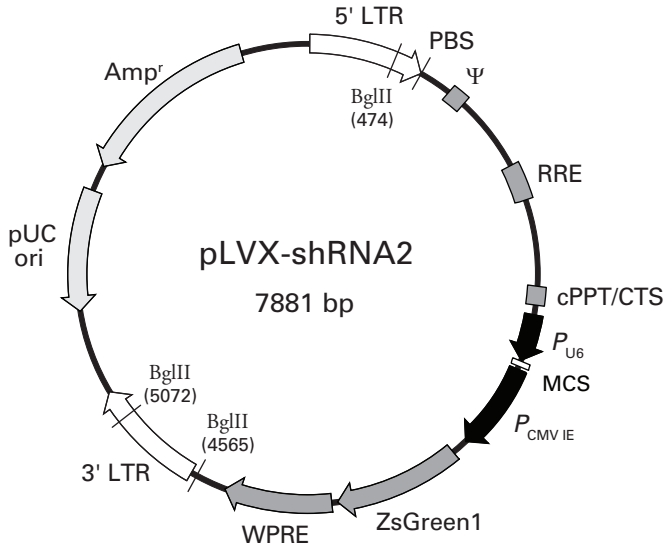
Vector Information

pLVX-shRNA2 Vector Information PT4052-5

Catalog No. 632179



BamHI EcoRI

2431 AAGGACGAGG ATCCGGACAA GCTTCGAATT CTAGTTATTA
TTCCTGCTCC TAGGCCTGTT CGAAGCTTAA GATGGCCCAT

pLVX-shRNA2 Vector Map and Multiple Cloning Site (MCS).

Description

pLVX-shRNA2 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-shRNA2 can 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.

In addition to expressing shRNAs, pLVX-shRNA2 also expresses the fluorescent protein ZsGreen1, a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein, ZsGreen (1). Expression of ZsGreen1 is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), allowing it to be used as an indicator of transfection or transduction efficiency, as well as a marker for cell sorting.

pLVX-shRNA2 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 (2), 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 (3). Finally, pLVX-shRNA2 also contains a central polypurine tract/central termination sequence element (cPPT/CTS). During target cell infection, this

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element creates a central DNA flap that increases nuclear import of the viral genome, resulting in improved vector integration and more efficient transduction (4). The vector also contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp^r) for propagation and selection in bacteria.

Use

Before pLVX-shRNA2 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 (5).

ZsGreen1 is the brightest commercially available green fluorescent protein. The presence of this protein allows transductants to be sorted by flow cytometry with standard FITC filter sets (ZsGreen1 has an excitation maximum of 493 nm and an emission maximum of 505 nm).

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
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2462–3050
- ZsGreen1 (*Zoanthus sp.* green fluorescent protein): 3074–3769
- WPRE (woodchuck posttranscriptional regulatory element): 3803–4394
- 3' LTR: 4598–5234
- pUC origin of replication: 5704–6374 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 6519–7515 (complementary)

Selection of Transductants

- Marker: ZsGreen1

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. Reef Coral Fluorescent Protein Vectors (July 2003) *Clontechniques* **XVIII**(3):6–7.
2. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4):2886–2892.
3. Cochrane, A. W. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**(3):1198–1202.
4. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
5. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.

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