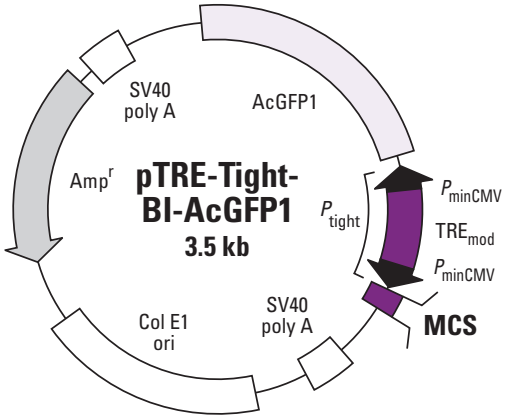
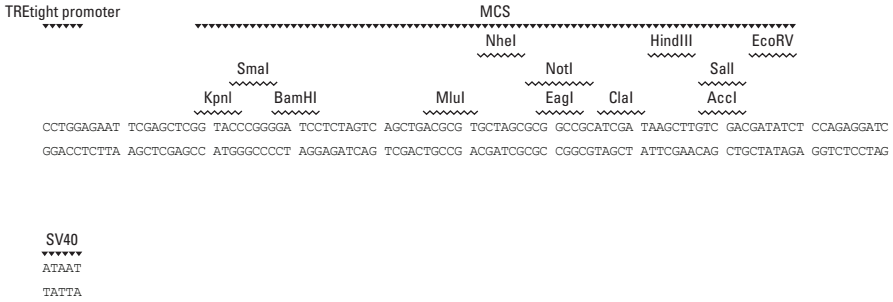
Vector Information

pTRE-Tight-BI-AcGFP1 Vector Information PT3874-5

Catalog No. 631066





Vector Map and Multiple Cloning Site (MCS) of pTRE-Tight-AcGFP1 Vector. All sites shown are unique.

Description

pTRE-Tight-BI-AcGFP1 is a bidirectional TRE-Tight plasmid that can be used to inducibly express a reporter green fluorescent protein (AcGFP1) along with a gene of interest with 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-AcGFP1 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. pTRE-Tight-BI-AcGFP1 encodes a variant of wild-type *Aqueorea coerulescens* green fluorescent protein (AcGFP1) (excitation maximum = 475 nm; emission maximum = 505 nm). PTRE-Tight-BI-AcGFP1 contains a multiple cloning site (MCS) downstream of the BI-Tet-responsive *P*tight promoters. Both the cDNAs or genes inserted into the MCS and AcGFP1 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-AcGFP1 plasmid 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). pTRE-Tight-BI-AcGFP1 was derived from pTRE, (originally described as pUHD10-3 (7)) and pTREtight (5).

The pTRE-Tight-BI-Luc Control Vector, packaged with the pTRE-Tight-BI-AcGFP1 Vector, contains an additional 1,649 bp encoding firefly luciferase inserted into the MCS I. This vector can be used as a reporter of induction efficiency using standard luciferase detection reagents.

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Location of features

- *P*tight Tet-responsive promoter: –70–318
  - 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 $P_{minCMV-2}$: 3533–3464
    - TATAA box-2: 3502-3496
- Multiple cloning site 1(MCS): 335–405
- Fragment containing SV40 polyA signal-1: 417–617
- AcGFP1 gene: 3453–2734; start codon: 3453–3450; stop codon: 2736–2734
- Fragment containing SV40 polyA signal-2: 2725–2528
- 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: 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**(12):5547–5551.
3. Gossen, M. *et al.* (1995) *Science* **268**(5218):1766–1769.
4. pTREtight vectors (April 2003) Clontechniques **XVIII** (2):10–11.
5. Kozak, M. (1987) *Nucleic Acids Res.* **15**(20):8125–8148.
6. Linear selection markers (April 2003) Clontechniques **XVIII**(2):11:
7. Resnitzky, D. *et al.* (1994) *Mol. Cell. Biol.* **14**:1669–1679.

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.