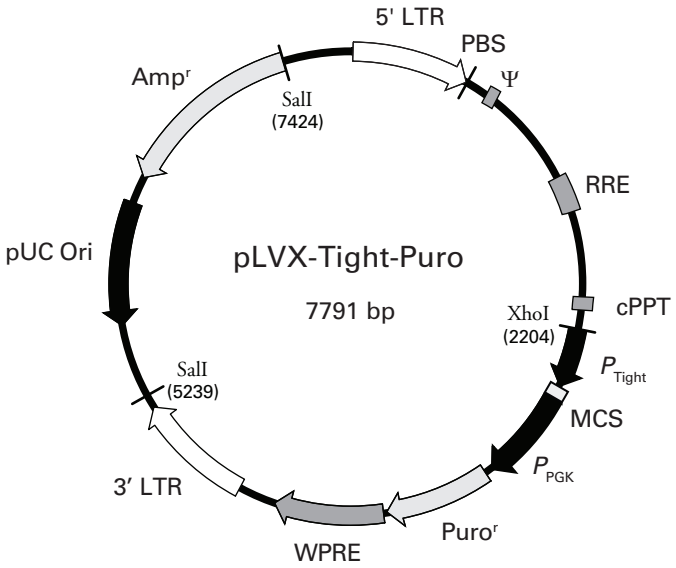
Vector Information

pLVX-Tight-Puro Vector Information

PT3996-5

Catalog Nos. 632162
632163



BamHI NotI XbaI* MluI EcoRI

```
2521  TGGAGAAGGA TCCGCGGCCG CGCCGGCTCT AGATCGCGAA CGCGTGAATT CTACCGGGTA
      ACCTCTTCCT AGGCGCCGGC GCGGCCGAGA TCTAGCGCTT GCGCACTTAA GATGGCCCAT
```

Xba I site (*) is methylated in the DNA provided by Clontech Laboratories, Inc. If you wish to digest the vector with Xba I enzyme, you will need to transform the vector into a dam- host and make fresh DNA.

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

Description

pLVX-Tight-Puro is a tetracycline (Tet)-inducible, lentiviral expression vector designed to express a gene of interest under the control of P_{Tight}, a modified Tet-responsive promoter. P_{Tight} consists of a modified minimal CMV promoter, and seven direct repeats of a 36 bp regulatory sequence that contains the 19 bp tet operator sequence (*tetO*; 1). This vector is designed to be used with our Lenti-X™ Tet-On® Advanced and Tet-Off® Advanced Inducible Expression Systems (Cat. Nos. 632162 and 632163). These systems provide the inducible gene expression strategy of Gossen & Bujard, with major improvements described by Urlinger, *et al.* (2-6), in a lentiviral format.

pLVX-Tight-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 (7), 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 (8). Finally, pLVX-Tight-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 (9).

(PR063561; published 30 June 2010)

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

In addition to lentiviral elements, pLVX-Tight-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.

Use

pLVX-Tight-Puro is a lentiviral expression vector that allows tightly regulated, doxycycline-controlled expression of a gene of interest. In order to function, the system requires the presence of a tetracycline-controlled transcriptional activator (rtTA Advanced or tTA Advanced) supplied by either pLVX-Tet-On Advanced or pLVX-Tet-off Advanced lentiviruses. Before pLVX-Tight-Puro can be transduced into cells, 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 infectious, replication-incompetent, VSV-G pseudotyped lentiviral particles that can infect a wide range of cell types, including non-dividing and primary cells (10).

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_{Tight} (modified Tet-responsive promoter): 2205–2520
- MCS (multiple cloning site): 2528–2571
- P_{PGK} (phosphoglycerate kinase promoter): 2572–3075
- Puro^r (puromycin resistance gene): 3096–3695
- WPRE (woodchuck hepatitis virus posttransctiptional regulatory element): 3713–4304
- 3' LTR: 4508–5144
- pUC origin of replication: 5614–6284 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 6429–7425 (complementary)

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to puromycin.

Propagation in *E. coli*

- Suitable host strains: DH5α™ and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- 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. pTRE-Tight Vectors (April 2003) *Clontechniques* **XVIII**(3):13–14.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci USA* **89**(12):5547–5551.
3. Gossen, M., *et al.* (1995) *Science* **268**(5218):1766–1769.
4. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.
5. Inducible Gene Expression Systems (January 2007) *Clontechniques* **XXII**(1):1–2.
6. Tet-On Advanced Inducible Gene Expression System (2006) *Clontechniques* **XXI**(2):1–3.
7. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4):2886–2892.
8. Cochrane, A. W. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**(3):1198–1202.
9. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
10. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.