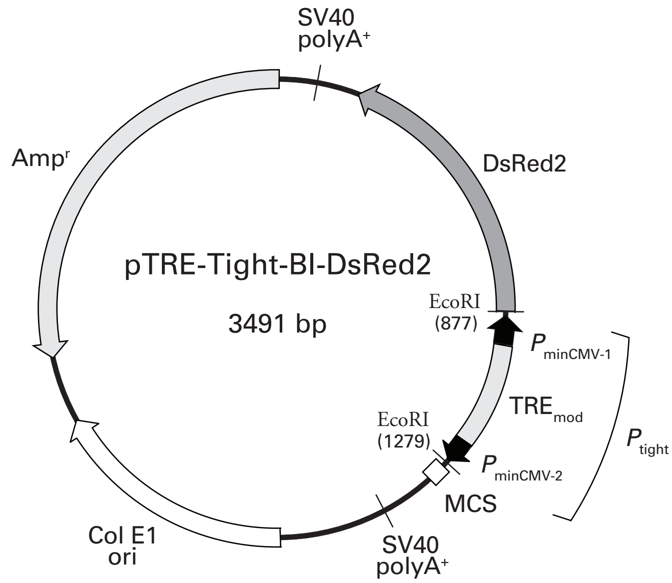
pTRE-Tight-BI-DsRed2 Vector Information

PT3873-5

Catalog No. 631064

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                      SmaI                                         NheI      NotI
               KpnI          BamHI                    MluI                   EagI      ClaI
1281 TTCGAGCTCG GTACCCGGGG ATCCTCTAGT CAGCTGACGC GTGCTAGCGC GGCCGCATCG
     AAGCTCGAGC CATGGGCCCC TAGGAGATCA GTCGACTGCG CACGATCGCG CCGGCGTAGC

          HindIII   SalI
     ClaI          AccI    EcoRV
1341 ATAAGCTTGT CGACGATATC
     TATTCGAACA GCTGCTATAG
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pTRE-Tight-BI-DsRed2 Vector Map and Multiple Cloning Site (MCS).

Description

pTRE-Tight-BI-DsRed2 is a bidirectional TRE-Tight plasmid that can be used to inducibly express a red fluorescent protein (DsRed2) 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-DsRed2 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). The two mini CMV promoters (P_{minCMV}), which lack the enhancer that is part of the complete CMV promoter, flank the TREmod. pTRE-Tight-BI-DsRed2 encodes a variant of wild-type *Discosoma sp*. red fluorescent protein (drFP583; excitation maximum = 558 nm; emission maximum = 583 nm) that can be detected with rapid appearance of red fluorescence within 24 hr of transfection. pTRE-Tight-BI-DsRed2 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 DsRed2 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-DsRed2 should be cotransfected with either our Linear Hygromycin Marker (Cat. No. 631625, not included) or our Linear Puromycin Marker (Cat. No. 631626, not included) to permit selection of stable transfectants (7). pTRE-Tight-BI-DsRed2 was derived from pTRE (originally described as pUHD10-3; 8) and pTREtight (5).

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The pTRE-Tight-BI-Luc Control Vector, packaged with pTRE-Tight-BI-DsRed2, lacks the DsRed2 gene, but contains a 1,649 bp firefly luciferase gene inserted into the MCS. This vector can be used as a reporter of induction efficiency using standard luciferase detection reagents.

Location of features

- SV40 polyA$^+$ signal 1: 1–188 (complementary)
- DsRed2 gene: 198–875 (complementary)
  start codon: 875–873; stop codon; 200–198
- P_{tight} (Tet-responsive promoter): 887–1276
  $P_{minCMV-1}$: 887–955 (complementary)
  TATAA box-1: 918–924 (complementary)
  Tet response element (TREmod): 958–1207
  Location of seven *tetO* 19-mers:
  967–985; 1003–1021; 1038–1056; 1074–1092; 1110–1128; 1145–1163 & 1181–1199
  $P_{minCMV-2}$: 1213-1272
  TATAA box-2: 1235-1241
- MCS (Multiple cloning site): 1290–1360
- SV40 polyA$^+$ signal 2: 1372–1572
- Col E1 origin of replication: 1735–2334
- Amp^r (Ampicillin resistance gene; β-lactamase): 2496–3491 (complementary)

Sequencing primer locations

pTRE-Tight Sequencing Primer:

Reverse primer (1638–1615): 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. 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.
7. Linear selection markers (April 2003) Clontechniques **XVIII**(2):11.
8. Resnitzky, D. *et al.* (1994) *Mol. Cell. Biol.* **14**(3):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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