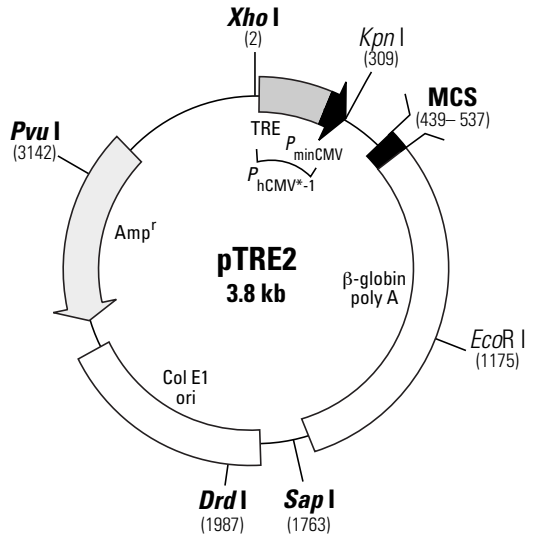

439

CCGCGGCCCCGAATTCGAGCTCGGTACCCGGGGATCCTCTAGTCAGCTG

Sac II *Eco*R I *Kpn* I **Bam**H I **Pvu II**

488

ACGCGTGCTAGCGCGGCCGCATCGATAAGCTTGTCGACGATATCTCTAGA

Mlu I **Nhe I** **Eag I** **Not I** **Cla I** **Hin**d III **Sal I** **Acc I** **Eco**R V **Xba I**

Map and Multiple Cloning Site (MCS) of pTRE2 Vector. Unique restriction sites are in bold.

Description

pTRE2 is a response plasmid that can be used to express a gene of interest (Gene X) in Clontech's Tet-On™, Tet-Off™, RevTet-On™, and RevTet-Off™ Gene Expression Systems and 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). pTRE2 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 42-bp tet operator sequence (*tetO*). The TRE is just upstream of the minimal CMV promoter ($P_{min\ CMV}$), 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. pTRE2-Gene X plasmids should be cotransfected with the pTK-Hyg Vector (not included) to permit selection of stable transfectants.pTRE2 was originally described as pUHD10-3 in reference 5.

The pTRE2-Luc Control Vector, packaged with the pTRE2 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.

(PR651807; published 19 May 2006)

Location of features

- $P_{hCMV*-1}$ Tet-responsive promoter: 7–438
  - Tet response element (TRE): 7–318
    - Location of seven *tetO* 18-mers: 15–33; 57–75; 99–117; 141–159; 183–201; 225–243 & 257–275
  - Fragment containing $P_{min\ CMV}$: 319–438
    - TATAA box: 341–348
- Multiple cloning site (MCS): 470–537
- Fragment containing β-globin poly A signal: 538–1705
- Fragment containing Col E1 origin of replication: 1907–2550
- Ampicillin resistance gene (β-lactamase): 3558–2697

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1

Primer Locations

- pTRE2 5' Sequencing/PCR Primer (#9130-1): 376–392
- pTRE2 3' Sequencing/PCR Primer (#9130-1): 630–649

References

1. HT1080 Cell Line & pTRE2 Vector (January 1999) *CLONTECHniques* **XIV**(1):23.
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.

Note:

The attached sequence file has been 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.