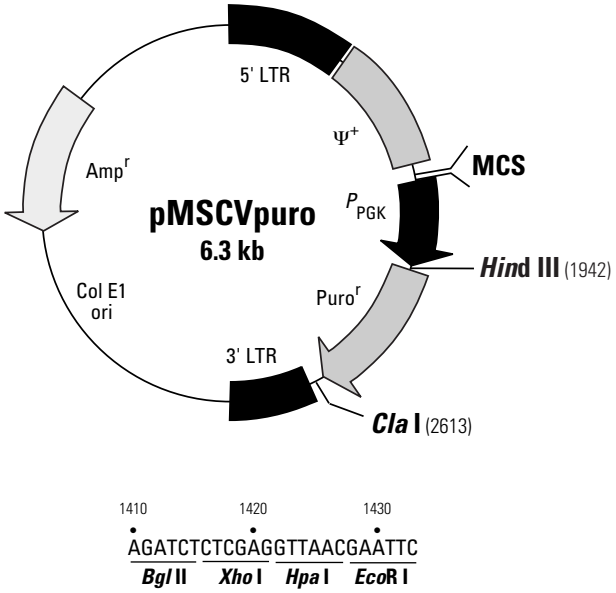
Vector Information

pMSCVpuro Vector Information PT3303-5

GenBank Accession #: Submission in progress. Sold as part of Cat. No. 634401



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

Description

The Murine Stem Cell Virus (MSCV) vectors were derived from the Murine Embryonic Stem Cell Virus (MESV) and the LN retroviral vectors (1,2). Upon transfection into a packaging cell line, pMSCVpuro transiently expresses, or integrates and stably expresses, a transcript containing the extended viral packaging signal Ψ^+, the puromycin resistance gene, and a gene of interest. The vectors achieve stable, high-level gene expression in hematopoietic and embryonic stem cells through a specifically designed 5' long terminal repeat (LTR). This LTR is from the murine stem cell PCMV virus, and it differs from the MoMuLV LTR used in other retroviral vectors by several point mutations and a deletion. These changes enhance transcriptional activation and prevent transcriptional suppression in embryonic stem and embryonal carcinoma cells. As a result, the LTR drives high-level constitutive expression of a target gene in stem cells or other mammalian cell lines (3). A gene can be cloned into the multiple cloning site immediately downstream of this LTR. The murine phosphoglycerate kinase (PKG) promoter (P_{PKG}) controls expression of the puromycin resistance gene (Puro^r) for antibiotic selection in eukaryotic cells. pMSCVpuro also contains the pUC origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

pMSCVpuro 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. pMSCVpuro does not contain the *gag*, *pol*, and *env* structural genes necessary for particle formation and replication: these genes are stably integrated into the PT67 genome (4–7). Introduction of pMSCVpuro into a packaging cell line results in production of high-titer, replication-incompetent infectious virus particles. These particles can infect target cells and transmit the gene of interest to them. However, they cannot replicate because the target cells lack viral structural genes. The process of separately introducing and integrating the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

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Location of features:

- 5' PCMV LTR: 1–515
- Ψ^+ (extended packaging signal): 516–1404
- Puromycin resistance gene (Puro^r): 1958–2557
- PKG promoter ($P_{CMV\ IE}$): 1429–1937
- Multiple Cloning Site: 1410–1433
- 3' PCMV LTR: 2687–3170
- Col E1 origin of replication:
  Site of replication initiation: 3741
- Ampicillin resistance gene (β-lactamase): 5361–4504

Sequencing primer locations:

- pMSCV Primers:
  5' primer (1333–1355): 5'-CCCTTGAACCTCCTCGTTCGACC-3'
  3' primer (1660–1682): 5'-GAGACGTGCTACTTCCATTTGTC-3'

Propagation in *E. coli:*

- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

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. Grez, M., *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**:9202–9206.
2. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**:980–990.
3. Hawley, T.S. *et al.* (1994) *Gene Ther.* **1**:136–138.
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.

Note: 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.