



Map and Multiple Cloning Site (MCS) of pTRE2hyg Vector. Unique restriction sites are in bold.

### Description

pTRE2hyg is a response plasmid that expresses a gene of interest (Gene X) in Clontech's Tet-On<sup>®</sup> and Tet-Off<sup>®</sup> Gene Expression Systems and Tet-On and Tet-Off Cell Lines (1). The Tet Expression Systems and Cell Lines give researchers ready access to the tetracycline-regulated expression systems described by Gossen & Bujard (2; Tet-Off) and Gossen *et al.* (3; Tet-On). pTRE2hyg contains an MCS immediately downstream of the Tet-responsive  $P_{hCMV^{*1}}$  promoter. cDNAs or genes inserted into the MCS will be responsive to the tTA and rtTA regulatory proteins in the Tet-Off and Tet-On systems, respectively.  $P_{hCMV^{*1}}$  contains the Tet response element (TRE), which consists of seven copies of the 19-bp tet operator sequence (*tetO*). The TRE element is just upstream of the minimal CMV promoter ( $P_{minCMV}$ ), which lacks the enhancer that is part of the complete CMV promoter. Consequently,  $P_{hCMV^{*1}}$  is silent in the absence of binding of TetR or rTetR to the *tetO* sequences. Note that the cloned insert must have an initiating ATG codon. In some cases, addition of a Kozak consensus ribosome binding site (4) may improve expression levels; however, many cDNAs have been efficiently expressed in Tet systems without the addition of a Kozak sequence. pTRE2hyg also contains the hygromycin resistance gene for direct selection of stable transformants. The parental vector pTRE2 was originally described as pUHD10-3 in reference 5.

The pTRE2hyg-Luc Control Vector, packaged with the pTRE2hyg Vector, contains an additional 1649 bp encoding firefly luciferase inserted into the MCS. This vector can be used as a reporter of induction efficiency using standard luciferase detection reagents. It is not intended as a cloning vector.

(PR752241; published 7 May 2007)



**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

**Location of features**

- $P_{hCMV^{*-1}}$  Tet-responsive promoter: 7–439
  - Tet response element (TRE): 7–319
  - Location of seven *tetO* 19-mers: 15–33; 57–75; 99–117; 141–159; 183–201; 225–243 & 257–275
  - Fragment containing  $P_{min\ CMV}$ : 320–439
  - TATAA box: 342–349
- Multiple cloning site (MCS): 471–532
- Fragment containing  $\beta$ -globin poly-A signal: 539–1706
- Fragment containing Col E1 origin of replication: 1908–2551
- Ampicillin resistance gene ( $\beta$ -lactamase):
  - Start codon (ATG): 3559–3557; stop codon: 2701–2698
- Hygromycin resistance gene: 5312–3765
  - $P_{SV40}$  promoter: 5312–5045
  - Hygromycin coding sequence: 4988–3963
  - SV40 poly-A signal: 3815–3765

**Propagation in *E. coli***

- Suitable host strains: DH5 $\alpha$  and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1

**References**

1. NewTet Vectors: pTRE2pur & pTRE2hyg (October 2000) *Clontechniques* **XV**(4):20.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci USA* **89**:5547–5551.
3. Gossen, M., *et al.* (1995) *Science* **268**:1766–1769.
4. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
5. Resnitzky, D., *et al.* (1994) *Mol. Cell. Biol.* **14**:1669–1679.

**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 Holding 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 IP Merchandisers in order to use the Tet Technology. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. TET Systems Holding 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 Holding 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 IP Merchandisers, Inc. For license information, please contact:

Hans Peter Kneubuehl  
TET Systems Holding GmbH & Co. KG  
Im Neuenheimer Feld 582  
69120 Heidelberg  
Germany  
Tel +49 6221 588 04 00  
Fax +49 6221 588 04 04  
eMail: kneubuehl@tet-systems.de  
or use our electronic licensing request form via  
[http://www.tetsystems.com/main\\_inquiry.htm](http://www.tetsystems.com/main_inquiry.htm)

Clontech, the Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc., unless noted otherwise. Clontech is a Takara Bio Company. ©2007