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pSV00CAT: low background CAT plasmid

Eiichi Araki[1,2], Fumio Shimada[1], Motoaki Shichiri[2], Masataka Mori[1] and Yousuke Ebina[1]

[1]Institute for Medical Genetics and [2]Department of Metabolic Medicine, Kumamoto University Medical School, Kumamoto 862, Japan
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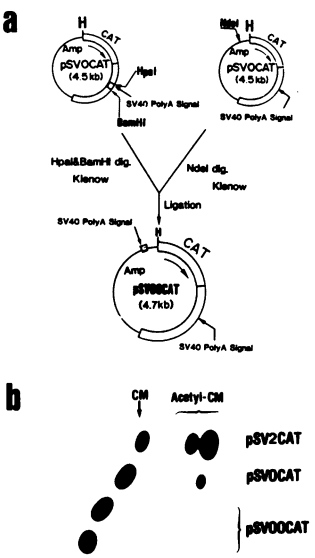
pSV0CAT plasmid (1) is widely used for the tool to assess eukaryotic promoter activities. However, the back ground of chloramphenicol acetyltransferase (CAT) activity in the cells transfected with pSV0CAT sometimes interferes with the assessment of weak promoter activities. This problem is thought to be due to cryptic promoters in pBR322 sequence of pSV0CAT which work to transcribe the CAT gene in eukaryotic cells (2). To prevent this problem, we have constructed the **pSV00CAT** plasmid which shows lower back ground of CAT activity in the transfected cells than pSV0CAT.



As shown in Fig.(a), a *Hpa*I-*Bam*HI (135 bp) fragment of pSV0CAT, which contains polyA signal of SV40 early region (3) was isolated. Both ends of this fragment were modified by incubation with Klenow fragment of DNA polymerase I to create blunt ends. This fragment was inserted into *Nde*I site of pSV0CAT whose ends were created blunt ends with Klenow fragment. This improved CAT plasmid, **pSV00CAT**, has a transcription termination signal of SV40 just before CAT gene in order to terminate the transcriptions which are initiated from cryptic promoters in pBR322 sequence in eukaryotic cells. As shown in Fig.(b), the **pSV00CAT** showed one-fifth CAT activity of that of pSV0CAT in transfected Chinese hamster ovary (CHO) cells. Almost the same results were obtained using COS cells (data not shown). We have successfully used this plasmid in the study of the promoter activity of the human insulin receptor gene (unpublished).

Figures: (a) Construction of **pSV00CAT**. (b) CAT activities in the CHO cells transfected with pSV2CAT, pSV0CAT and **pSV00CAT**.

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