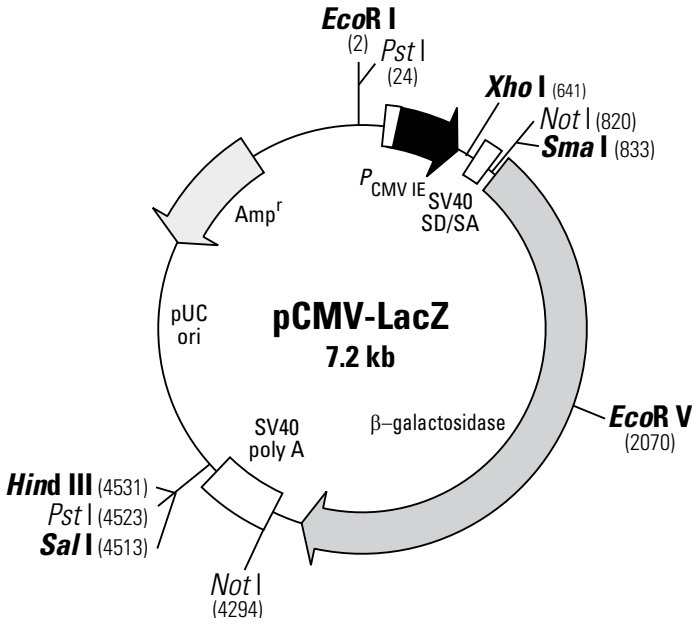
Vector Information

pCMV-LacZ Vector Information PT2004-5

GenBank Accession No. U02451 Cat. No. 631719



Restriction Map of pCMV-LacZ. Unique restriction sites are in bold.

Description

pCMV-LacZ is a mammalian reporter vector designed to expression β-galactosidase in mammalian cells from the human cytomegalovirus immediate early gene promoter (1). pCMV-LacZ contains an intron (splice donor/splice acceptor; 2) and polyadenylation signal from SV40, and the full-length *E. coli* β-galactosidase gene with eukaryotic translation initiation signals (3). pCMV-LacZ expresses high levels of β-galactosidase and can be used as a reference (control) plasmid when transfecting other reporter gene constructs and can be used to optimize transfection protocols by employing standard assays or stains to assay β-galactosidase activity. Alternatively, the β-galactosidase gene can be excised using the *Not* I sites at each end to allow other genes to be inserted into the pCMV-LacZ vector backbone for expression in mammalian cells or to insert the β-galactosidase fragment into another expression vector.

Location of features

- Immediate early cytomegalovirus promoter ($P_{CMV\ IE}$)
  - Enhancer region: 27–431
  - TATA box: 520–526
  - Transcription start point: 549
- Intron (SV40 splice donor/splice acceptor)
  - SV40 late 19s mRNA intron: 672–702
  - Modified SV40 late 16s mRNA intron (2): 672–768
- β-galactosidase gene with eukaryotic initiation signals (3)
  - Eukaryotic translation initiation signal: 867–876
  - β-galactosidase fusion protein: start codon (ATG): 873–875; stop codon: 4014–4016
  - Amino acids from *D. melanogaster* alcohol dehydrogenase: 873–965
  - Amino acids from *E. coli* β-galactosidase: 969–4013
  - C→A (Phe→Leu) mutation removing *Eco*R I site: 3965
- SV40 polyadenylation signal
  - Polyadenylation signal: 4426–4431
  - mRNA 3' end: 4445
- pUC origin of replication: 4918–5561

(032712)

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Location of features (continued)

- Ampicillin resistance (β-lactamase) gene
  Promoter: –35 region: 6639–6634; –10 region: 6616–6611
  Transcription start point: 6604
  Ribosome binding site: 6581–6577
  β-lactamase coding sequences:
  start codon (ATG): 6569–6567; stop codon: 5711–5709
  β-lactamase signal peptide: 6569–6501
  β-lactamase mature polypeptide: 6500–5712

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

References

1. MacGregor, G. R. & Caskey, C.T. (1989) *Nucleic Acids Res.* **17**:2365.
2. Okayama, H. & Berg, P. (1983) *Mol. Cell Biol.* **3**:280–289.
3. MacGregor, G. R., *et al.* (1987) *Somat. Cell Mol. Genet.* **13**:253–265.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.