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An *E. coli* β-galactosidase cassette suitable for study of eukaryotic expression

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The E. coli lacZ gene and its product, β-galactosidase, are useful for the study of gene regulation because of the availability of sensitive enzymatic assays, histochemical stains, and commercially available antibodies. Since we are unaware of an available DNA cassette containing a functional lacZ with an initiator ATG codon, but without any regulatory elements or other DNA sequences, we have prepared such a construction.

The lacZ gene lacking 8 non-essential, N-terminal amino acids was isolated as a BamHI fragment from the plasmid pMC1871 provided by M. Casadaban (1). The fragment was ligated to a preannealed mixture of two synthetic complimentary 17-mers as shown in Fig. 1. One end of the oligomer is complimentary to the cleaved BamHI site in lacZ, but has a nucleotide alteration such that the restriction site is not regenerated. The linkers also provide an inframe ATG and new unique BamHI and SalI cloning sites on both ends of the lacZ fragment. The modified fragment was inserted into the SalI site of pBR322 and used to transform MC1061 competent bacteria which are deleted for the lac operon (2). Blue transformants containing a functional lacZ were selected on media containing ampicillin and X-gal. Restriction mapping confirmed the presence of the appropriate fragment with an orientation compatible with expression from the tet gene promoter of pBR322. The construction is designated pGH101, and the cassette can be excised with either BamHI or SalI.

The lacZ fragment from pGH101 was inserted into a mammalian retroviral expression vector derived from pZIP-NeoSV(B) (3). Chinese hamster fibroblasts ($3x10^5$ cells/100 mM dish) were transfected with 10 ug of plasmid DNA and cells harvested at 72 hr. In a colorimetric assay (4) performed for 3.5 hr., we obtained β-galactosidase activity of 190 pmol of o-nitrophenol formed per min per mg of protein compared to ≤10 units in mock transfected cells. The pGH101 construction should be useful for a variety of research applications.

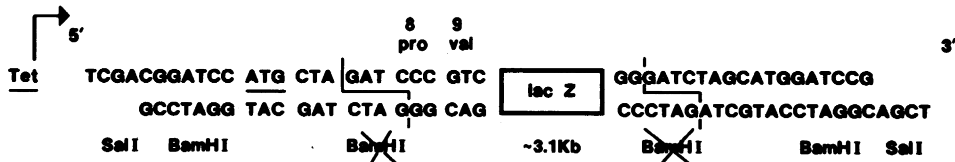

Fig. 1

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3. Cepko, C.L., et al. (1984) Cell, 37, 1053-62.
4. Nielsen, D.A., et al. (1983) Proc. Natl. Acad. Sci. USA, 80, 5198-5202.

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