



|     |             |                                 |         |         |      |
|-----|-------------|---------------------------------|---------|---------|------|
|     | End of DD   | BamHI                           |         | MluI    | NheI |
| 659 | AAA CCG GAA | GGA TCC TCT AGT CAG CTG         | ACG CGT | GCT AGC |      |
|     | TTT GGC CTT | CCT AGG AGA TCA GTC GAC         | TGC GCA | CGA TCG |      |
|     | NotI        | HindIII                         | SalI    |         |      |
|     | EagI        | ClaI                            | EcoRV   |         |      |
| 698 | GCG GCC GCA | TCG ATA AGC TTG TCG ACG ATA TCT |         |         |      |
|     | CGC CGG CGT | AGC TAT TCG AAC AGC TGC TAT AGA |         |         |      |

**pTRE-Cycle2 Vector Map and Multiple Cloning Site (MCS).**

**Description**

pTRE-Cycle2 is a bidirectional, mammalian expression vector that lets you cycle the amount of your protein of interest in cells. Protein expression is tightly regulated by a bidirectional, tetracycline(Tet)-responsive promoter. Once expression is induced, protein levels can be rapidly reduced by simultaneously shutting down transcription and inducing rapid proteasomal degradation. This process can be reversed at any time, allowing the protein of interest to rapidly accumulate once again. In addition, the bidirectional promoter provides concurrent, Tet-regulated coexpression of the red fluorescent protein mCherry.

pTRE-Cycle2 contains two main features that make such precise control over protein levels possible. First, expression of the gene of interest is tightly controlled by  $P_{Tight-BI}$ , a bidirectional, Tet-responsive promoter.  $P_{Tight-BI}$  consists of two minimal CMV promoters ( $P_{minCMV1}$  and  $P_{minCMV2}$ ) and a modified Tet response element ( $TRE_{mod}$ ) that consists of seven direct repeats of a 36 bp regulatory sequence containing the 19 bp tet operator sequence (*tetO*; 1). Second, the vector encodes a ProteoTuner™ destabilization domain (DD; 2). This domain is located between  $P_{Tight}$  and the multiple cloning site (MCS), allowing the addition of an N-terminal DD tag to your protein of interest. The DD tag causes the rapid degradation of any protein to which it is fused. This degradation can be prevented by the addition of Shield1 stabilizing ligand to the culture medium. Shield1 'shields' the fusion protein from proteasomal degradation, allowing the rapid accumulation of the tagged protein. When Shield1 is removed from the medium, the tagged protein is rapidly degraded.

(PR083629; published 7 September 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

mCherry, a mutant red fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (3), is positioned downstream of  $P_{\text{minCMV2}}$ . As a result, mCherry is coexpressed with the DD-tagged protein of interest. 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

pTRE-Cycle2 allows tightly regulated, doxycycline(Dox)-controlled coexpression of a DD-tagged protein of interest, and the fluorescent protein mCherry. To create your DD-tagged protein of interest, your gene of interest must be cloned into the MCS in the same reading frame as the DD tag sequence. Dox-regulated expression of the proteins requires the presence of a tetracycline-controlled transcriptional activator, supplied by a stable Tet-On<sup>®</sup> Advanced or Tet-Off<sup>®</sup> Advanced cell line that can be created with our Tet-On Advanced or Tet-Off Advanced Inducible Gene Expression Systems (Cat. Nos. 630930 and 630934). These systems provide the inducible gene expression strategy of Gossen & Bujard, with major improvements described by Urlinger, *et al.* (4–8).

The effects of Dox and Shield1 are concentration-dependent and reversible. Therefore, it is possible to fine-tune: a) the amount of both the DD-tagged protein of interest and mCherry present in the cells by adjusting the concentration of Dox in the medium; or b) the amount of just the DD-tagged protein of interest by adjusting the concentration of Shield1 in the medium.

Dox-regulated expression of mCherry allows the use of fluorescence microscopy or flow cytometry to easily monitor and/or select cells expressing the gene of interest (mCherry has an excitation maximum of 587 nm and an emission maximum of 610 nm).

### Location of features

- $P_{\text{Tight-BI}}$  (bidirectional, Tet-responsive promoter):
  - TRE<sub>mod</sub> (modified Tet-response element): 3–252
  - $P_{\text{minCMV1}}$  (minimal CMV promoter 1): 258–317
  - $P_{\text{minCMV2}}$  (minimal CMV promoter 2): 3816–3884 (complementary)
- DD (ProteoTuner destabilization domain): 344–667
- MCS (multiple cloning site): 668–729
- SV40 polyA signal: 741–928
- ColE1 origin of replication: 1104–1703
- Amp<sup>r</sup> (ampicillin resistance gene;  $\beta$ -lactamase): 1865–2860 (complementary)
- SV40 polyA signal: 2861–3048 (complementary)
- mCherry (human codon-optimized): 3058–3774 (complementary)

### Propagation in *E. coli*

- Recommended host strain: DH5 $\alpha$ <sup>™</sup>, HB101, 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
- Plasmid incompatibility group: pMB1/ColE1

### Excitation and emission maxima of mCherry

- Excitation maximum = 587 nm
- Emission maximum = 610 nm

### References

1. pTRE-Tight Vectors (April 2003) *Clontechniques XVIII*(3):13–14.
2. Banaszynski, L. *et al.* (2006) *Cell* **126**(5):995–1004.
3. Shaner, N. C. *et al.* (2004) *Nature Biotech.* **22**(12):1567–1572.
4. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci USA* **89**(12):5547–5551.
5. Gossen, M., *et al.* (1995) *Science* **268**(5218):1766–1769.
6. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.
7. Inducible Gene Expression Systems (January 2007) *Clontechniques XXII*(1):1–2.
8. Tet-On Advanced Inducible Gene Expression System (2006) *Clontechniques XXI*(2):1–3.

**Note:** 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.

**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)

The DsRed-Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,005,511; 7,157,566; 7,393,923 and 7,250,298.

**Living Colors Fluorescent Protein Products:**

**Not-For-Profit Entities:** Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at [licensing@clontech.com](mailto:licensing@clontech.com).

**For-Profit Entities** wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at [licensing@clontech.com](mailto:licensing@clontech.com).

DH5 $\alpha$ <sup>™</sup> is a trademark of Invitrogen 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.