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Brief Notes

Reporter constructs with low background activity utilizing the *cat* gene

(Recombinant DNA; chloramphenicol acetyltransferase assay; gene transfer; promoter/enhancer analysis; transcriptional regulation; cryptic promoter/enhancer)

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SUMMARY

Reporter plasmids utilizing the *cat* gene for the analysis of promoter and enhancer sequences in vertebrate cells, were constructed. These plasmids minimize the background of transcription derived from cryptic promoters or cryptic regulatory elements within the vector.

Reporter genes allowing functional analysis of eukaryotic promoters and regulatory *cis*-elements by a simple enzymatic assay (CAT, luciferase, β-galactosidase, etc.) are widely used. A major caveat of these assays is that expression of enzymatic activity may result from transcription initiation within the vector. Also, vector sequences may affect the activity of the promoter under test. The former situation can be controlled by RNA start point mapping whereas exclusion of the latter possibility requires preparative removal of vector sequences before gene transfer. Both methods are impractical for routine transient transfection assays.

We have previously described the reporter plasmids pBLCAT2 and pBLCAT3 with convenient restriction sites 5′ and 3′ of the *cat* transcription unit (Luckow and Schütz, 1987). In a few cell lines we noted *cat* expression even from the promoterless construct pBLCAT3 and strong activation of expression after cAMP induction. Also, cotransfection of an expression vector encoding the transcription factor C/EBP led to strong activation of *cat* expression from pBLCAT3. We and others have attempted to identify and delete the respective prokaryotic sequences (Schüle et al., 1988) giving rise to high background of *cat* expression in some cell lines. Also, insertion of 'terminator' sequences has been suggested to prevent initiation of *cat* transcripts at cryptic promoters in the vector (Heard et al., 1987). To improve pBLCAT2 and pBLCAT3, we have combined both strategies: 467 bp of vector sequence immediately upstream from the polylinker were deleted and replaced by two fragments, each containing the polyadenylation signal of the SV40 large T encoding gene. As a result, transcripts derived from vector starts should be 3′ processed efficiently and no mature mRNA encoding CAT should be generated. The resulting *cat* reporter plasmids pBLCAT5 (with HSV *tk* promoter) and pBLCAT6 (promoterless) have polylinkers 5′ and 3′ to the *cat* transcription unit (Fig. 1) and give high plasmid yields. 'Vector effects' on *cat* expression are abolished or strongly reduced.

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Abbreviations: *bla*, gene encoding β-lactamase; bp, base pair(s); CAT, Cm acetyltransferase; *cat*, gene encoding CAT; C/EBP, CCAAT box/enhancer-binding protein; Cm, chloramphenicol; HSV, herpes simplex virus; kb, kilobase(s) or 1000 bp; nt, nucleotide(s); SV40, simian virus 40; *tk*, HSV gene encoding thymidine kinase.

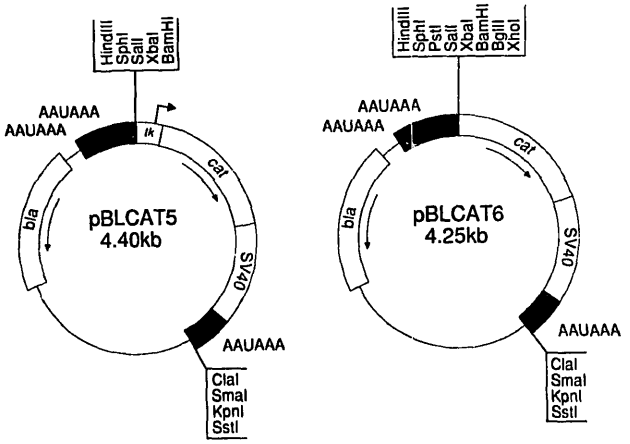

Fig. 1. Maps of pBLCAT5 and pBLCAT6. The plasmids contain SV40 polyadenylation signals (black boxes, AAUAAA), the *cat* gene, the SV40 small t intron sequences (SV40), the β-lactamase encoding gene (*bla*) and pBLCAT5 contains the *tk* promoter (open box, *tk*). For construction of pBLCAT5, the sequence from nt 4428 (*Aat*II site) to nt 398 (5′ of *Hin*dIII site) of pBLCAT2 (Luckow and Schütz, 1987) was deleted and replaced by two fragments from SV40 (Buchman et al., 1981) inserted in tandem (*Hpa*I-*Bam*HI, nt 2668–2534 and *Bcl*I-*Bam*HI, nt 2770–2534). Plasmid pBLCAT6 was similarly derived from pBLCAT3. The modified parts of the vectors were verified by sequencing. Compiled sequences for pBLCAT5 and pBLCAT6 are available (GenBank accession Nos. M80483 and M80484, respectively).

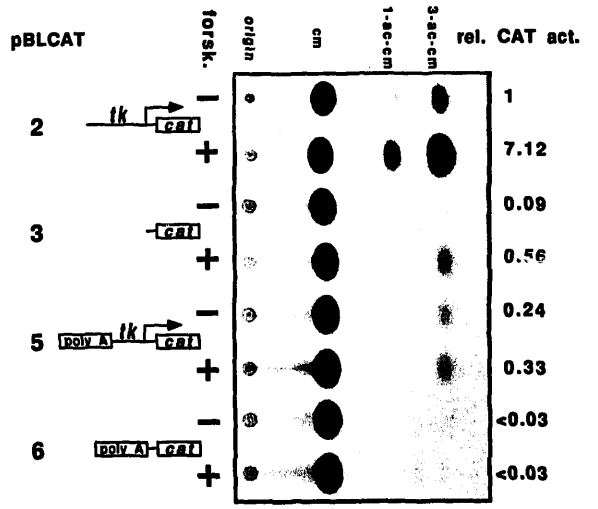

Fig. 2. Comparison of pBLCAT reporter constructs in PC12 cells. PC12 cells were electroporated as described (Boshart et al., 1990) with pBLCAT2 and 3 (Luckow and Schütz, 1987) and pBLCAT5 and 6 (see Fig. 1) and induced 4 h later with forskolin (forsk., which activates adenylate cyclase; Seamon et al., 1981) as indicated by + symbols. After 24 h of expression, extracts were prepared and assayed for CAT activity and protein as described (Boshart et al., 1990). CAT activity was quantified, corrected for protein and is given as relative activity, setting the value of uninduced pBLCAT2 to 1. An autoradiogram of the thin layer chromatogram used to separate chloramphenicol from its acetylated derivatives (1-ac and 3-ac) is shown.

As an example, Fig. 2 shows that in PC12 cells high basal expression (even from the promoterless construct) and response to cAMP induction is abolished. In FT02B rat hepatoma cells, transactivation of vector derived *cat* expression by cotransfected C/EBP is reduced about eightfold with the modified versions pBLCAT5 and pBLCAT6 (Ruppert et al., 1990) (data not shown). In human melanoma cells (SKMel25 and SKMel28), vector derived expression from pBLCAT6 is more than fivefold reduced compared to pBLCAT3 (data not shown). In conclusion, a substantial improvement of the pBLCAT reporter constructs, now used in many laboratories, has been achieved.

REFERENCES

Boshart, M., Weih, F., Schmidt, A., Fournier, R.E.K. and Schütz, G.: A cyclic AMP response element mediates repression of tyrosine aminotransferase gene transcription by the tissue specific extinguisher locus Tse-1. Cell 61 (1990) 905–916.

Buchman, A.R., Burnett, L. and Berg, P.: Appendix A/The SV40 nucleotide sequence. In: Tooze, J. (Ed.), DNA Tumor Viruses, Molecular Biology of Tumor Viruses, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1981, pp. 799–841.

Heard, J.-M., Herbomel, P., Ott, M.-O., Mottura-Roller, A., Weiss, M.C. and Yaniv, M.: Determinants of rat albumin promoter tissue specificity analysed by an improved transient expression system. Mol. Cell. Biol. 7 (1987) 2425–2434.

Luckow, B. and Schütz, G.: CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements. Nucleic Acids Res. 15 (1987) 5490.

Ruppert, S., Boshart, M., Bosch, F.X., Schmid, W., Fournier, R.E.K. and Schütz, G.: Two genetically defined trans-acting loci coordinately regulate overlapping sets of liver-specific genes. Cell 61 (1990) 895–904.

Schüle, R., Muller, M., Otsuka-Murakami, H. and Renkawitz R.: Cooperativity of the glucocorticoid receptor and the CACCC-box binding factor. Nature 322 (1988) 87–90.

Seamon, K.B., Padgett, W. and Daly, J.W.: Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc. Natl. Acad. Sci. USA 78 (1981) 3363–3367.