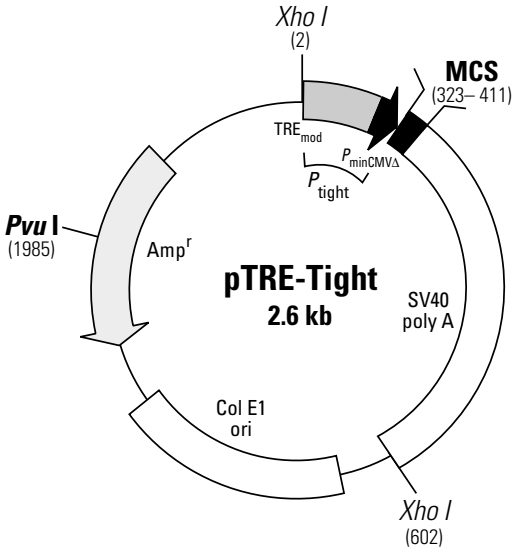

323
•
GAATTCGAGCTCGGTACCCGGGGATCCTCTAGTCAGCTGACGCGT
*Eco*R I *Sac* I *Kpn* I *Bam*H I *Pvu* II *Mlu* I
Sma I
368
•
GCTAGCGCGGCCGCATCGATAAGCTTGTCGACGATATCTCTAGA
Nhe I *Eag* I *Cla* I *Hin*d III *Sal* I *Eco*R V *Xba* I
Not I *Acc* I

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

Description

pTRE-Tight is a response plasmid that can be used to express a gene of interest (Gene X) in our BD™ Tet-On and BD™ Tet-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). pTRE-Tight contains an MCS immediately downstream of the Tet-responsive P_{tight} 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_{tight} contains a modified Tet response element (TRE_{mod}), which consists of seven direct repeats of a 36-bp sequence that contains the 19-bp tet operator sequence (*tetO*). The TRE_{mod} is just upstream of the minimal CMV promoter ($P_{minCMV\Delta}$), which lacks the enhancer that is part of the complete CMV promoter. Consequently, P_{tight} 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. pTRE-Tight-Gene X plasmids should be cotransfected with the Linear Hygromycin Marker (#631625 or #6202-1, not included) or Linear Puromycin Marker (#631626 or #6203-1, not included) to permit selection of stable transfectants. pTRE-Tight was derived from pTRE, originally described as pUHD10-3 in reference 5.

The pTRE-Tight-Luc Control Vector, packaged with the pTRE-Tight Vector, contains an additional 1,649 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.

(PR32437; published 21 February 2003)

Location of features

- P_{tight} Tet-responsive promoter: 3–318
  Tet response element (TRE_{mod}): 3–252
  Location of seven *tetO* 19-mers: 12–30; 48–66; 83–101; 119–137; 155–173; 190–208 & 226–244
  Fragment containing $P_{minCMV\Delta}$: 258–317
  TATAA box: 280–286
- Multiple cloning site (MCS): 323–411
- Fragment containing SV40 poly A signal: 406–606
- Fragment containing Col E1 origin of replication: 780–1379
- Ampicillin resistance gene (β-lactamase): 2536–1541

Propagation in *E. coli*

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

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 BD Biosciences Clontech. This vector has not been completely sequenced.