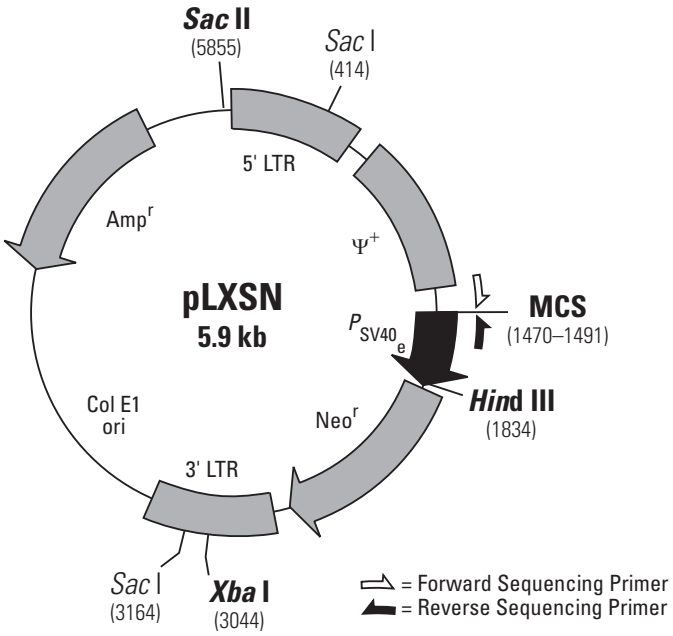
Vector Information

pLXSN Retroviral Vector Information PT3134-5

GenBank Accession No.: M28248 Sold as part of Catalog No. 631509



Restriction Map and Multiple Cloning Site (MCS) of pLXSN Retroviral Vector. Unique restriction sites are in bold.

Description

pLXSN 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, pLXSN can transiently express, or integrate and stably express, a transcript containing Ψ+ (the extended viral packaging signal) the gene of interest, and a selectable marker. The 5′ viral LTR in this vector contains promoter/enhancer sequences that control expression of the gene of interest in the multiple cloning site. The SV40 early promoter (P_{SV40e}) controls expression of the neomycin resistance gene (Neo^r), which allows antibiotic selection in eukaryotic cells. pLXSN also includes the Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

As a part of the Retro-X System (Cat. No. 631508), pLXSN 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. pLXSN 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 pLXSN, containing Ψ+ (psi), transcription and processing elements, and the gene of interest produces high-titer, replication-incompetent infectious virus. That is, 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.

(PR99509; published 17 January 2000)

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Location of Features

- 5′ MoMuSV LTR: 1–589
- Ψ^+ (extended packaging signal): 659–1468
  Mutated *gag* (ATG->TAG): 1049–1051
- Multiple Cloning Site (MCS): 1470–1491
- Early SV40 promoter (P_{SV40e}): 1481–1846
- Neomycin resistance gene (Neo^r):
  Start codon: 1892–1894; stop codon: 2684–2686
- 3′ MoMuLV LTR: 2746–3339
- Col E1 origin of replication:
  Site of replication initiation: 3875
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5495–5493; stop codon: 4637–4635

Sequencing Primer Locations

- pLXSN Seq/PCR Primers (#K1060-E):
  5′ primer (1398–1420): 5′-CCCTTGAACCTCCTCGTTCGACC–3′
  3′ primer (1537–1515): 5′-GAGCCTGGGGACTTTCCACACCC–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.