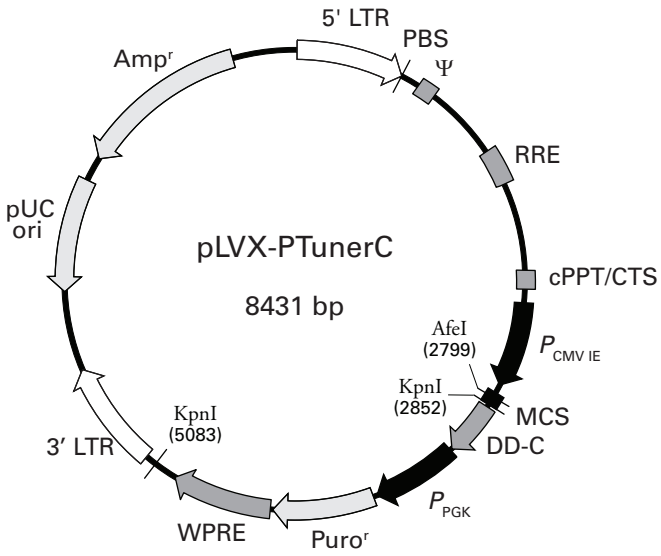
Vector Information

**pLVX-PTunerC Vector Information** PT4438-5

Catalog No. 631074




BstBI

AfeI XhoI EcoRI

2790 ATC GCT AGC GCT ACC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT

ApaI BamHI Start of DD-C tag

2838 GCA GTC GAC GGT ACC GCG GGC CCG GGA TCC CGC GAC TCT AGA GGA GTG

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

## Description

pLVX-PTunerC is a bicistronic, HIV-1-based, lentiviral expression vector that allows you to precisely regulate the amount of your protein of interest in virtually any mammalian cell type, including primary cells. The vector encodes a 12 kDa, FKBP-based destabilization domain (DD-C) that has been optimized for use as a C-terminal tag (1). This domain, located just downstream of the multiple cloning site (MCS), causes the rapid degradation of any protein to which it is fused. Degradation of the DD-C-tagged protein can be prevented by the addition of Shield1 stabilizing ligand to the medium. Shield1 is a membrane permeant molecule that binds to the DD-C tag, 'shielding' the fusion protein from proteasomal degradation, causing the rapid accumulation of the C-terminally tagged protein of interest.

pLVX-PTunerC 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 (2), 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 (3). Finally, pLVX-PTunerC 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 (4).

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In addition to lentiviral elements, pLVX-PTunerC contains a puromycin resistance gene (Puro$^r$) under the control of the murine phosphoglycerate kinase promoter ($P_{PGK}$). Turnover of the Puro$^r$ gene product is unaffected by the DD-C tag, so puromycin resistance can be used as an indicator of transduction efficiency and a marker for selection. 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

pLVX-PTunerC is available in the Lenti-X™ ProteoTuner™ Shield System C (Cat. No. 631074). The vector is designed to constitutively coexpress a C-terminally DD-C-tagged protein of interest and puromycin resistance when transduced into mammalian cells. To create your DD-C-tagged protein of interest, your gene of interest must contain no in-frame stop codons, and must be cloned into the MCS in the same reading frame as the DD-C tag sequence. Before it can be transduced into target cells, the vector must be cotransfected into 293T cells with our Lenti-X™ HT Packaging System (Cat. Nos. 632160 and 632161) 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).

When cells expressing a DD-C-tagged protein of interest are grown in medium containing Shield1, the ligand binds to the DD-C tag and protects the fusion protein from degradation. As a result, the protein quickly accumulates inside the cells in amounts that are directly proportional to the concentration of Shield1 in the medium. If the cells are subsequently grown in medium lacking Shield1, the DD-C tag is no longer stabilized and the fusion protein is rapidly degraded. Because the effects of Shield1 are concentration-dependent and reversible, it is possible to fine-tune the amount of fusion protein present in the cells simply by adjusting the concentration of the ligand in the medium (6).

## 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): 2031–2151
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2185–2787
- MCS (multiple cloning site): 2815–2867
- DD-C (destabilization domain, optimized for use as a C-terminal tag): 2880–3200
- $P_{PGK}$ (phosphoglycerate kinase promoter): 3211–3719
- Puro$^r$ (puromycin resistance gene; puromycin N-acetyltransferase): 3740–4339
- WPRE (woodchuck posttranscriptional regulatory element): 4353–4944
- 3' LTR: 5148–5784
- pUC origin of replication: 6254–6924 (complementary)
- Amp$^r$ (ampicillin resistance gene; β-lactamase): 7069–8065 (complementary)

## Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to puromycin.

## Propagation in *E. coli*

- Suitable host strains: Stellar™ Competent Cells.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

## References

1. Chu, B. W. *et al.* (2008) *Bioorg. Med. Chem. Lett.* **18**(22):5941–5944.
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.
6. Banaszynski, L. *et al.* (2006) *Cell* **126**(5):995–1004.

**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.