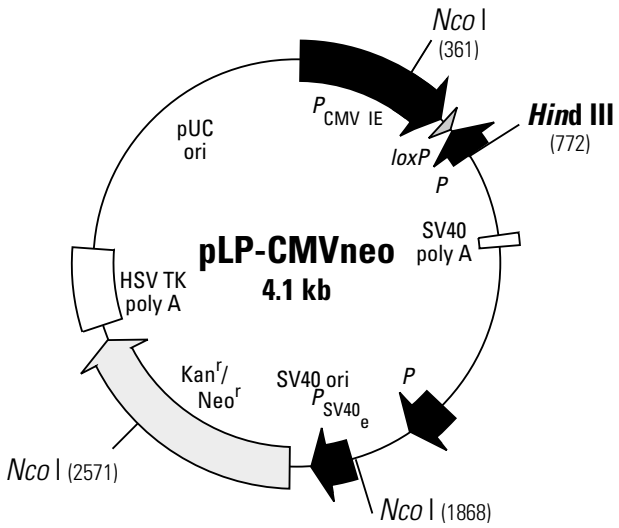

Restriction Map of pLP-CMVneo. Unique restriction sites are in bold.

Description

The pLP-CMVneo Acceptor Vector is designed to be used with CLONTECH's Creator™ Cloning Kit to rapidly generate high-level, constitutive expression constructs in mammalian cells. Instead of a multiple cloning site (MCS), pLP-CMVneo contains the *loxP* sequence from the P1 bacteriophage (1). In the presence of Cre Recombinase, the *loxP* site allows rapid transfer of the gene of interest from any Creator System donor vector into pLP-CMVneo through Cre-mediated recombination (1). Genes transferred from the donor vector should include an initiating ATG codon for proper expression.

High-level expression in mammalian cells is driven by the human cytomegalovirus immediate early promoter/enhancer ($P_{CMV\,IE}$). SV40 polyadenylation signals downstream of the *loxP* site direct proper processing of the 3' end of the mRNA of the transferred gene. The pLP-CMVneo backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418 (2). A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

pLP-CMVneo contains a bacterial promoter adjacent to the *loxP* site. Upon transfer of the gene of interest from the donor to pLP-CMVneo, the Cm^r gene present in the donor cassette becomes activated. The separation of the promoter and the coding sequence on the two parent vectors (pLP-CMVneo and the donor vector), ensures that only recombinant pLP-CMVneo vectors containing the transferred fragment in the correct orientation will be propagated in the presence of chloramphenicol. The inclusion of sucrose in the medium provides further selection against the parent donor vector.

(PR18940; published 10 August 2001)

Use

pLP-CMVneo can be used to express a gene of interest at a high constiutive level in mammalian cells. The recombinant pLP-CMVneo vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (2). Be sure to establish a kill curve for each lot of G418 to determine optimal selection concentration.

Location of Features

- $P_{CMV\ IE}$ promoter: 1–589
- *loxP* site: 597–630
- Bacterial promoter: 757–632
- SV40 polyadenylation signal:
  Polyadenylation signal: 922–956
- Bacterial promoter for expression of Kan^r gene: 1483–1603
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 1659–1730 & 1731–1802
  21-bp repeats: 1806–1826, 1827–1847 & 1849–1869
  Early promoter element: 1882–1888
  Major transcription start points: 1878, 1916, 1922 & 1927
- SV40 origin of replication: 1826–1961
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 2010–2012; stop codon: 2802–2804
  G→A mutation to remove *Pst* I site: 2192
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 2538
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signal: 3040–3058
- pUC plasmid replication origin: 3389–4032

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains for propagation of the plasmid.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500

References

1. Sauer, B. (1994) *Curr. Opin. Biotechnol.* **5**:521–527.
2. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.