CAT constructs with convenient sites for cloning and generating deletions

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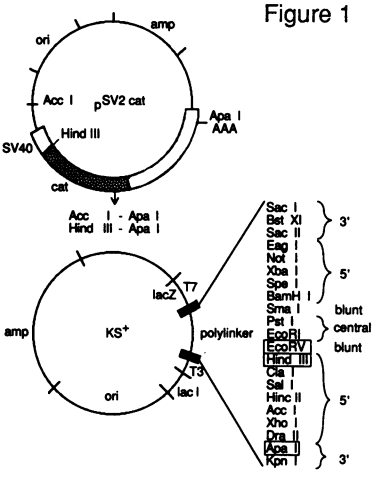
The chloramphenicol acetyl transferase (CAT) gene is often used as a reporter gene to test the activity of a putative promoter region. In the original pSV-OCAT plasmid, a promoter region is inserted in a unique Hind III site 5' to the CAT gene (Gorman et. al., Mol. Cell Biol. 2, 1044-1051, 1982). We have devised a new construct making use of the plasmid Bluescript (KS^+) (Stratagene), which has the following advantages: a) a polylinker region providing several sites for subcloning a putative promoter upstream from the structural CAT gene; b) sites for using the ExoIII - mung bean nuclease procedure to generate nested deletions of the promoter, and; c) the availability of universal primers upstream from the intact, as well as deleted, promoter for easy sequencing. The outline of constructing the new CAT plasmids using KS^+ is shown in Fig. 1. An AccI-ApaI fragment from the original pSV2CAT plasmid was blunt-ended (because the AccI sites are not compatible in the two plasmids) and inserted in the EcoRV site of the KS^+ in order to create the KS^+-SV2CAT construct, containing the SV40 promoter (positive control). A HindIII-ApaI fragment (lacking the SV40 promoter) from the pSV2CAT was inserted in the HindIII-ApaI sites of KS^+ in order to create the KS^+-SVOCAT (negative control). This vector will be the recipient of putative promoter elements. Attention should be drawn to the fact that two of the KS^+ polylinker region sites, BamHI and EcoRI, are present in the HindIII-ApaI fragment. Therefore, if a putative promoter is to be cloned in either of these sites it should be cloned before the HindIII-ApaI fragment is inserted. We have transfected these new constructs into human pancreatic carcinoma cells, HeLa cells, COS cells, NIH 3T3 cells, human intestinal (INT 407 ATCC) cells, chick sternal chondrocytes and chick limb bud mesenchymal cells. In all the assays the KS^+-SV2CAT functions as well as the original pSV2CAT (Fig. 2). In some cell lines, we noticed, however, some very low CAT activity when transfected with KS^+-SVOCAT, (Fig. 2, Lane 4), probably due to the unspecific firing of the T7 promoter. This work was supported by NIH grant HD 22016 to PFG and CA 42595 to JLM. PAT is a recipient of an Arthritis Investigator Award.

Figure 1



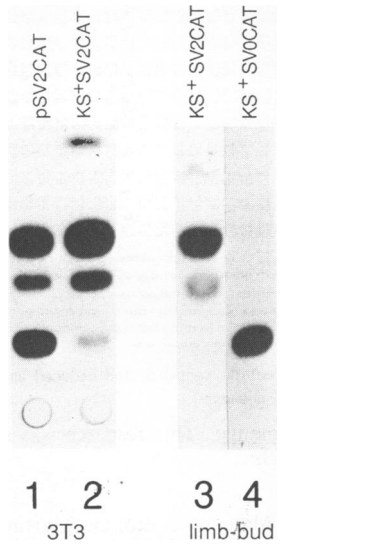


Figure 2