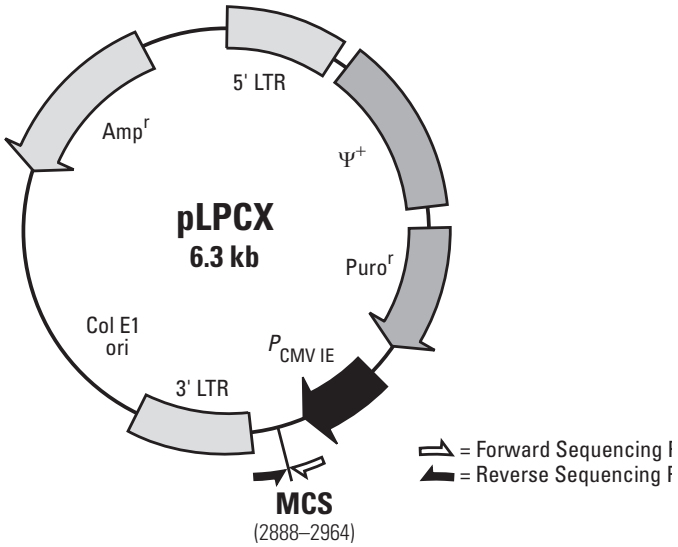
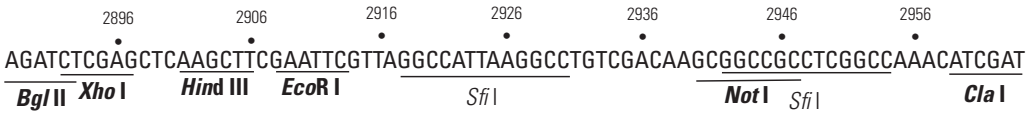
Vector Information

pLPCX Vector Information PT3299-5

GenBank Accession No.: Submission in progress. Sold as part of Catalog No. 631511





Restriction Map and Multiple Cloning Site (MCS) of pLPCX. Unique restriction sites are shown in bold.

Description

pLPCX contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is designed for retroviral gene delivery and expression (1–3). Upon transfection into a packaging cell line, pLPCX transiently expresses, or integrates and stably expresses, a transcript containing Ψ^+ (the extended viral packaging signal) the puromycin resistance gene, and the gene of interest. The 5' viral LTR in this vector contains promoter/enhancer sequences that control expression of the puromycin resistance (Puro^r) gene for antibiotic selection in eukaryotic cells. A gene of interest can be cloned into the multiple cloning site immediately downstream of the human cytomegalovirus (CMV) immediate early promoter ($P_{CMV\ IE}$). pLPCX also includes the Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

pLPCX can be transfected into a packaging cell line such as the RetroPack™ PT67 Cell Line (Cat. No. 631510). Once in the cell, RNA from the vector is packaged into infectious, replication-incompetent retroviral particles. pLPCX does not contain the structural genes (*gag*, *pol*, and *env*) necessary for particle formation and replication; however, these genes are stably integrated into PT67 (4–7). Subsequent introduction of pLPCX, containing Ψ^+, transcription and processing elements, and the gene of interest produces high-titer, replication-incompetent infectious virus. These retroviral particles can infect target cells and transmit the gene of interest (which is cloned between the viral LTR sequences), but cannot replicate within these cells since the cells lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

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Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Location of Features

- 5' MoMuSV LTR: 1–589
- Ψ^+ (extended packaging signal): 659–1468
  Mutated *gag* (ATG->TAG): 1049–1051
- Puromycin resistance gene (Puro^r):
  Start codon: 1566–1568; stop codon: 2163–2165
- Immediate early CMV promoter ($P_{CMV\ IE}$): 2338–2868
- Multiple Cloning Site (MCS): 2888–2964
- 3' MoMuLV LTR: 3004–3597
- Col E1 origin of replication:
  Site of replication initiation: 4266
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5886–5884; stop codon: 5028–5026

Sequencing Primer Locations

- pLNCX Seq/PCR Primers (#K1060-F)
  5' primer (2844–2868): 5'-AGCTCGTTTAGTGAACCGTCAGATC-3'
  3' primer (3026–3001): 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'

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: Col E1
- Copy number: low

References

1. Coffin, J. M. & Varmus, H. E., Eds. (1996) *Retroviruses* (Cold Spring Harbor Laboratory Press, NY).
2. Ausubel, F. M., *et al*. (1994) *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. & John Wiley & Sons, Inc.).
3. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**:980–990.
4. Mann, R., *et al*. (1983) *Cell* **33**:153–159.
5. Miller, A. D. & Buttimore, C. (1986) *Mol. Cell. Biol*. **6**:2895–2902.
6. Morgenstern, J. P. & Land, H. (1990) *Nucleic Acids Res.* **18**:3587–3590.
7. Miller, A. D. & Chen, F. (1996) *J. Virol.* **70**:5564–5571.

Notes: The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

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.