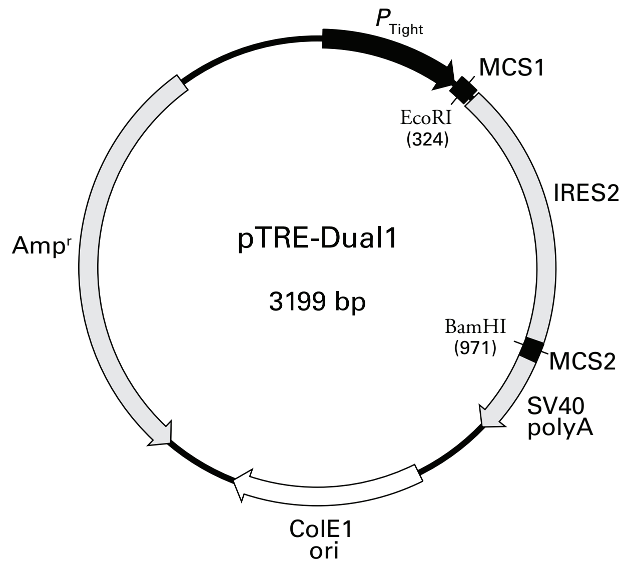
pTRE-Dual1 Vector Information

PT5040-5

Catalog No. 631114



MCS1

```
                    PstI                      PvuII
         EcoRI              XmaI       PacI              MluI       NheI
321  GAGAATTCCT GCAGCCCGGG TTAATTAACA GCTGACGCGT GCTAGCGCC
     CTCTTAAGGA CGTCGGGCCC AATTAATTGT CGACTGCGCA CGATCGCGG
```

MCS2

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                             NotI
     End of IRES2            EagI     BglII     BamHI     ClaI      SalI
943  ATG GCC ACA ACC GCG GCC GCT AGA TCT GGA TCC ATC GAT GTC GAC
     TAC CGG TGT TGG CGC CGG CGA TCT AGA CCT AGG TAG CTA CAG CTG

     EcoRV    NdeI     XbaI
988  GAT ATC CAT ATG TCT AGA GGA
     CTA TAG GTA TAC AGA TCT CCT
```

pTRE-Dual1 Vector Map and Multiple Cloning Sites (MCS1 and MCS2). The internal start site (ATG) at the IRES2/MCS2 junction is indicated in bold.

Description

pTRE-Dual1 is a tetracycline (Tet)-regulatable, mammalian expression vector designed to coexpress two genes of your choice under the control of P_{Tight}, a modified Tet-responsive promoter. P_{Tight} consists of a modified minimal CMV promoter, and seven direct repeats of a 36 bp regulatory sequence that contains the 19 bp tet operator sequence (*tetO*; 1). This vector is designed to be used with our Tet-On® Advanced and Tet-Off® Advanced Inducible Gene Expression Systems (Cat. Nos. 630930 and 630934). These systems provide the inducible gene expression strategy of Gossen & Bujard, with major improvements described by Urlinger, *et al.* (2–6).

pTRE-Dual1 allows inducible co-expression of two genes cloned into multiple cloning sites 1 and 2 (MCS1 and MCS2), respectively. An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES2), positioned between the two MCSs, facilitates cap-independent translation of the gene cloned into MCS2, from an internal start site at the IRES2/MCS2 junction (7). The vector also contains a ColE1 origin of replication and an ampicillin resistance gene (Amp^r) to allow for propagation and selection in *E. coli*.

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Use

pTRE-Dual1 is a mammalian expression vector that allows tightly regulated, doxycycline-controlled coexpression of two genes of your choice. Each gene must have both a start and a stop codon. For enhanced expression, the gene cloned into MCS2 should also be cloned in-frame with the start codon at the IRES2/MCS2 junction (this codon is shown in bold in the MCS2 sequence on page 1).

In order to function, the system requires the presence of a tetracycline-controlled transcriptional activator (Tet-On Advanced or Tet-Off Advanced), supplied by a stable Tet-On Advanced or Tet-Off Advanced cell line that can be created with our Tet-On Advanced or Tet-Off Advanced Inducible Gene Expression Systems (Cat. Nos. 630930 and 630934).

Location of features

- P_{Tight} (modified Tet-responsive promoter): 8–321
- MCS1 (multiple cloning site 1): 323–366
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 367–951
- MCS2 (multiple cloning site 2): 955–1005
- SV40 polyA signal: 1012–1194
- ColE1 origin of replication: 1370–1794
- Amp^r (ampicillin resistance gene; β-lactamase): 1956–2879 (complementary)

Propagation in *E. coli*

- Recommended host strain: DH5α™, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Plasmid incompatibility group: pMB1/ColE1

References

1. pTRE-Tight Vectors (April 2003) *Clontechniques* **XVIII**(3):13–14.
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. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.
5. Inducible Gene Expression Systems (January 2007) *Clontechniques* **XXII**(1):1–2.
6. Tet-On Advanced Inducible Gene Expression System (2006) *Clontechniques* **XXI**(2):1–3.
7. Jang, S. K. *et al.* (1988) *J. Virol.* **62**(8):2636–2643.

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.