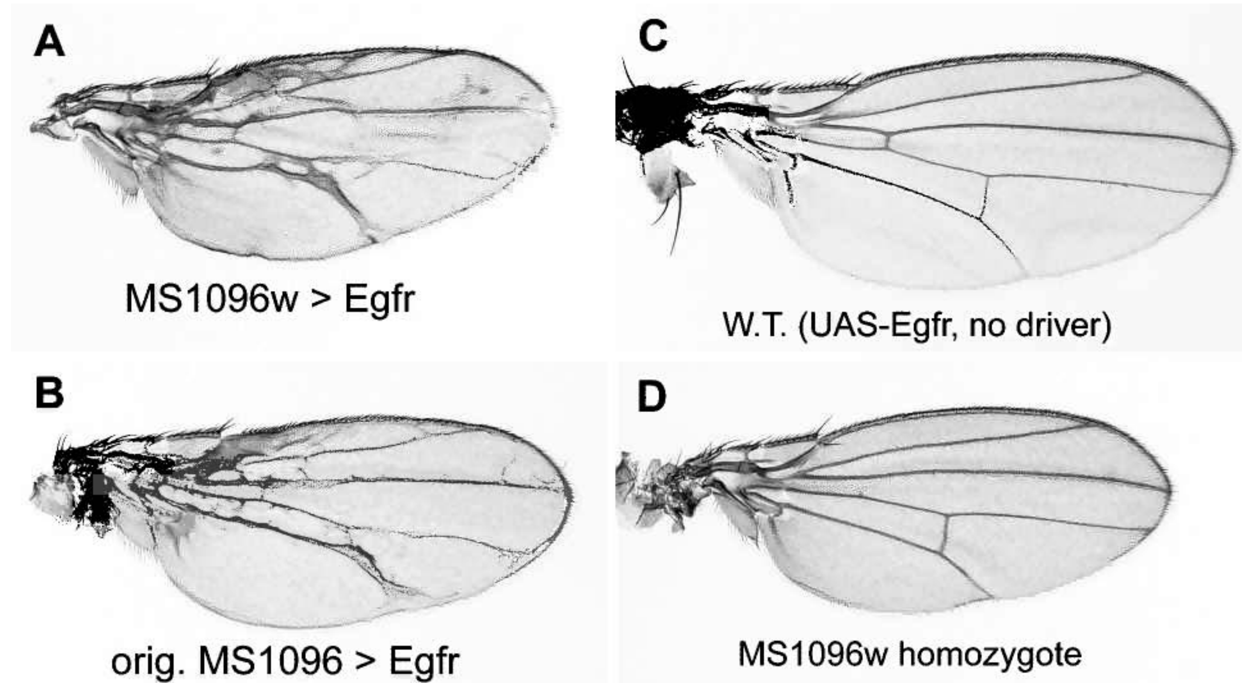

Figure 2. Wing phenotypes of *MS1096w*. A, C; Progeny of a cross between *MS1096w*/*FM7c* and *UAS-Egfr*. A, *MS1096w*/+ ; *UAS-Egfr*. C, *FM7c* /+ ; *UAS-Egfr* (wild type). B, *MS1096*/+ ; *UAS-Egfr*. A-C raised at 26°C. D, *MS1096w* homozygous female with anterior crossveinless phenotype.

The *MS1096* insert was shown to be located in the second intron of *Beadex*/*dLMO* (*Bx*), and it acts as a mild, loss-of-function *Bx* allele (Milan, *et al*., 1998). Thus *MS1096* males exhibit mild vein defects even with no responder present. To determine if the *MS1096w* lesion has altered this phenotype, we examined wings of *MS1096w* males and homozygous females. *MS1096w* does not fully revert the *MS1096* phenotype, as would be expected for a precise excision, confirming that part of *P[GawB]* remains in *MS1096w*. Loss of *miniwhite* does not worsen the *MS1096* phenotype either, suggesting it is an internal deletion of *P[GawB]* (or an external deletion of limited extent, that does not eliminate *Bx* expression). However, *MS1096w* slightly differs from *MS1096*: most *MS1096w* mutants have a normal vein pattern, and the most common defect is loss of the anterior crossvein, seen in ~30% of mutants (Figure 2D, compare to Milan, *et al*., 1998; and Brentrup, *et al*., 2000). Thus it appears the deletion of *miniwhite* has slightly altered *Bx* expression. Both *MS1096w* and its progenitor are fully recessive, and so for use as wing drivers they should be employed only in a heterozygous state (unless the goal is to study interactions with *Bx*).

In conclusion, we describe a simple, efficient F_1 screen that could be adapted to remove *miniwhite* from any GAL4 or *UAS* transgene, provided the GAL4 or *UAS* portion can produce a dominant phenotype that does not interfere with fertility. We used this screen to obtain a w^- derivative of *MS1096*, named *MS1096w*, which has been submitted to the Bloomington Drosophila Stock Center. Since *MS1096* is such a powerful driver, *miniwhite* is usually not required to detect its presence. Our derivative thus allows scoring of multiple transgenes in an *MS1096* background.

Materials and Methods

Flies were reared on Jazz-Mix (Fisher) at room temperature unless stated. *MS1096* was a gift from Dr. S. Newfeld. *UAS-Pez* was generated in our lab (K. Vadali, C. Weddle, and K. Edwards, in preparation). All other stocks were obtained from the Bloomington Drosophila Stock Center. Wings were mounted in Euparal and photographed with an Olympus DP10 digital camera mounted on a Leica MZ9.5 dissecting microscope.

Acknowledgments: This work was funded by NIH R15-GM62185 to K.E.

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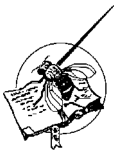

pUASTET: A dually inducible Gal4 – Tet-activator transformation vector.

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The UAS-Gal4 activation system of Perrimon and Brand (1993) has proven to be an extremely robust system for tissue-specific activation of specific genes in *Drosophila*, largely because of the wide array of Gal4 driver lines that have been developed. In contrast, mammalian expression systems tend to rely on the Tet-activator system of Gossen and Bujard (1992), in which a synthetic activator consisting of the *E. coli* TetR protein fused to the VP16 activation domain is used to activate expression from genes under the control of a multimerized *tet* operator (*tetO*). The Tet-activator system has the advantage that it can be regulated by the administration of tetracycline or doxycycline, which binds to the synthetic activator. Two forms of the synthetic activator have been developed (Gossen *et al*., 1995), that are either inactivated by doxycycline (tTA) or activated by doxycycline (rtTA), such that the target genes can be regulated in either way by the administration of doxycycline. Construction of similar transformants for *Drosophila* indicated that the Tet-activation system can also be used in *Drosophila*, where gene activation can be regulated simply by the addition of tetracycline or doxycycline to the feeding media (Bello *et al*., 1998; Bieschke *et al*., 1998). This inducible system seems especially advantageous for the study of adult physiology and behavior.

A major limitation in the use of the Tet-activation system in *Drosophila* is the limited number of driver lines available to drive Tet-responsive constructs, in contrast to the plethora of lines available for the Gal4 system. In this report, I describe a new transformation vector, pUASTET,