

Vermilion as a small selectable marker gene for *Drosophila* transformation

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The technique of P element-mediated germ line transformation is an invaluable tool for the study of gene regulation and function in *Drosophila melanogaster* (1). The most widely used transformation vectors contain the *rosy* (*ry*) gene as a selectable marker to screen for transformants (2, 3) largely because of the convenience of the easily identifiable eye color change produced by the *ry* gene. However, since the *ry* fragment is 7.2 kb in length, the large size of *ry* vectors often makes the cloning and transformation of large fragments difficult. Other transformation vectors, which utilize as selectable markers the *Drosophila white* gene or genes encoding either specific enzymatic activity or antibiotic resistance, have also been constructed (4).

We have constructed a germ line transformation vector that utilizes *vermilion* (*v*) as the selectable marker gene. This gene encodes the enzyme tryptophan oxygenase, required for brown eye pigment synthesis in *Drosophila*. Thus, like *ry*, the *v* gene controls a visible eye color phenotype. However, since *v* is considerably smaller than *ry*, this new vector should facilitate transformation experiments involving large fragments. The *v* gene has been cloned and characterized in detail at the molecular level (5). Germ line transformation studies have delineated sequences required for *v* gene expression (6). A 1.8 kb SspI fragment completely rescues the *v* mutant phenotype although it produces only 30% of the wild-type level of *v* RNA (data not shown). After addition of HindIII linkers to this 1.8 kb fragment, it was substituted for the 7.2 kb *ry* HindIII fragment in pDM30 (7). The resulting plasmid, pYC1.8, is 5.4 kb in length and has available for cloning unique NotI and SalI sites (Figure 1).

We have successfully injected this construction into v^{36f}; ry^{506} embryos and obtained transformants. The v^{36f} allele, caused by a roo/B104 insertion into the fourth *v* intron, produces a very low level of nonfunctional *v* RNA which terminates within roo/B104 sequences (5). In the transformation experiment, a mixture containing 240 μg/ml pYC1.8 and 70 μg/ml 'wings clipped' helper plasmid pπ25.7wc was injected into the v^{36f}; ry^{506} embryos as described by Karess (1). Of the 128 embryos injected, 23% survived to adulthood and 80% of the survivors were fertile. Twenty-five percent of the survivors were transformants. The peach eye color of v^{36f}; ry^{506} flies is converted to dull red in flies transformed with the pYC1.8 DNA. Although like *ry*, expression of *v* is non-autonomous, transient expression is rarely seen in Go flies. Presumably that is because the level of *v* transient expression is not sufficient to rescue the mutant phenotype. Second and third chromosome balancer stocks have been constructed in a v^{36f}; ry^{506} background. These stocks can be used to determine the chromosomal location of the transduced DNA and to make homozygous stocks.

ACKNOWLEDGEMENT

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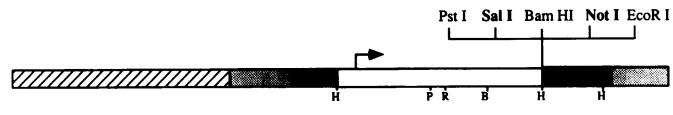

Figure 1. The map of pYC1.8. The open box indicates the 1.8 kb *v* fragment, and the direction of *v* transcription is indicated by the arrow. Bacterial pUC8 sequences are depicted by the hatched box. Solid boxes are P element sequences. The *white* gene sequences are depicted by stippled boxes. Unique cloning sites in the polylinker are highlighted. Restriction enzyme abbreviations are B—BamHI, H—HindIII, P—PstI, R—EcoRI.

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