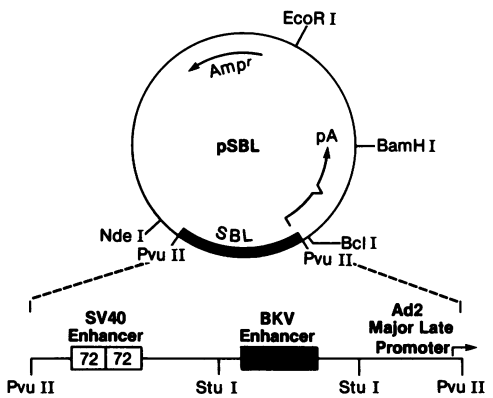
Volume 16 Number 4 1988 Nucleic Acids Research

Tandem promoter/enhancer units create a versatile regulatory element for the expression of genes in mammalian cells

David T.Berg, Patrick Q.Mooney, Melvyn Baez and Brian W.Grinnell*

Department of Molecular Biology, Lilly Research Laboratories, Indianapolis, IN 46285, USA
Submitted December 18, 1987

We have constructed a hybrid transcriptional unit, designated SBL, in which the enhancer-containing regulatory regions from SV40 and BK virus were inserted, in tandem, upstream of the adenovirus major late promoter. This hybrid promoter was used to construct a eucaryotic shuttle vector, pSBL, comprised of the amp^r gene from pBR322, the SBL promoter, and 3' regulatory sequences of SV40 containing the small t splice site and polyadenylation signals. The hybrid promoter can be excised from pSBL on a Pvu.II cassette. A unique Bcl.1 site was included to allow any gene of interest to be inserted downstream of the SBL promoter. To assess the strength and utility of the SBL promoter, a chloramphenicol acetyltransferase (CAT) expression vector, pSBL-CAT, was constructed. Following transfection of this expression vector into a variety of mammalian host cells, the level of CAT activity was measured 48 to 72 h later as described previously (1). The level of CAT activity obtained from pSV2-CAT, in which the CAT gene is driven by the strong SV40 early promoter (1), was used for comparative purposes. The SBL promoter was 3 to 6 fold stronger than the SV40 early promoter in the following cell lines: BHK-21, HeLa, MK2, COS-1, 293, CHO (all available from the American Type Culture collection), UCLA-P3 (2), K816 (3), and an adenovirus-transformed Syrian hamster tumor line, AV12-664. In primary human embryonic kidney and liver cells, CAT activity was detected after transfection with pSBL-CAT, but not with pSV2-CAT. Although efficient expression from the MLP could be obtained with either the BK (pBL-CAT) or SV40 enhancer (pSL-CAT) (3), these single enhancer constructions did not function efficiently in all cells. For example, pSV2-CAT was 3 fold stronger than pSL-CAT in 293 cells and 10 fold stronger than pBL-CAT in HeLa cells. Thus, by using tandem enhancer sequences upstream of the MLP we have developed a strong and versatile promoter that displays little host cell dependence and can be used for the efficient expression of genes in a wide variety of mammalian cells.



*To whom correspondence should be addressed

REFERENCES

1. Gorman, C.M., Moffat, L.F. and Howard, B.H. (1982) Mol. Cell. Biol. 2, 1044-1051
2. Varki, N.M., Reisfeld, R.A. and Walker, L.E. (1984) Cancer Res. 44, 681-687
3. Grinnell, B.W., Berg, D.T. and Walls, J. (1986) Mol. Cell. Biol. 6, 3596-3605