the efficiency of transfection. In light of the fact that ECs are difficult to transfect, the success of this method is warranted for a wider range of cells with different origins. In fact, we have obtained similar results in temporally transfecting NIH 3T3 cells using this method (data not shown). In addition, this method of temporal transfection might have utility in reversing cellular abnormalities through genetic intervention of the temporally introduced gene.

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GFP and β-Galactosidase Transformation Vectors for Promoter/Enhancer Analysis in *Drosophila*

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Reporter genes are widely used in *Drosophila* to assay the expression patterns of enhancers and promoters in transgenic animals. The most commonly used reporter genes are the *E. coli lacZ* gene, which encodes β-galactosidase (β-gal), and the *GFP* gene, which encodes the green fluorescent protein of *Aequoria victoria.* One problem with current reporter gene transformation vectors is that the expression of the reporter can be strongly affected by the chromatin configuration at the site of insertion into the genome ("position effects") (13). This leads to variability in

expression among transgenic lines and requires that multiple lines be examined to accurately assay quantitative aspects of gene expression. Another problem is the limited number of restriction sites available for cloning, which can make the engineering of reporter constructs difficult.

We have constructed a series of improved P element-based *GFP* or *lacZ* transformation vectors for analysis of promoter and enhancer activity in transgenic *Drosophila*, which we call the Pelican vectors (P-element *lacZ*/GFP-insulated CaSpeR enhancer vectors) (Figure 1). These plasmid vectors, derived from the pCaSpeR vector (8,11), contain several features that we have found to be useful for promoter/enhancer analysis. To date, we have identified *cis*-regulatory regions in 11 genes using Pelican and its derivatives (Reference 4 and unpublished results).

The Pelican vectors are based on pCaSpeR-lacZ (5), which is derived from pCaSpeR (8) and pC4-AUG-βgal (14). Unlike pCaSpeR-AUG-βgal (14), pCaSpeR-lacZ was constructed with the reporter gene in tandem orientation with the *white* gene (Figure 1), so that *white* regulatory sequences do not inappropriately activate reporter gene expression, which is particularly useful when studying gene expression in imaginal discs because of *white* promoter activity in the developing eye. The following features of the Pelican vectors constitute further improvements over some earlier reporter vectors such as pCaSpeR-AUG-βgal (14).

Two copies of the insulator sequence from the *gypsy* transposon have been added, flanking the reporter gene (Figure 1). The 0.4-kb insulator, which contains 12 binding sites for the suppressor-of-Hairy-wing [su(Hw)] protein (12), has been shown to reduce chromatin effects when flanking a marker gene in transgenic flies (7,9,10) and to block some enhancers and silencers when placed between them and a promoter (1,2). We have found that line-to-line variation in expression levels is substantially reduced for Pelican constructs, relative to comparable constructs in non-insulated vectors, allowing us to generate and maintain fewer transgenic lines for each construct. Occasional enhancer-trap effects do occur in Pelican-derived lines, although they appear to be reduced in frequency.

The *gypsy* insulator sequences were amplified from *D. melanogaster* w^{1118} genomic DNA by PCR. A 432-bp product with *Pst*I ends was generated with primers (5′-CGGCCTGCAGATGCATCACGTAATAAGTGTGCGTTG-3′ and 5′-CGGCCTGCAGCATGCTGTTGCCGAGCACAATTGATCGG-3′) and cloned into the *Pst*I site of pCaSpeR-lacZ, upstream of *lacZ*. A 420-bp product with *Mfe*I ends was generated with primers (5′-CGGCCAATTGACTAGTCACGTAATAAGTGTGCGTTG-3′ and 5′-CGGCCAATTGATCGGCTAAATGGTATGG-3′) and cloned into the *Eco*RI site of pCaSpeR-lacZ, downstream of *lacZ*. Initial testing of an earlier version of pPelican, in which the upstream insulator was directly adjacent to the 3′ P-foot, showed an 85% lower transformation rate in *Drosophila*, relative to pCaSpeR. We theorized that this effect was due to interference with transposition by su(Hw) protein bound to the insulator in injected embryos. Insertion of a transcriptionally neutral 383-bp DNA fragment between the insulator and the P-foot restored the transformation rate to a level comparable to that of pCaSpeR. The "spacer" fragment was PCR-amplified from coding sequence of the neomycin phosphotransferase (*neo*) gene with primers (5′-GACCGGGTCAAGCTTCCTAGGTCAAGACCGACCTGTCCGGTGCCC-3′ and 5′-GACCGGGTCAAGCTTGGCGAACAGTTCGGCTGGCGCGAGCCCC-3′) and cloned into a *Hin*dIII site to make the final version

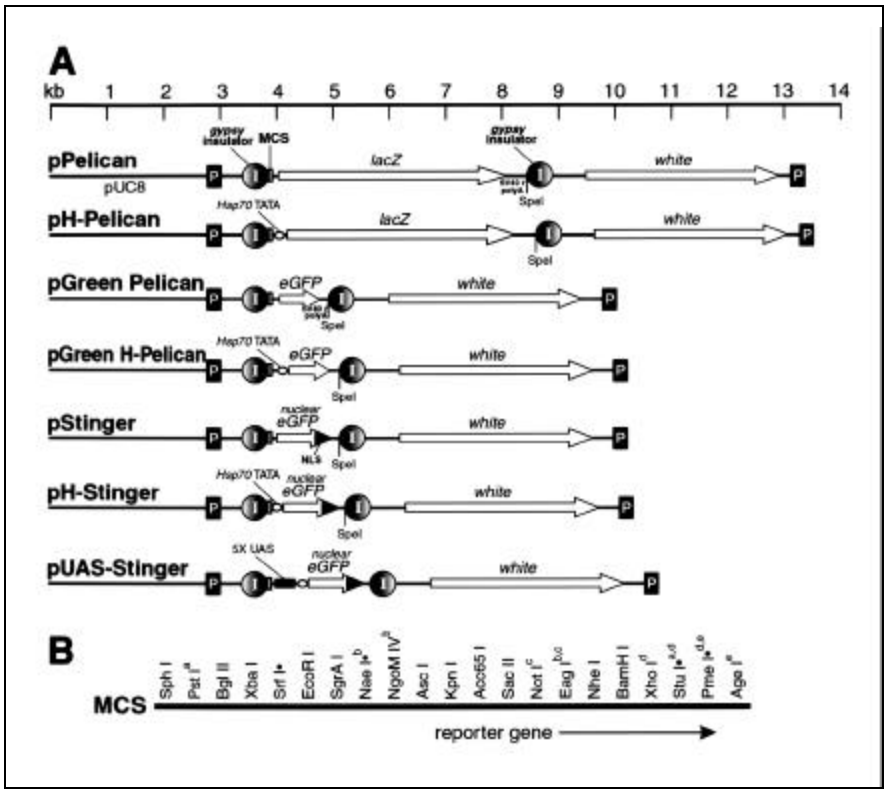

Figure 1. New "insulated" eGFP/lacZ *Drosophila* transformation vectors. (A) Diagram of pPelican and its derivatives. pPelican and pGreen Pelican express *lac*Z and eGFP, respectively, while pStinger expresses a nuclear-localized eGFP. pH-Pelican, pGreen H-Pelican and pH-Stinger contain a minimal *Hsp70* promoter driving the reporter gene. Black boxes labeled "P" represent P-element transposition sequences (P-feet). Shaded circles labeled "I" represent transcriptional insulator sequences isolated from the *gypsy* transposable element. Small white rectangles represent MCSs. Arrows indicate the direction of gene transcription. (B) Unique restriction sites in the MCS of pPelican and its derivatives. Arrow indicates the location and the direction of transcription of the reporter gene. Notes on restriction sites: [a]not present, or not unique, in vectors containing the *Hsp70* minimal promoter; [b]not unique in pPelican or pGreen H-Pelican; [c]not unique in pStinger or pH-Stinger; [d]not present in pStinger; [e]not present in lacZ vectors. Dots (•) indicate enzymes that generate blunt ends. Complete vector sequences and sample images are available at www.biology.ucsd.edu/labs/posakony.

of pPelican. Pelican vectors show a higher rate of recessive lethal insertions than that of uninsulated vectors. This mutagenic effect of insulated transgenes has been previously described (9) and is presumably due to enhancer-blocking effects at the insertion site. The same insulator and spacer sequences are used in all seven vectors described in this report.

Many transformation vectors have a limited number of unique restriction sites available for cloning upstream of the reporter gene. For example, the multiple cloning site (MCS) of pCaSpeR-AUG-βgal consists of *Eco*RI, *Bam*HI, and *Kpn*I/*Acc*65I sites (14). We have built an expanded MCS for the Pelican vectors by oligonucleotide synthesis, containing between 14 and 19 unique restriction sites upstream of the reporter gene, including sites for enzymes generating blunt ends (Figure 1), which we have found to facilitate the cloning of DNA fragments. The Pelican MCS is transcriptionally neutral, that is, it does not promote ectopic reporter gene expression. The MCSs for all seven vectors have been submitted to GenBank® (accession nos. AF242360–AF242366), and complete vector sequences and maps are available at www.biology.ucsd.edu/labs/posakony.

pPelican and pH-Pelican express the *lacZ* reporter; pGreen Pelican and pGreen H-Pelican express the enhanced F64L/S65T variant of GFP (eGFP) (16) and pStinger, pH-Stinger and pUAS-Stinger (Stable insulated nuclear eGFP vectors) express a novel nuclear-localized form of eGFP. In our studies of the development and morphogenesis of the mechanosensory bristle, we have found that nuclear eGFP is ideal for identifying the number of cells expressing the reporter, while non-nuclear eGFP is useful for determining cell shape and position. Transgenic lines carrying our GAL4-inducible nuclear eGFP construct (UAS-Stinger) are useful for visualizing the expression pattern of GAL4 constructs and GAL4 enhancer trap lines. See www.biology.ucsd.edu/labs/posakony for images of Pelican, Stinger and UAS-Stinger expression.

eGFP [including the simian virus 40 (SV40) *t* polyadenylation sequence] was excised from the plasmid pEGFP-1 (Clontech Laboratories, Palo Alto, CA, USA) and ligated into pPelican, replacing *lacZ*, to make pGreen Pelican. To make a nuclear-localized eGFP, a PCR product containing codons 84–122 of the female-specific transcript of the *D. melanogaster transformer* (*tra*) gene was ligated in frame into the *Bsr*GI site at the C-terminus of eGFP in pEGFP-1. This portion of the Tra protein has been shown to be a fully sufficient nuclear localization signal (3). The eGFP-*traNLS* chimeric reporter was then ligated into pPelican, replacing *lacZ*, to make pStinger.

When assaying genomic DNA fragments for transcriptional *cis*-regulatory activity, a minimal promoter, containing a TATA box and initiator sequence, is required for reporter gene activity in vivo. In some cases, such as when the

native promoter of the gene of interest is undefined, it is simpler to use a heterologous promoter. In other cases, particularly when a regulatory element has not been separated from its native promoter, or when an enhancer has a "preference" for its native promoter (6), it may be safer to use the native promoter of the gene of interest. The Pelican vectors are available with or without a minimal *Hsp70* promoter, which extends from -43 to +92 and includes a TATA box (15). Every enhancer that we have tested to date will drive transcription from the minimal *Hsp70* promoter.

The *Hsp70* minimal promoter (hs43) was isolated from the plasmid pCaSpeR-hs43-βgal3 (15,17) as an *Xho*I-*Sma*I fragment and cloned into the *Xho*I and *Stu*I sites of pPelican.

We have included a unique *Spe*I restriction site downstream of the reporter gene in the Pelican vectors, which may be useful for two purposes: (*i*) placing an enhancer downstream of the reporter and (*ii*) replacing the reporter with a gene of interest. This site was used to replace the *lacZ* gene in pPelican with *GFP* to make the pGreen Pelican and pStinger vectors. The Pelican vectors can therefore be modified to serve as insulated gene misexpression vectors, which would be expected to show less position-effect variability than other such vectors, and may therefore require analysis of fewer transgenic lines.

We believe that these insulated GFP and β-gal transformation vectors will prove to be useful to investigators who use reporter genes to assay transcriptional *cis*-regulatory sequences in transgenic flies.

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Treatment by Methyl Methanesulfonate Induces Up-Regulation of Cytomegalovirus Immediate/Early Promoter

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The major immediate early promoter/enhancer of human cytomegalovirus (CMV promoter) is one of the conventional promoters for expression of proteins in eukaryotic cells. The advantages of this promoter are a broad spectrum of host cells and a high level of constitutive transcription (1). However, modest levels of transgene expression from the CMV promoter have often been observed following integration of expression cassettes into chromosomal DNA (2,9). Here, we report that the CMV promoter may be dramatically up-regulated. Treatment of cells by less than 1 mM methyl methanesulfonate (Aldrich Chemical, Milwaukee, WI, USA) induces more than 100 times the stimulation of transcription from the CMV promoter.

This effect was observed during an attempt to express the fusion protein me(y)2-EGFP in mammalian cells. The me(y)2 is a small 101-amino acid protein, the mouse homologue of the *Drosophila melanogaster* e(y)2 transcription factor (12). The expression construct was prepared by subcloning the me(y)2 ORF into vector pEGFP-N1 (Clontech Laboratories, Palo Alto, CA, USA) to produce a fusion with the enhanced green fluorescent protein (EGFP). Correct in-frame insertion was verified by sequencing both strands of the resulting fusion ORF. The control pEGFP-N1 vector contains the CMV enhancer/promoter region (-582 to +7