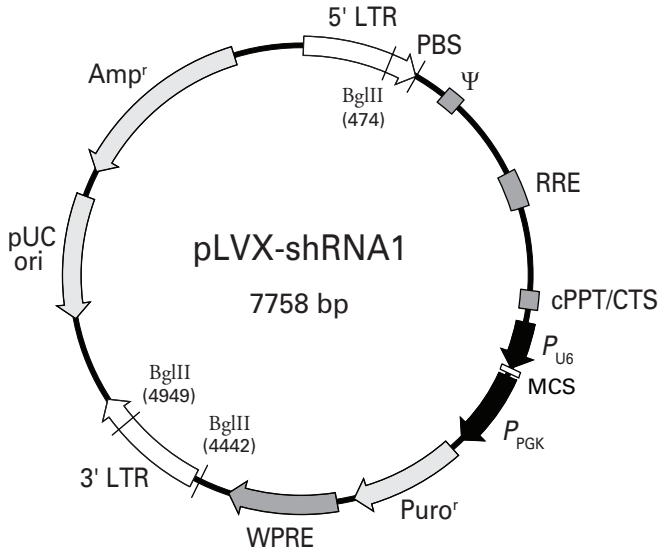
Vector Information

pLVX-shRNA1 Vector Information PT4051-5

Catalog No. 632177



BamHI EcoRI

2431 AAGGACGAGG ATCCGGACAA GCTTCGAATT CTACCGGGTA
TTCCTGCTCC TAGGCCTGTT CGAAGCTTAA GATGGCCCAT

pLVX-shRNA1 Vector Map and Multiple Cloning Site (MCS).

Description

pLVX-shRNA1 is an HIV-1-based, lentiviral expression vector designed to express a small hairpin RNA (shRNA) for RNA interference (RNAi) studies. Expression of your shRNA is driven by the RNA Pol III-dependent, human U6 promoter (P_{U6}), located just upstream of the MCS. pLVX-shRNA1 can either be used as a plasmid expression vector and transfected into cells, or it can be packaged into viral particles and transduced into cells. Lentiviral particles derived from the vector allow the expression of shRNAs in virtually any cell type, including primary cells.

pLVX-shRNA1 contains all of the viral processing elements necessary for the production of replication-incompetent lentivirus, as well as elements to improve viral titer and overall vector function. The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) promotes RNA processing events and enhances nuclear export of viral RNA (1), leading to increased viral titers from packaging cells. In addition, the vector includes a Rev-response element (RRE), which further increases viral titers by enhancing the transport of unspliced viral RNA out of the nucleus (2). Finally, pLVX-shRNA1 also contains a central polypurine tract/central termination sequence element (cPPT/CTS). During target cell infection, this element creates a central DNA flap that increases nuclear import of the viral genome, resulting in improved vector integration and more efficient transduction (3).

In addition to lentiviral elements, pLVX-shRNA1 contains a puromycin resistance gene (Puro^r) under the control of the murine phosphoglycerate kinase promoter (P_{PGK}) for the selection of stable transductants. The vector also contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp^r) for propagation and selection in bacteria.

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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

Use

pLVX-shRNA1 is available in the Lenti-X™ shRNA Expression System (Cat. No. 632177). Before it can be transduced into target cells, the vector must be cotransfected into 293T cells with our Lenti-X HTX Packaging System (Cat. Nos. 631247 and 631249) and packaged into viral particles. This packaging system allows the safe production of high titer, infectious, replication-incompetent, VSV-G pseudotyped lentiviral particles that can infect a wide range of cell types, including non-dividing and primary cells (4).

Location of Features

- 5' LTR: 1–635
- PBS (primer binding site): 636–653
- Ψ (packaging signal): 685–822
- RRE (Rev-response element): 1303–1536
- cPPT/CTS (central polypurine tract/central termination sequence): 2028–2151
- P_{U6} (human U6 promoter): 2187–2444
- MCS (multiple cloning site): 2440–2457
- P_{PGK} (phosphoglycerate kinase promoter): 2457–2965
- Puro^r (puromycin resistance gene; puromycin N-acetyltransferase): 2986–3585
- WPRE (woodchuck posttranscriptional regulatory element): 3680–4271
- 3' LTR: 4475–5111
- pUC origin of replication: 5581–6251 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 6396–7392 (complementary)

Selection of Stable Transductants

- Selectable marker: plasmid confers resistance to puromycin.

Propagation in *E. coli*

- Suitable host strains: DH5α™, DH10B™ and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Notes:

The vector sequence was 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.

The viral supernatants produced by this lentiviral vector could contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant lentivirus. Appropriate NIH, regional, and institutional guidelines apply.

References

1. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4):2886–2892.
2. Cochrane, A. W. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**(3):1198–1202.
3. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
4. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.

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