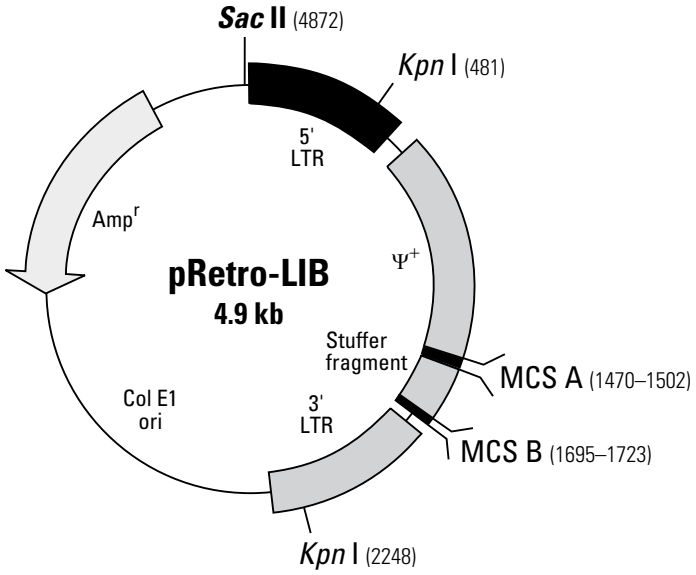
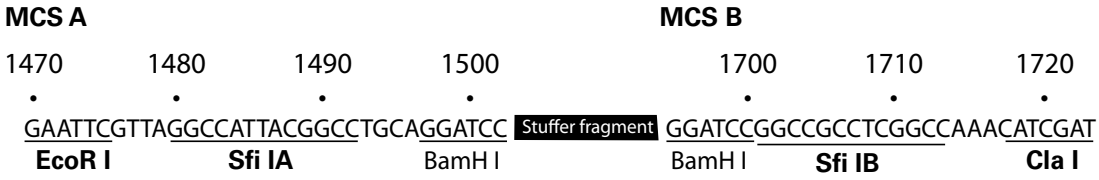
Vector Information

pRetro-Lib Vector Information

PT3627-5

Cat. No. 635002





Restriction map and multiple cloning site of pRetro-LIB Vector. Unique restriction sites are in bold.

Description

pRetro-Lib, derived from Moloney murine leukemia virus (MoMuLV), is designed for delivery and expression of retroviral libraries or genes (1–3). Upon transfection into a packaging cell line, pRetro-Lib can transiently express, or integrate and stably express, a transcript containing the gene of interest. The 5' viral LTR in this vector contains a viral promoter that controls expression of genes cloned into the MCS. pRetro-Lib also contains the Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria. pRetro-Lib can be used as an alternative vector in the construction of a cDNA library using the SMART™ cDNA Library Construction Kit (Cat. No. 634901). Retroviral expression libraries can be constructed by directionally cloning the SfiI(dT)$_{30}$ primed cDNA into the SfiIA/B sites of pRetro-Lib. Restriction digestion with SfiI will remove the Stuffer fragment, prior to cloning.

Use

pRetro-Lib facilitates expression cloning in combination with the benefits of retroviral gene delivery. Upon transfection into a high-titer packaging cell line, RNA from the vector is packaged into infectious, replication-incompetent retroviral particles. 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. pRetro-Lib does not contain the structural genes necessary for particle formation and replication, *gag*, *pol*, and *env*; these genes are stably integrated into the packaging line. Subsequent introduction of pRetro-Lib, containing Ψ+, transcription and processing elements, and the gene of interest produces high-titer, replication-incompetent infectious virus.

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Location of features:

- 5' MoMuLV LTR: 1–589
- Ψ^+ (extended packaging signal): 659–1468
- Multiple Cloning Site (MCS) A: 1470–1502
- Stuffer Fragment: 1503–1694
- Multiple Cloning Site (MCS) B: 1695–1723
- 3' MoMuLV LTR: 1763–2356
- Col E1 plasmid replication region
  Site of replication initiation: 2892
- Ampicillin resistance gene (β-lactamase): 4512–3652

Sequencing primer locations:

5' primer (1444–1463): 5'-AGCCCTCACTCCTTCTCTAG-3'
3' primer (1785–1760): 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'

Propagation in *E. coli*:

- Suitable host strains: DH5α, 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
- Plasmid incompatibility group: pBR322

NOTE: 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.

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.