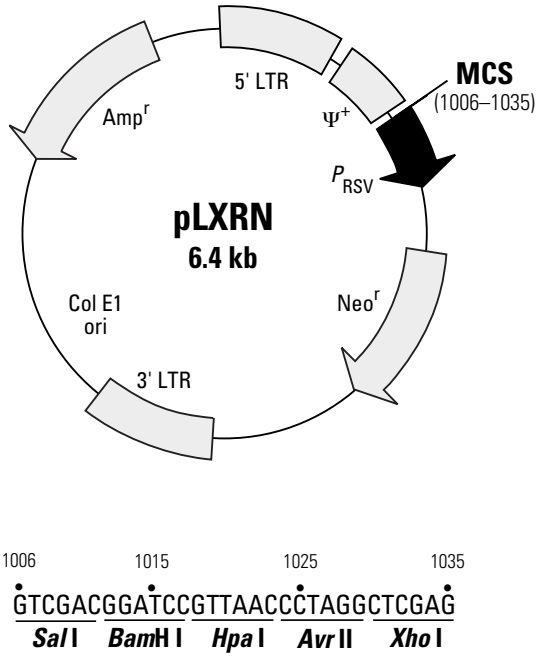
Vector Information

pLXRN Retroviral Vector Information PT3346-5

GenBank Accession No.: Submission in progress Sold as part of Cat. No. 631512



Restriction Map and Multiple Cloning Site (MCS) of pLXRN Retroviral Vector. All restriction sites shown are unique.

Description

pLXRN contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is designed for retroviral gene delivery and expression (1–4). Upon transfection into a packaging cell line, pLXRN can transiently express, or integrate and stably express, a transcript containing the viral packaging signal Ψ^+, a target gene, and the neomycin selection marker (Neo^r). The 5' viral LTR in this vector contains promoter/enhancer sequences that control expression of the target gene. The Rous sarcoma virus promoter (P_{RSV}) controls expression of the Neo^r gene, which allows antibiotic selection in eukaryotic cells. pLXRN also includes the Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

As part of the Pantropic Retroviral Expression System (Cat. No. 631512), pLXRN can be cotransfected with pVSV-G into the GP-293 Packaging Cell Line (5) to produce infectious, replication-incompetent retrovirus. pLXRN does not contain the structural genes necessary for viral particle formation and replication. The genes encoding the viral *gag* and *pol* proteins are stably integrated into GP-293. Because the VSV-G envelope protein is toxic, this protein is expressed transiently from pVSV-G (5). Although the virus can infect target cell lines and transmit a target gene, it cannot replicate because the target cell lines 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. By using the minimal viral sequences and separately introducing the structural genes into the packaging cell line, the chance of producing replication-competent virus due to recombination events is minimized. Alternatively, virus can be produced by transfecting pLNHX into either RetroPack™ PT67 (Cat. No. 631510) cells. Note, these packaging cell lines stably express the MoMuLV envelope and thus, do not require pVSV-G.

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Location of Features

- 5' MoMuSV LTR: 1–589
- Ψ^+ (packaging signal): 659–989
- Multiple Cloning Site (MCS): 1006–1035
- Rous sarcoma virus (RSV) promoter (P_{RSV}): 1039–1350
- Neomycin resistance gene (Neo^r):
  Start codon: 1704–1706; stop codon: 2496–2498
- 3' MoMuLV LTR: 3290–3883
- Col E1 origin of replication
  Site of replication initiation: 4419
- Ampicillin resistance gene (β-lactamase):
  Start codon: 6039–6037; stop codon: 5181–5179

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.*, Eds. (1995) *Current Protocols in Molecular Biology* (John Wiley & Sons, Inc.).
3. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**:980–990.
4. Xu, L., *et al.*, (1989) *Virology* **171**:331–341.
5. Burns, J. C., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90:**8033–8037.

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.