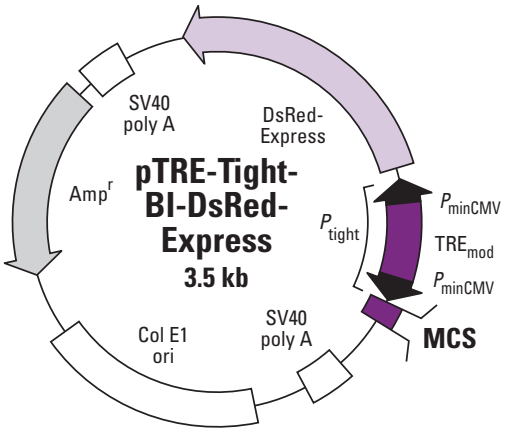
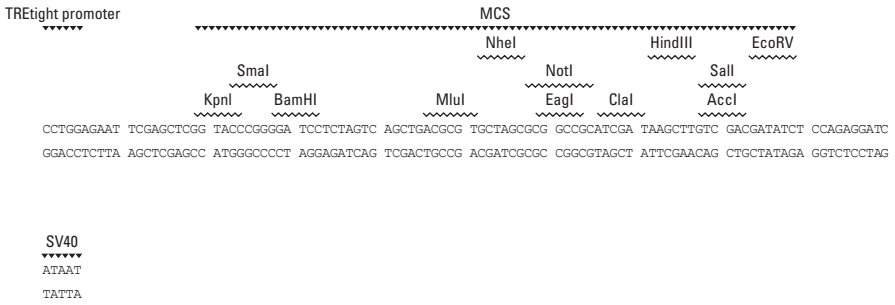
Vector Information

# pTRE-Tight-BI-DsRed-Express Vector Information

PT3876-5

Catalog No. 631065




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

## Description

pTRE-Tight-BI-DsRed-Express is a bidirectional TRE-Tight plasmid that can be used to express a red fluorescent protein (DsRed-Express) 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-DsRed-Express 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-DsRed-Express encodes a variant of wild-type *Discosoma sp.* red fluorescent protein (DsRed) (excitation maximum = 557 nm; emission maximum = 583 nm) which can be detected with rapid appearance of red fluorescence within 8–12 hrs of transfection (6). pTRE-Tight-BI-DsRed-Express 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 DsRed-Express 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 (7) may improve expression levels; however, many cDNAs have been efficiently expressed in Tet systems without the addition of a Kozak sequence. pTRE-Tight-BI-DsRed-Express 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 (8). pTRE-Tight-BI-DsRed-Express was derived from pTRE, (originally described as pUHD10-3 (9)) and pTREtight(5).

The pTRE-Tight-BI-Luc Control Vector, packaged with the pTRE-Tight-BI-DsRed-Express 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: –68–318
  - 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 $P_{minCMV-2}$: 3491–3424
    - TATAA box-2: 3460–3454
- Multiple cloning site (MCS): 335–405
- Fragment containing SV40 polyA signal-1: 417–617
- DsRed-Express gene: 3411–2734; start codon: 3411–3409; stop codon; 2736–2734
- Fragment containing SV40 polyA signal-2: 2726–2532
- 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: 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. Bevis, B. J. & Glick B. S. (2002) *Nature Biotech.* **20**:83-87
7. Kozak, M. (1987) *Nucleic Acids Res.* **15**(20):8125–8148.
8. Linear selection markers (April 2003) Clontechniques **XVIII**(2):11:
9. 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.

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The RCFPs (including DsRed-Express and DsRed-Express2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.

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