



BamHI
NotI
XbaI\*
MluI
EcoRI

```

2521  TGGAGAAGGA  TCCGCGGCCG  CGCCGGCTCT  AGATCGCGAA  CGCGTGAATT  CTACCGGGTA
      ACCTCTTCT  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<sup>r</sup>) 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<sup>r</sup>) 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
- $\Psi$  (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<sup>r</sup> (puromycin resistance gene): 3096–3695
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 3713–4304
- 3' LTR: 4508–5144
- pUC origin of replication: 5614–6284 (complementary)
- Amp<sup>r</sup> (ampicillin resistance gene;  $\beta$ -lactamase): 6429–7425 (complementary)

## Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to puromycin.

## Propagation in *E. coli*

- Suitable host strains: DH5 $\alpha$ <sup>TM</sup> and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/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.

## Notice to Purchaser

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. Patent Nos. 5,464,758 and 5,814,618, which are proprietary to TET Systems GmbH & Co. KG. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet Technology only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet Technology or its component parts to third parties. Notwithstanding the above, academic and not-for profit research institutions whose research using the Tet Technology is sponsored by for profit organizations, which shall receive ownership to all data and results stemming from the sponsored research, shall need a commercial license agreement from TET Systems in order to use the Tet Technology. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. TET Systems GmbH & Co. KG makes no warranties, express or implied or of any kind, and hereby disclaims any warranties, representations, or guarantees of any kind as to the Tet Technology, patents, or products. All others are invited to request a license from TET Systems GmbH & Co. KG prior to purchasing these reagents or using them for any purpose. Clontech is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression system to TET Systems. For license information, please contact: GSF/CEO, TET Systems GmbH & Co. KG, Im Neuenheimer Feld 582, 69120 Heidelberg, Germany Tel: +4962215880400, Fax: +4962215880404 eMail: info@tetsystems.com or use the electronic licensing request form via [http://www.tetsystems.com/main\\_inquiry.htm](http://www.tetsystems.com/main_inquiry.htm)

Clontech has a license to sell products containing WPRE, under the terms described below. Any use of WPRE outside of Clontech's product or the product's intended use, requires a license as detailed below. Before using the product containing WPRE, please read the following license agreement. If you do not agree to be bound by its terms, contact Clontech within 10 days for authorization to return the unused product containing WPRE and to receive a full credit.

Patents: The WPRE technology is covered by one or more of the following U.S. Patents and corresponding patent claims outside the U.S.: 6,136,597; 6,284,469; 6,312,912; 6,287,814, issued to The Salk Institute for Biological Studies

Individual License Agreement: Clontech grants you a non-exclusive license to use the enclosed product containing WPRE in its entirety for its intended use. The product is being transferred to you in furtherance of, and reliance on, such license. Any use of WPRE outside of Clontech's product or the product's intended use, requires a license from the Salk Institute for Biological Studies.

Termination of License: This license agreement is effective until terminated. You may terminate it at any time by destroying all products containing WPRE in your control. It will also terminate automatically if you fail to comply with the terms and conditions of the license agreement. You shall, upon termination of the license agreement, destroy all products containing WPRE in your control, and so notify Clontech in writing. This License shall be governed in its interpretation and enforcement by the laws of the State of California.

Contact for WPRE Licensing: The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, Attn.: Office of Technology Management, Phone: 858.453.4100 ext. 1275

This product and its use are the subject of U.S. Pat. No. 6,682,907. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot disclose information, sell or otherwise transfer this product, its components or materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for any commercial purposes. If the buyer is not willing to accept the limitations of this limited use statement, Clontech is willing to accept return of the product with a full refund. For information on purchasing a license to the DNA-Flap technology for purposes other than research, contact the Transfer of Technology Office, Institut Pasteur, 28 rue du Docteur Roux, 75 724 Paris Cedex 15 ([www.pasteur.fr](http://www.pasteur.fr)).

DH5 $\alpha$ <sup>TM</sup> is a trademark of Life Technologies Corporation.

Clontech, the Clontech Logo and all other trademarks are the property of Clontech Laboratories, Inc., unless noted otherwise. Clontech is a Takara Bio Company. ©2010 Clontech Laboratories, Inc.