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The construction of a highly efficient and versatile set of mammalian expression vectors

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We have developed a set of highly efficient and versatile vectors for the expression of heterologous genes in mammalian cells. These vectors allow the rapid analysis of gene expression in the transient COS cell system, and the subsequent establishment of stable cell lines in a variety of host cells.The vectors consist of bacterial plasmid sequences which facilitate growth and selection in *Escherichia coli*, a polylinker for the insertion of heterologous genes and regulatory sequences which allow gene expression in mammalian cells.

The start point for the vector construction was the ColE1 based plasmid PCT54 (1). pEE6 (fig.1) was designed for optimal expression in COS cells and consequently possesses a "poison minus" deletion (2) which removes most of the non-essential sequences from the bacterial replicon.This deletion was introduced by replacing the *Bam*HI to *Bgl* I fragment encoding the origin of replication with that from pBR328 (nucleotides 375 to 2422). Following the addition of a *Sal* I linker at the *Ava* I site a 774 nucleotide *Sal* I fragment was removed thus extending the deletion of bacterial sequences in this vector. The sequence of the *β-lactamase* gene is derived from pSP64 by an exchange of the *Bgl* I to *Xmn* I fragment thereby removing the *Pst* I site from the vector. The polylinker from pSP64 was inserted into the vector between the *Hin*dIII and *Eco*RI sites .The *Bam*HI and *Sal* I sites were subsequently removed from the polylinker by cutting and filling in with Klenow. Finally the SV40 early polyadenylation signal sequence was inserted between the *Bcl* I and *Bam*HI sites.

Various mammalian promoters have been inserted at the *Hin*dIII site in the polylinker resulting in the formation of a variety of vectors. A comparison of the transient expression of the tissue plaminogen activator (tPA)gene from some of these vectors in COS-1 cells is shown in Table 1.This indicates that pEE6, which has the largest deletion of bacterial sequences, represents a significant improvement compared with other bacterial plasmids. In addition we have demonstrated that the human cytomegalovirus major immediate early (HCMV) and the SV40Late(L) promoters appear to be the most efficient in COS-1 cells. These constructs can be readily adapted for the establishment of stable cell lines by the insertion of a selectable marker gene or an amplifiable gene at the *Bam*HI site located downstream of the polyadenylation sequence. The vectors and details of their nucleotide sequence are available from the authors upon request.

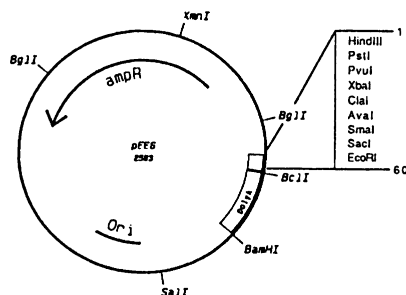

Fig 1 pEE6 Expression vector

Table 1 Production of tPA in COS-1 cells

Vector	Promoter	Bacterial Replicon	Production of tPA ng/ml
pEE3	SV40L	pAT153	250
pEE4	SV40L	pBR328	300
pSP64L	SV40L	pSP64	150
pEE6	HCMV	pBR328+S-A Δ	500
pEE6	SV40L	pBR328+S-A Δ	500

All the vectors possess the *Hin*dIII - *Pvu* II fragment from SV40 which contains the origin of replication.

S-AΔ = the *Sal* I - *Ava* I deletion described in the text.

References

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