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pChs-Gal4, a vector for the generation of *Drosophila* Gal4 lines driven by identified enhancer elements.

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A P-element vector was constructed that allows the direct insertion of identified enhancer fragments in front of a Gal4 sequence without the need for its own promoter sequences. The vector pChs-Gal4 is a derivative of pCaSpeR-hs43-AUG-lacZ (Thummel and Pirrotta, 1992; kindly provided by V. Pirrotta) and pGaTB (Brand and Perrimon, 1993; kindly provided by A. Brand).

Construction of pChs-Gal4

In a *Pst*I digestion the lacZ sequence was removed from pCaSpeR-hs43-AUG-lacZ. After removal of the overhangs, the vector fragment was ligated to the Gal4 sequence which was obtained in a *Bam*HI/*Not*I digestion of pGaTB and a subsequent Klenow fill in reaction. The resulting vector pChs-Gal4 contains P-element inverted repeats, pUC backbone, *white* marker gene, MCS and hs minimal promoter from pCaSpeR-hs43-AUG-lacZ followed by a short hsp70 sequence, Gal4 sequence and hsp70 polyA signal derived from pGaTB (see Figure 1).

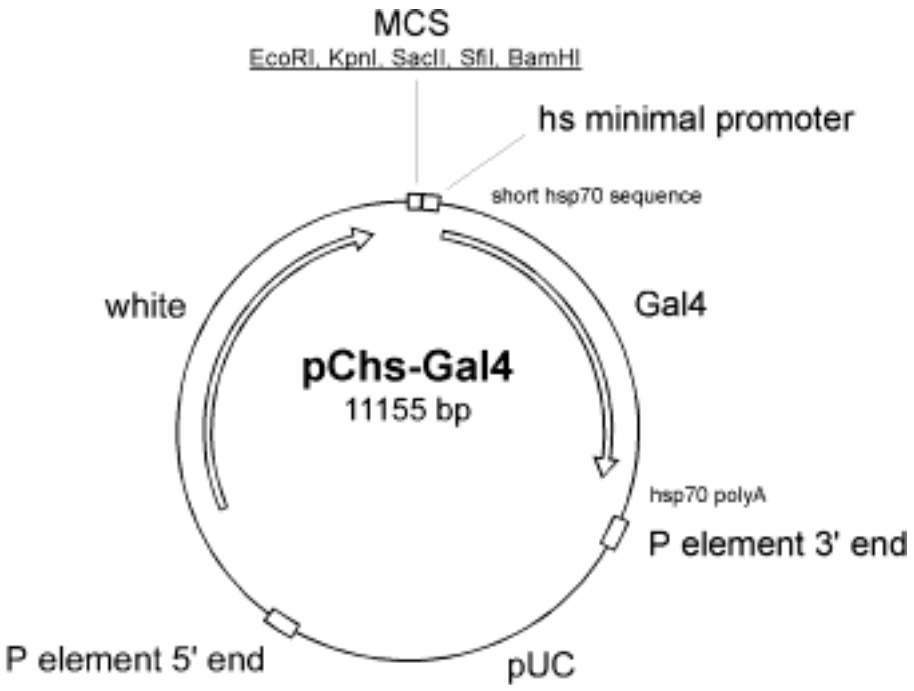

Figure1. Structure of pChs-Gal4

Testing of pChs-Gal4

In pChs-Gal4 the hs promoter sequence is 5 bp shorter at the 3' end than the hs43 minimal promoter in pCaSpeR-hs43-AUG-lacZ due to the removal of the *Pst*I overhangs. This does not interfere with the activity of the hs minimal promoter sequence as we showed with *rst* enhancer sequences (see below).

The correct ligation of both fragments at the hs minimal promoter site was confirmed by sequencing. All single cutter sites in the MCS were verified by

restriction digests. Finally, the functionality of the vector was tested by transforming flies with five different *rst* enhancer pChs-Gal4 constructs and subsequent βGal antibody stainings of *rst*-Gal4 × UAS-lacZ progenies. With all constructs we obtained a strong and reliable reporter gene expression (Apitz and Fischbach, unpublished results).

pChs-Gal4 has several advantages compared to pGaTB. It is a vector for generating Gal4 P-element constructs of identified enhancers in a single ligation step. There are five unique restriction sites in the MCS and there is no need for providing an additional promoter sequence behind the enhancer fragment.

The complete sequence of pChs-Gal4 obtained by virtual cloning of the pCaSpeR-hs43-AUG-lacZ and pGaTB sequences is available at the following hyperlink: http://filab.biologie.uni-freiburg.de/pchsgal4.

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References: Brand, A.H., and N. Perrimon 1993, Development 118: 401-415; Thummel, C.S., and V. Pirrotta 1992, Dros. Inf. Serv. 71: 150.