Position-independent germline transformation in *Drosophila* using a cuticle pigmentation gene as a selectable marker

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P element mediated germline transformation is an essential part of the analysis of gene function in *Drosophila melanogaster*. This method allows for efficient introduction of single copies of genes into random positions within the genome (1). The generation of multiple transformed lines is required, as some genes display variable expression patterns depending on the insertion site (2, 3). Elimination of chromosomal position-effects would be of utility since it would remove the necessity to study a large number of independent transformed lines. Recently, the *suppressor of Hairy-wing* [*su(Hw)*] protein was found to isolate the mini-white gene of the CaSpeR transformation vector from chromosomal position-effects when binding sites for this protein flank the white gene (data submitted but not shown). These effects result from inactivation of distal enhancer and silencer elements (4, 5). Incorporation of *su(Hw)* binding regions into a P element transformation vector should confer position-independent expression to the experimental gene.

Germline transformation relies on a series of vectors which carry a selectable marker gene and a multiple cloning site placed within the ends of a P element. Two classes of selectable markers are available. The first class of vectors carries genes which allow transformants to grow on selective media; these include the alcohol dehydrogenase and the bacterial neomycin genes (6). The usefulness of vectors in this class suffers from the necessity to make separate selection media and the increased selection of false positives. The second class of vectors provides phenotypic recognition of transformation by carrying genes encoding proteins required for eye pigmentation; the most widely used genes being *rosy* and *white* (6). These vectors permit easy recognition of transformants. In many cases, however, it would be useful to have a selectable marker that is not involved in eye pigmentation. Among the advantages of a second type of phenotypic marker would be facilitation of the construction of strains carrying multiple transformed genes and the creation of defined deletion endpoints using P elements (7).

We constructed a transformation vector which insulates the experimental gene from chromosomal position-effects by providing binding sites for the *su(Hw)* protein and uses the yellow gene to confer a non-eye color phenotype for selection of transformants (Figure 1). To create this vector, the P element vector Carnegie 4 (9) was altered by blunt-end ligation of a NotI linker into the EcoRI site. Next, a 5.2 kb Sail fragment containing the intronless yellow gene (8) was inserted into the Sail site of pCar-y. The 3' flanking DNA and directs pigmentation of larval mouth parts and denticle belts, as well as adult body and wing cuticle (8). The binding regions for the *su(Hw)* protein were inserted flanking the yellow gene and most of the multiple cloning site. These 430 bp regions were isolated from the gypsy retrotransposon and contain 12 binding sites for *su(Hw)* protein (10, 11). One binding region was blunt-end ligated at the 5' end of the yellow gene into the Sail site of pCar-y. The second binding region was inserted at the 3' end of this gene as a XbaI–NotI fragment. The 3' *su(Hw)* binding region is inserted between the unique Sstl and NotI sites, so an experimental gene inserted in the NotI site will not be protected from position-effects. We call this transformation vector Y.E.S., for yellow, enhancers suppressed. The Y.E.S. vector contains six unique restriction endonuclease sites, SalI, XbaI, XhoI, SmaI, SstI and NotI.

We have successfully used the Y.E.S. vector for germline transformation. Flies carrying an X chromosome deletion for the yellow and achaete genes and the *w*^118^ mutation (*y-ac-w*^118^) were used as the parental strain for germline transformation. Cuticle pigmentation of adult flies is restored to a wild-type level (Figure 2). The size of the yellow fragment used in this vector is smaller than that of the *rosy* gene and comparable to that of the mini-white gene in CaSpeR (6). Thus, this vector should accommodate large fragments for germline transformation. Furthermore, the yellow protein is cell autonomous, providing a suitable marker for mosaic analysis of the experimental gene.

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REFERENCES


Figure 2. Phenotype of transformed flies containing the Y.E.S. vector. Shown are a male and female fly from the parental strain (y-ac-w¹¹¹₈), a wild-type strain (Canton S) and transformants containing the Y.E.S. vector (Y-ac-w¹¹¹₈; P[Y.E.S.]). Note the dark cuticle color in the transformants.

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