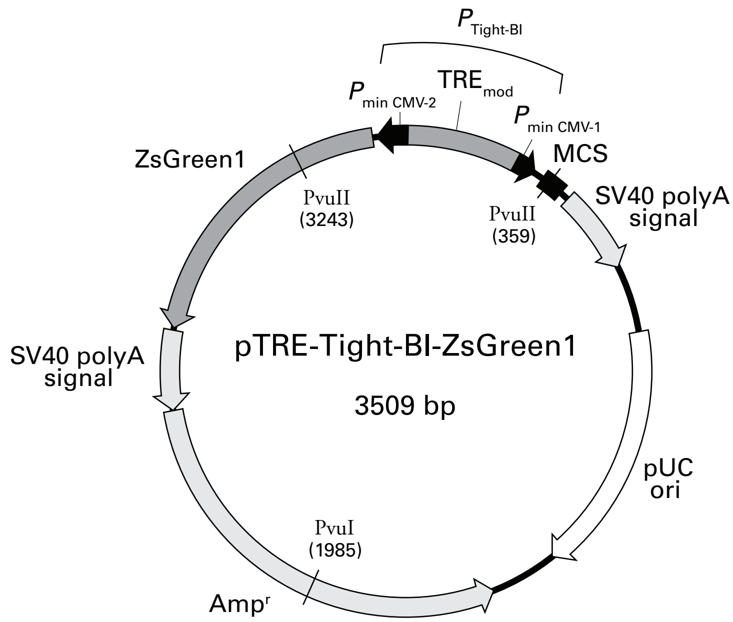
pTRE-Tight-BI-ZsGreen1 Vector Information

PT3875-5

Catalog No. 631067

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                 SmaI                                   NheI
         KpnI           BamHI                   MluI
331  GCTCGGTACC CGGGGATCCT CTAGTCAGCT GACGCGTGCT
     CGAGCCATGG GCCCCTAGGA GATCAGTCGA CTGCGCACGA

            NotI              HindIII    SalI
     NheI   EagI      ClaI               AccI
371  AGCGCGGCCG CATCGATAAG CTTGTCGACG
     TCGCGCCGGC GTAGCTATTC GAACAGCTGC
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pTRE-Tight-BI-ZsGreen1 Vector Map and Multiple Cloning Site (MCS). All sites shown are unique.

Description

pTRE-Tight-BI-ZsGreen1 is a bidirectional TRE-Tight plasmid that can be used to inducibly express a green fluorescent protein (ZsGreen1) 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-ZsGreen1 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 (P_{minCMV}), which lack the enhancer that is part of the complete CMV promoter, flank the TREmod. pTRE-Tight-BI-ZsGreen1 encodes a variant of wild-type *Zoanthus sp*. green fluorescent protein (ZsGreen1) (excitation maximum = 493 nm; emission maximum = 505 nm). pTRE-Tight-BI-ZsGreen1 contains a multiple cloning site (MCS) downstream of the bidirectional, Tet-responsive $P_{Tight-BI}$ promoter. Both ZsGreen1 and genes inserted into the MCS will be responsive to the tTA and rtTA regulatory proteins in the

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Tet-Off and Tet-On systems, respectively. Note that the cloned insert must have an initiation codon (ATG). 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-ZsGreen1 should be cotransfected with a Linear Hygromycin or Puromycin Marker (Cat. Nos. 631625 and 631626, respectively; not included) to permit selection of stable transfectants (7). pTRE-Tight-BI-ZsGreen1 was derived from pTRE, (originally described as pUHD10-3 (8)) and pTREtight (5).

The pTRE-Tight-BI-Luc Control Vector, packaged with the pTRE-Tight-BI-ZsGreen1 Vector, lacks the ZsGreen1 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

- $P_{\text{Tight-BI}}$ (bidirectional, Tet-responsive promoter):
  - TRE_{mod} (modified Tet-response element): 3–252
  - $P_{\text{minCMV-1}}$ (minimal CMV promoter 1): 258–317
  - $P_{\text{minCMV-2}}$ (minimal CMV promoter 2): 3440–3509 (complementary)
- MCS (multiple cloning site): 335–399
- SV40 polyA signal: 417–617
- pUC origin of replication: 780–1379
- Amp^{r} (ampicillin resistance gene; β-lactamase): 1540–2536 (complementary)
- SV40 polyA signal: 2538–2728 (complementary)
- ZsGreen1 gene: 2734–3429 (complementary)

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: pUC

Excitation and emission maxima of ZsGreen1

- Excitation maximum = 493 nm
- Emission maximum = 505 nm

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**:1669–1679.

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