

**MCS1**

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

MCS2

	End of IRES2	NotI	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.



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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: DH5 α TM, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/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.

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