

pEF-BOS, a powerful mammalian expression vector

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Polypeptide chain elongation factor 1 α (EF-1 α) is an eukaryotic counterpart of *E. coli* EF-Tu which promotes the GTP-dependent binding of an aminoacyl-tRNA to ribosomes. EF-1 α is one of the most abundant proteins in eukaryotic cells, and expressed in almost all kinds of mammalian cells. Recently, we have isolated human chromosomal gene coding for EF-1 α , and shown that the promoter of EF-1 α chromosomal gene very efficiently stimulates the *in vitro* transcription (1). In this report, we have constructed a powerful mammalian expression vector, pEF-BOS, using the promoter of human EF-1 α chromosomal gene.

As shown in Fig. 1, pEF-BOS carries the SV40 replication origin (311 bp of *EcoRI* G fragment), the promoter region of human EF-1 α chromosomal gene (1.2 kb), the stuffer fragment (450 bp) from CDM8 vector (2) and poly(A) adenylation signal from human G-CSF cDNA (700 bp *EcoRI* ~ *EcoRI* DNA fragment) (3) in *HindIII-EcoRI* site of pUC119. The promoter region of EF-1 α gene is from nucleotide position 373 to 1561 (1) which includes 203 bp 5' flanking region, 33 bp first exon, 943 bp first intron and 10 bp of the part of the second exon located at 20 bp upstream of the ATG initiation codon. The size of pEF-BOS is 5.8 kb, and the cDNA to be expressed can be inserted at *BstXI* site using *BstXI* adapter, or *XbaI* site using *XbaI* linker.

Human G-CSF cDNA (4) was inserted into *BstXI* site of pEF-BOS or CDM8, or into *BamHI* site of pKCR vector containing SV40 early promoter (5). As shown in Table 1, when these plasmids were transfected into COS cells by DEAE-dextran/chloroquine method, the construct in pEF-BOS has directed the synthesis of human G-CSF about 20 times more efficiently than the construct in CDM8, and 50 ~ 200 times more efficiently than the construct in pKCR. In addition, when *E. coli* chloramphenicol acetyltransferase (CAT) gene was inserted into pEF-BOS, the CAT activities observed with pEF-BOS-CAT were 1.5 ~ 50 times higher than that of pSV2-CAT or pRSV-CAT after transfection into various cell lines including murine L929, human HeLa, CHU-2 and simian COS cells (Table 2). The pEF-BOS vector, therefore, will be used to produce a large amount of growth factors and proteins in mammalian cells, to express a high level of anti-sense RNA. Furthermore, the pEF-BOS-CAT will be an ideal positive control for CAT assay in various cell types.

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REFERENCES

1. Uetsuki, T., Naito, A., Nagata, S. and Kaziro, Y. (1989) *J. Biol. Chem.* **264**, 5791-5798.
2. Seed, B. (1987) *Nature* **329**, 840-842.
3. Nagata, S., Tsuchiya, M., Asano, S., Kaziro, Y., Yamazaki, Y., Yamamoto, O., Hirata, Y., Kubota, N., Oheda, M., Nomura, H. and Ono, M. (1986) *Nature* **319**, 415-418.
4. Kim, D. W., Uetsuki, T., Kaziro, Y., Yamaguchi, N. and Sugano, S. *Gene* in press.
5. O'Hare, K., Benoist, C. and Breathnach, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1527-1531.

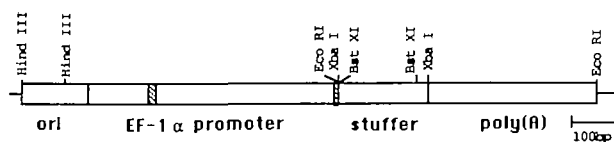


Figure 1. The structure of pEF-BOS. The boxes indicate SV40 origin, human EF-1 α promoter region, stuffer from CDM8 and poly(A) adenylation site, respectively. The slashed areas in the EF-1 α promoter region are first exon and the part of second exon, respectively. The lines flanking boxes are the sequence of pUC119. Major recognition sites for restriction enzymes are shown.

Table 1. Production of human G-CSF in COS cells

| Vector | G-CSF activity in medium ^a (units ^b /ml) | |
|---------|---|--------|
| pEF-BOS | 33,000 | 12,000 |
| CDM8 | 1,600 | 1,200 |
| pKCR | 160 | 160 |

^aAt 72 hrs post transfection, the G-CSF activity in the medium was assayed.

^b1 unit of the activity corresponds to about 62 pg of human G-CSF.

Table 2. Promoter activities in various cells

| Vector | Relative CAT activities ^a | | | |
|---------|--------------------------------------|------|-------|------|
| | L929 | HeLa | CHU-2 | COS |
| pSV2 | 2.0 | 74.3 | 82.7 | 10.2 |
| pRSV | 8.0 | 8.5 | 19.2 | 22.5 |
| pEF-BOS | 100 | 100 | 100 | 100 |
| CDM8 | n.t. ^b | n.t. | n.t. | 25.4 |

^aCAT activities are presented as a percentage of that of the pEF-BOS

^bn.t., not tested.

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