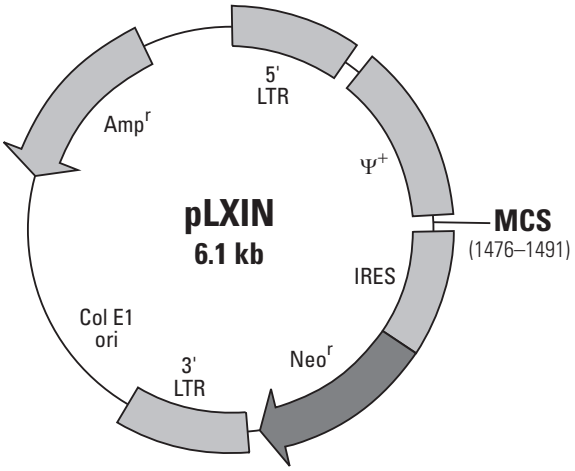
Vector Information

pLXIN Vector Information PT3155-5

GenBank Accession No.: Submission in progress. Catalog No. 631501



Restriction Map and Multiple Cloning Site (MCS) of pLXIN Retroviral Vector.

Description

pLXIN contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is a bicistronic retroviral vector designed for retroviral gene delivery and expression (1–3). This vector contains an internal ribosome entry site (IRES) between the MCS and the neomycin resistance (Neo^r) gene. Thus, the 5' viral LTR promoter in this vector controls expression of both the gene of interest and Neo^r. pLXIN also possesses a Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

The use of this bicistronic vector poses two primary advantages: Upon transfection into a packaging cell line, selection for antibiotic resistance allows for the simultaneous selection of high-titer, virus-producing lines. Later, upon infection of target cells with this virus, neomycin resistance correlates to expression of the gene of interest.

pLXIN 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. pLXIN 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 pLXIN, containing Ψ$^+$ (psi; the extended viral packaging signal), 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.

(PR99510; 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): 1476–1491
- IRES: 1514–2089
- Neomycin resistance gene (Neo^r):
  Start codon: 2090–2092; stop codon: 2891–2893
- 3' MoMuLV LTR: 2953–3546
- Col E1 origin of replication:
  Site of replication initiation: 4082
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5702–5700; stop codon: 4844–4842

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

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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.
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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.