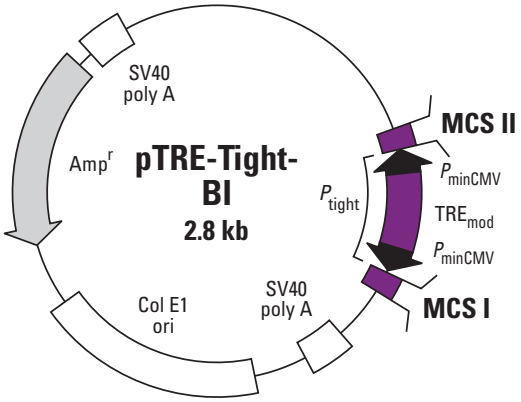
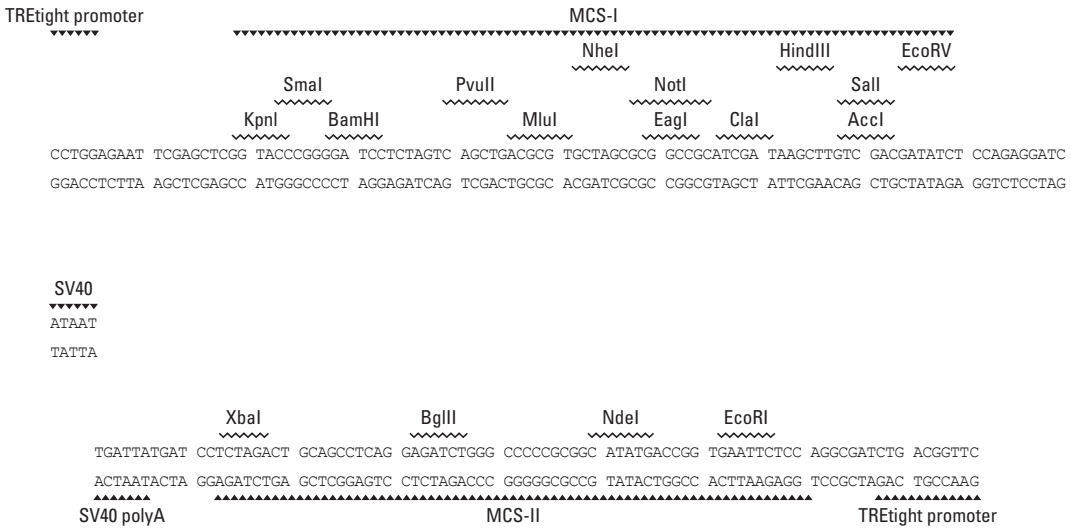
Vector Information

**pTRE-Tight-BI Vector Information** PT3893-5

Catalog No. 631068




**Vector Map and Multiple Cloning Site (MCS) of pTRE-Tight-BI Vector.** All sites shown are unique.

## Description

pTRE-Tight-BI is a bidirectional TRE-Tight plasmid that can be used to inducibly express two genes (a combination of a reporter with a gene of interest or two genes of interest) simultaneously in our Tet-On and Tet-Off Gene Expression Systems and Cell Lines (1, 2). The Tet Expression Systems and Cell Lines provide researchers ready access to the tetracycline-regulated expression systems described by Gossen & Bujard (3; Tet-Off) and Gossen *et al.* (4; Tet-On).

pTRE-Tight-BI vector contains a modified Tet response element (TREmod), which consists of seven direct repeats of a 36 bp sequence that contains the 19 bp tet operator sequence (*tetO*) (5; pTREtight). The two mini CMV promoters, which lack the enhancer that is part of the complete CMV promoter, flank the TREmod. The multiple cloning sites (MCS) I and MCS II flank the BI-Tet-responsive *P*tight promoters on either side. Both genes inserted into MCS I and MCS II will be responsive to the tTA and rtTA regulatory proteins in the Tet-Off and Tet-On systems, respectively. Note that the cloned insert must have an initiating ATG codon. In some cases, addition of a Kozak consensus sequence (6) may improve expression levels; however, many cDNAs have been efficiently expressed in Tet systems without the addition of a Kozak sequence. pTRE-Tight-BI should be cotransfected with the Linear Hygromycin Marker (Cat. No. 631625, not included) or Linear Puromycin Marker (Cat. No. 631626, not included) to permit selection of stable transfectants (7).

(PR083611; published 16 August 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

The pTRE-Tight-BI-Luc Control Vector, packaged with the pTRE-Tight-BI vector, contains an additional 1,649 bp encoding firefly luciferase inserted into MCS I. This vector can be used as a reporter of induction efficiency using standard luciferase detection reagents with the gene of interest cloned into MCS II. pTRE-Tight-BI was derived from pTRE, (originally described as pUHD10-3 (5)) and pTREtight.

### Location of features

- *P*tight Tet-responsive promoter: –70–322
  - Tet response element (TREmod): 3–252
    - Location of seven *tetO* 19-mers: 12–30; 48–66; 83–101; 119–137; 155–173; 190–208 & 226–244
  - Fragment containing $P_{minCMV-1}$: 258–317
    - TATAA box-1: 280–286
  - Fragment containing $_{PminCMV-2}$: 2856–2788
    - TATAA box-2: 2825–2819
- Multiple cloning site I (MCS I): 335–405
- Multiple cloning site II (MCS II): 2782–2726
- Fragment containing SV40 polyA signal-1: 417–617
- Fragment containing SV40 polyA signal-2: 2726–2533
- Fragment containing ColE1 origin of replication: 780–1379
- Ampicillin resistance gene (β-lactamase): 2536–1540

### Sequencing primer locations

pTRE-Tight Sequencing Primer:

Reverse primer (683–660): 5'–TAT TAC CGC CTT TGA GTG AGC TGA–3'

### 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: ColE1

### References

1. HT1080 Cell Line & pTRE2 Vector (January 1999) *Clontechniques* **XIV**(1):23.
2. Matz,M.V., *et al* (1999) *Nature Biotech.* **17**(10):969-973
3. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci USA* **89**(12):5547–5551.
4. Gossen, M., *et al.* (1995) *Science* **268**(5218):1766–1769.
5. pTREtight vectors (April 2003) *Clontechniques* **XVIII** (2):10–11.
6. Kozak, M. (1987) *Nucleic Acids Res.* **15**(20):8125–8148.5.
7. Linear selection markers (April 2003) *Clontechniques* **XVIII**(2):11.

**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.