



	XhoI		EcoRI		SmaI	
	~~~~~		~~~~~		~~~~~	
2811	CAGATCTCGA	GCTCAAGCTT	CGAATTCTGC	AGTCGACGGT	ACCGCGGGCC	CGGGATCCCG
	GTCTAGAGCT	CGAGTTCGAA	GCTTAAGACG	TCAGCTGCCA	TGGCGCCCCG	GCCCTAGGGC
	XbaI					
	~~~~~					
2871	CGACTCTAGA					
	GCTGAGATCT					

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

Description

pLVX-Puro is an HIV-1-based, lentiviral expression vector. Lentiviral particles derived from the vector allow you to express your gene of interest in virtually any cell type, even primary cells. Expression of your gene is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV IE}$), located just upstream of the multiple cloning site (MCS), allowing constitutive, high level expression of your protein of interest.

pLVX-Puro contains all of the viral processing elements necessary for the production of replication-incompetent lentivirus, as well as elements to improve viral titer, transgene expression, and overall vector function. The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) promotes RNA processing events and enhances nuclear export of viral and transgene RNA (1), leading to increased viral titers from packaging cells, and enhanced expression of your gene of interest in target 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-Puro also contains a central polypurine tract (cPPT) element that increases nuclear importation of the viral genome during target cell infection, resulting in improved vector integration and more efficient transduction (3).

In addition to lentiviral elements, pLVX-Puro contains a puromycin resistance gene (Puro^r) under the control of the murine phosphoglycerate kinase (PGK) 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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Use

pLVX-Puro constitutively expresses your gene of interest from P_{CMVIE} when transduced into target cells. Before the vector can be transduced into cells, however, it must be transfected into 293T packaging cells with our Lenti-X™ HTX Packaging System (Cat. Nos. 631247 and 631249). This packaging system allows you to safely produce 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 (central polypurine tract): 2028–2151
- P_{CMVIE} (human cytomegalovirus immediate early promoter): 2185–2788
- MCS (multiple cloning site): 2816–2880
- P_{PGK} (phosphoglycerate kinase promoter): 2882–3390
- Puro^r (puromycin resistance gene): 3411–4010
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 4024–4615
- 3' LTR: 4819–5455
- pUC origin of replication: 5925–6595 (complementary)
- Amp^r (ampicillin resistance gene (β -lactamase): 6740–7736 (complementary)

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to puromycin.

Propagation in *E. coli*

- Suitable host strains: DH5 α ™ and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/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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