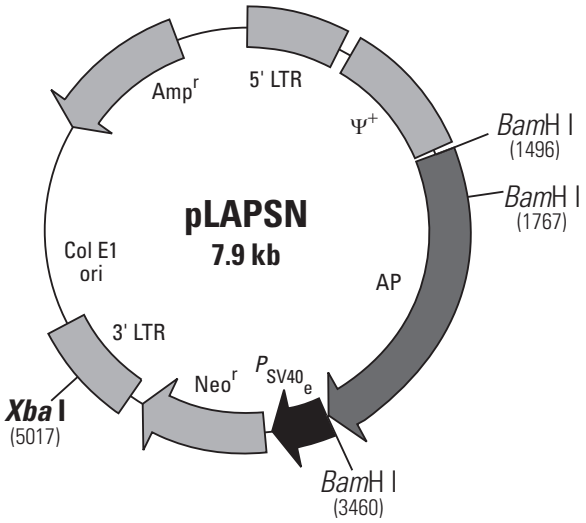
Vector Information

pLAPSN Retroviral Vector Information PT3135-5

GenBank Accession No.: Submission in progress. Sold as part of Catalog No. K1060-A



Restriction Map of pLAPSN Retroviral Vector. Unique restriction sites are in bold.

Description

pLAPSN contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is a control vector for establishing a retroviral gene delivery and expression system (1–3). This vector was created by cloning the gene for human placenta alkaline phosphatase (AP) into the *Xho* I site in the multiple cloning site of the pLXSN Retroviral Vector (Cat. No. 631509). Upon transfection into a packaging cell line, pLAPSN can transiently express, or integrate and stably express, a transcript containing Ψ^+ (the extended viral packaging signal) the human placenta alkaline phosphatase gene, and a selectable marker. The 5′ viral LTR in this vector contains promoter/enhancer sequences that control expression of the alkaline phosphatase gene. The SV40 early promoter (P_{SV40e}) controls expression of the neomycin resistance gene (Neo^r), which allows antibiotic selection in eukaryotic cells. pLAPSN also includes the Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

pLAPSN can be used in control experiments to establish retroviral gene transduction procedures. After being transfected into a packaging cell line (such as the RetroPack PT67 Cell Line, Cat. No. 631510), RNA from the vector is packaged into infectious, replication-incompetent retroviral particles. pLAPSN 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 pLAPSN, containing Ψ^+ (psi), transcription and processing elements, and the alkaline phosphatase gene, produces high-titer, replication-incompetent infectious virus. That is, these retroviral particles can infect target cells and transmit the alkaline phosphatase gene, 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. The expression of alkaline phosphatase can be confirmed using a standard assay.

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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
- Fragment containing the alkaline phosphatase (AP) gene: 1558–3150
- Early SV40 promoter (P_{SV40e}): 3467–3819
- Neomycin resistance gene (Neo^r):
  Start codon: 3865–3867; stop codon: 4657–4659
- 3′ MoMuLV LTR: 4719–5312
- Col E1 origin of replication:
  Site of replication initiation: 5848
- Ampicillin resistance gene (β-lactamase):
  Start codon: 7468–7466; stop codon: 6610–6608

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.