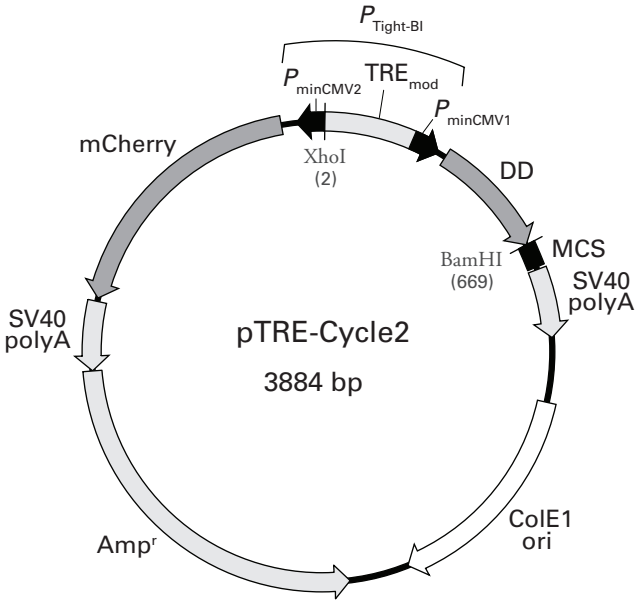
Vector Information

pTRE-Cycle2 Vector Information PT5046-5

Catalog No. 631116



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 $P_{Tight\text{-}Bl}$, a bidirectional, Tet-responsive promoter. $P_{Tight\text{-}Bl}$ 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.

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mCherry, a mutant red fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (3), is positioned downstream of $P_{minCMV2}$. 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^r) 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® Advanced or Tet-Off® 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

- $P_{Tight\text{-}Bl}$ (bidirectional, Tet-responsive promoter):
  - TRE_{mod} (modified Tet-response element): 3–252
  - $P_{minCMV1}$ (minimal CMV promoter 1): 258–317
  - $P_{minCMV2}$ (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^r (ampicillin resistance gene; β-lactamase): 1865–2860 (complementary)
- SV40 polyA signal: 2861–3048 (complementary)
- mCherry (human codon-optimized): 3058–3774 (complementary)

Propagation in *E. coli*

- Recommended host strain: DH5α™, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/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.