Vector Information

pMSCVhyg Vector Information PT3302-5

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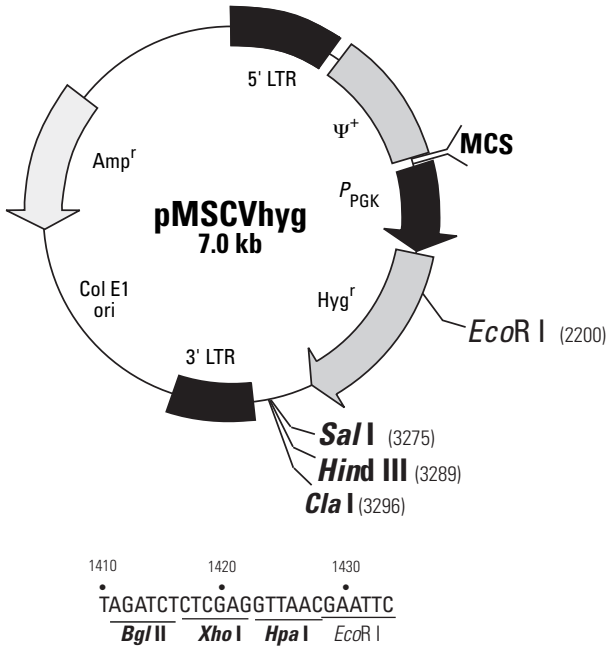

Restriction Map and Multiple Cloning Site (MCS) of pMSCVhyg. Unique restriction sites are in bold.

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, pMSCVhyg transiently expresses, or integrates and stably expresses, a transcript containing the extended viral packaging signal Ψ+, the hygromycin 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), when cloned into the multiple cloning site downstream of the 5' LTR. The murine phosphoglycerate kinase (PKG) promoter (P_{PKG}) controls expression of the hygromycin resistance gene (Hyg^r) for antibiotic selection in eukaryotic cells. pMSCVhyg also contains the pUC origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

pMSCVhyg 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. pMSCVhyg 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 pMSCVhyg 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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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' PCMV LTR: 1–515
- Ψ^+ (extended packaging signal): 516–1405
- Multiple Cloning Site: 1410–1433
- PKG promoter: 1430–1938
- Hygromycin resistance gene (Hyg^r): 1956–2982
- 3' PCMV LTR: 3370–3853
- Col E1 origin of replication:
  Site of replication initiation: 4424
- Ampicillin resistance gene (β-lactamase): 6044–5187

Sequencing primer locations:

- pMSCV Primers:
  5' primer (1333–1355): 5'-CCCTTGAACCTCCTCGTTCGACC-3'
  3' primer (1661–1683): 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:

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