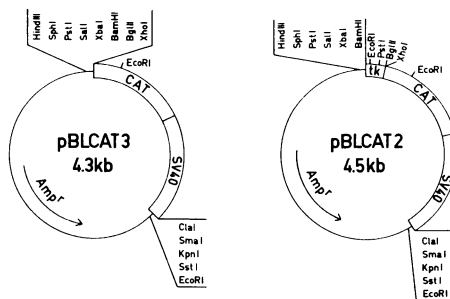


CAT constructions with multiple unique restriction sites for the functional analysis of eukaryotic promoters and regulatory elements

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The coding region of the bacterial chloramphenicol acetyltransferase (CAT) gene is widely used as an indicator gene in gene transfer experiments dealing with regulation of transcription in eukaryotes. Chimaeric CAT fusion genes are especially useful because no endogenous CAT activity is present in eukaryotic cells and because CAT enzyme activity can be monitored by a rapid and sensitive assay (1). In order to simplify the construction of hybrid CAT genes, we have constructed the plasmids pBLCAT2 and pBLCAT3. The coding region of the CAT gene as well as the small t intron and polyadenylation signals from SV40 were inserted into the polylinker region of the high copy number plasmid pUC18 (2). Unique BglII and XhoI restriction sites were introduced upstream of the CAT coding region by insertion of synthetic linkers. A BamHI site at the 3' end of the transcription unit was converted into a dam methylation sensitive ClaI site by partial digestion with BamHI, filling-in and re-ligation. In the promoterless construction pBLCAT3 eight unique restriction sites are suitable for insertion of different eukaryotic promoters at the 5' end of the CAT gene. Four additional unique restriction sites make the insertion of regulatory signals 3' of the CAT gene possible and enable the excision of the intact fusion gene from the prokaryotic vector. The presence of the Herpes simplex virus tk promoter in pBLCAT2 permits the analysis of the effects of putative regulatory elements on a heterologous eukaryotic promoter. A BamHI/BglII fragment from the HSV tk linker scanning mutant LS-115/-105 (3) spanning the promoter from -105 to +51 was inserted into the corresponding restriction sites of pBLCAT3 thereby generating pBLCAT2. The modified polylinker regions at the 5' and the 3' ends have been sequenced and compiled sequences for both plasmids are available on request.


References:

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