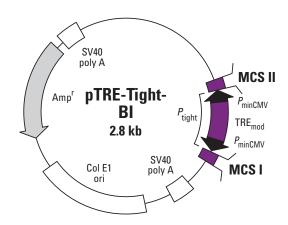
Catalog No. 631068



CCTGGAGAAT TCGAGCTCGG TACCCGGGGA TCCTCTAGTC AGCTGACGCG TGCTAGCGC GCCGCATCGA TAAGCTTGTC GACGATATCT CCAGAGGATCGGACCCTTA AGCTCGAGCC ATGGGCCCCT AGGAGTCAG TCGACTCGCA CCGCCTAGCT ATTCGAACAG CTGCTATAGA GGTCTCTAGA

SV40 ATAAT

	Xbal		BgIII		Ndel	EcoRI			
	~~~~		~~~~	~	~~~~	<b>~~~~~</b>			
TGATTATGAT	CCTCTAGACT	GCAGCCTCAG	GAGATCTGGG	CCCCCGCGGC	ATATGACCGG	TGAATTCTCC	AGGCGATCTG	ACGGTTC	
							TCCGCTAGAC		
							*******		
SV40 polyA	MCS-II					TREtight promoter			

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 Ptight 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)



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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

• PtightTet-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<sub>minCMV-1</sub>: 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 Col E1 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: DH5 $\alpha^{\text{\tiny TM}}$  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.
- 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.

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