

Tet-On[®] 3G Vector Set (with mCherry)

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Catalog No.

631160 (Not sold separately)

Lot Number

Specified on product label.

Product Information

The Tet-On 3G Vector Set (with mCherry) is used to create tightly regulated and highly responsive tetracycline (Tet)-inducible mammalian expression systems that are turned on by the addition of doxycycline to the culture medium. The Tet-On 3G Vector Set (with mCherry) allows the simultaneous expression of a gene of interest and a red fluorescent protein marker.

Package Contents

- 20 µl pCMV-Tet3G Vector (500 ng/µl)
- 20 µl pTRE3G-mCherry Vector (500 ng/µl)
- 20 µl pTRE3G-Luc Control Vector (500 ng/µl)
- 40 µl Linear Hygromycin Marker (50 ng/µl)
- 40 µl Linear Puromycin Marker (50 ng/µl)

Storage Conditions

- Store plasmids at -20°C .
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Plasmid Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Shipping Conditions

- Domestic: Dry ice (-70°C)
- International: Dry ice (-70°C)

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Product User Manuals

User manuals for Clontech[®] products are available for download at www.clontech.com/manuals.

The following user manual applies to this product:

- Tet-On 3G Expression Systems User Manual (PT5148-1)

pCMV-Tet3G Vector Information

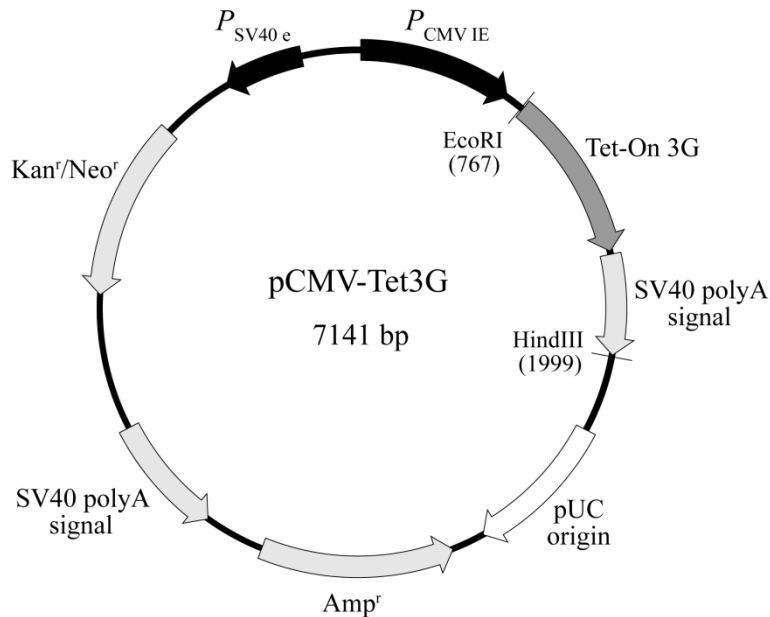


Figure 1. pCMV-Tet3G Vector Map.

Description

The pCMV-Tet3G Vector expresses Tet-On 3G, a tetracycline-controlled transactivator that exhibits high activity in the presence of the inducer doxycycline (Dox), and exceptionally low activity in its absence. Tet-On 3G results from the fusion of amino acids 1–207 of a mutant Tet repressor (TetR) to 39 amino acids that form three minimal "F"-type transcriptional activation domains from the herpes simplex virus VP16 protein. Tet-On 3G was derived from Tet-On Advanced (1–4); as a result, it's fully synthetic, lacks cryptic splice sites, and is codon-optimized for stable expression in mammalian cells. Compared to both of its predecessors, however, this 3rd generation Tet-On transactivator demonstrates increased sensitivity to Dox (1). Constitutive expression of Tet-On 3G is driven by the human cytomegalovirus immediately early promoter (P_{CMVIE}).

Location of Features in pCMV-Tet3G

- P_{CMVIE} (human cytomegalovirus immediate early promoter): 2–688
- Tet-On 3G (transactivator gene): 775–1521
- SV40 polyA signal: 1536–1991
- pUC origin of replication: 2342–2996
- Amp^r (ampicillin resistance gene; β -lactamase): 3144–4004 (complementary)
- SV40 polyA signal: 4275–4809 (complementary)
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 5417–6211 (complementary)

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- P_{SV40e} (SV40 early promoter): 6532–6891 (complementary)

Additional Information

pCMV-Tet3G is used to develop stable Tet-On 3G cell lines, which are hosts for Tet-inducible gene expression systems. To create a Tet-inducible expression system, a vector containing a gene of interest under the control of the Tet-inducible TRE3G promoter (P_{TRE3G}) is transfected into a Tet-On 3G cell line. The addition of Dox to the system causes Tet-On 3G to undergo a conformational change that allows it to bind to P_{TRE3G} , activating transcription of the gene of interest in a highly dose-dependent manner. Additional information on TRE-containing vectors, and protocols describing the construction of Tet-On 3G cell lines can be found in the Tet-On 3G Expression Systems User Manual (PT5148-1).

Propagation in *E. coli*

- Suitable host strain: Stellar[™] Competent Cells
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

pTRE3G-mCherry Vector and pTRE3G-Luc Control Vector Information

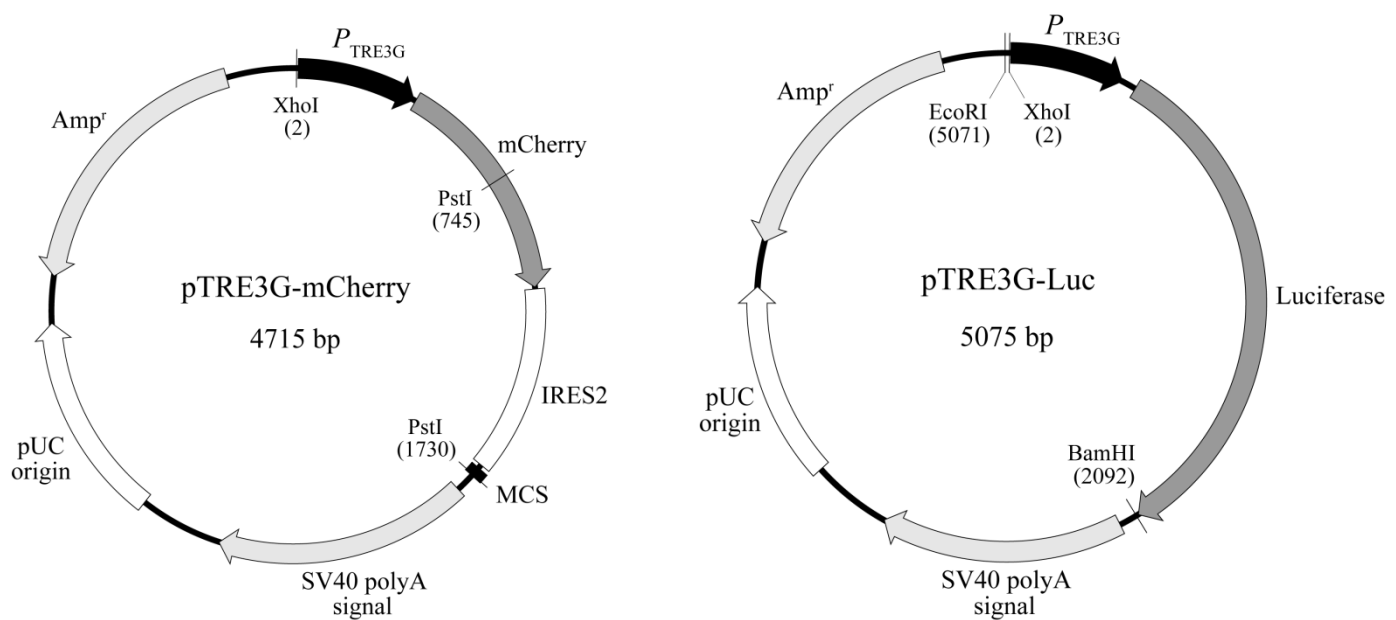


Figure 2. pTRE3G-mCherry Vector and pTRE3G-Luc Control Vector Maps.

	<u>End of IRES2</u>		<u>EcoRV</u>	<u>MluI</u>	<u>NdeI</u>	<u>NheI</u>	<u>BamHI</u>
1683	ATG GCC ACA ACC GGG CCG	GAT ATC	ACG CGT	CAT ATG	GCT AGC	CTG CAG	GGA TCC
	TAC CGG TGT TGG CCC GGC	CTA TAG	TGC GCA	GTA TAC	CGA TCG	GAC GTC	CCT AGG

Figure 3. pTRE3G-mCherry Vector Multiple Cloning Site. The internal start site (ATG) at the IRES2/MCS junction is indicated in bold.

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Description

pTRE3G-mCherry is a Tet-inducible, mammalian expression vector designed to coexpress a gene of interest and the red fluorescent protein mCherry under the control of the Tet-responsive promoter P_{TRE3G} . This promoter consists of a highly optimized Tet-responsive element (TRE) just upstream of a minimal CMV promoter (5). P_{TRE3G} exhibits exceptionally low basal activity; it's induced by the binding of Tet-On 3G, but is virtually silent in its absence. The vector is designed to be used as part of our Tet-On 3G Inducible Expression System (Cat. No. 631165).

mCherry is a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (excitation and emission maxima: 587 nm and 610 nm, respectively; 6). pTRE3G-mCherry allows Dox-inducible coexpression of mCherry and a gene of interest from a bicistronic mRNA transcript. An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES2), positioned between mCherry and the gene of interest, facilitates cap-independent translation of the gene of interest from an internal start site at the IRES2/MCS junction (7). This ensures that a high percentage of mCherry-expressing clones also express the gene of interest, allowing mCherry to be used as an indicator of inducibility and transfection efficiency, as well as a marker for selection by flow cytometry. The vector also contains a pUC origin of replication and an ampicillin resistance gene (Amp^r) to allow for propagation and selection in *E. coli*.

The pTRE3G-Luc is a Tet-inducible control vector that expresses firefly luciferase under the control of P_{TRE3G} . When used with standard luciferase detection reagents, this vector can be used as a reporter of induction efficiency (see User Manual for protocol). pTRE3G-Luc is not intended to be used as a cloning vector.

Location of Features in pTRE3G-mCherry

- P_{TRE3G} (3rd generation Tet-responsive promoter): 7–382
- mCherry: 389–1099
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 1106–1688
- MCS (multiple cloning site): 1701–1736
- SV40 polyA signal: 1791–2588
- pUC origin of replication: 2853–3496
- Amp^r (ampicillin resistance gene; β -lactamase): 3644–4504 (complementary)

Location of Features in pTRE3G-Luc

- P_{TRE3G} (3rd generation Tet-responsive promoter): 7–382
- Luciferase: 432–2084
- SV40 polyA signal: 2151–2948
- pUC origin of replication: 3213–3856
- Amp^r (ampicillin resistance gene; β -lactamase): 4004–4864 (complementary)

Additional Information

pTRE3G-mCherry is a mammalian expression vector that allows tightly regulated, Dox-controlled coexpression of a gene of interest and mCherry. The gene of interest must have both a start and a stop codon. The gene of interest should be cloned in-frame with the start codon at the IRES2/MCS junction (this codon is shown in bold in the MCS sequence in Figure 3, page 3; see the User Manual for details on how to use In-Fusion[®] to simplify your cloning). Cotransfection of pTRE3G-mCherry constructs with Linear Hygromycin or Puromycin Markers allows antibiotic selection of stable

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transfectants. In order to function, the system requires the presence of the Tet-On 3G transactivator protein, supplied by a stable Tet-On 3G cell line created with our Tet-On 3G Inducible Expression System (Cat. No. 631165).

Propagation in *E. coli*

- Suitable host strain: Stellar[™] Competent Cells
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Excitation and Emission Maxima of pTRE3G-mCherry

- Excitation: 587 nm
- Emission: 610 nm

References

1. Zhou, X. *et al.* (2006) *Gene Ther.* **13**(19):1382-1390.
2. Urlinger, S. *et al.* (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.
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. Löw, R., Heinz, N., Hampf, M., Bujard, H., & Gossen, M. Ameliorating the dynamic properties of the Tet system by altered minimal promoter design. Manuscript submitted for publication.
6. Shaner, N. C. *et al.* (2004) *Nature Biotech.* **22**(12):1567-1572.
7. Jang, S. K. *et al.* (1988) *J. Virol.* **62**(8):2636–2643.

Quality Control Data

Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Vector	Enzyme(s)	Fragment(s)
pCMV-Tet3G	EcoRI	7.1 kb
	EcoRI & HindIII	1.2 & 5.9 kb
pTRE3G-mCherry	XhoI	4.7 kb
	PstI	1.0 & 3.7 kb
pTRE3G-Luc	XhoI	5.1 kb
	EcoRI & BamHI	2.1 & 3.0 kb
Linear Hygromycin Marker	HindIII & XbaI	0.5, 0.6 & 1.1 kb
Linear Puromycin Marker	HindIII & XbaI	0.45, 0.6, & 0.75 kb

- Vector identity was confirmed by sequencing.
- A₂₆₀/A₂₈₀: 1.8–2.0

Functional Testing of Linear Markers

- HEK 293 cells were transfected with 200 ng of either the Linear Hygromycin Marker or the Linear Puromycin Marker. After 5 hr at 37°C, the transfection solution was removed and the cells were given fresh medium. 48 hr later, the cells were plated in two 10 cm plates. 48 hr after plating, medium containing either hygromycin or

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puromycin (depending on the linear marker used to transfect the cells) was added to the plates. After 2–3 weeks, >20 clones were identified.

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Use of the Tetracycline controllable expression systems (the "Tet Technology") is covered by a series of patents including U.S. Patent Nos. 6087166, 6271341, 7541446, 8383364, European Patents: EP 0990030, 1954811, 2050818, 2352833 and corresponding patent claims outside these regions which are proprietary to TET Systems GmbH & Co. KG. Academic research institutions are granted an automatic license with the purchase of this product to use the Tet Technology only for internal, academic research purposes, which license specifically excludes the right to sell, or otherwise transfer, the Tet Technology or its component parts to third parties. Notwithstanding the above, academic and not-for profit research institutions whose research using the Tet Technology is sponsored by for profit organizations, which shall receive ownership to all data and results stemming from the sponsored research, shall need a commercial license agreement from TET Systems in order to use the Tet Technology. In accepting this license, all users acknowledge that the Tet Technology is experimental in nature. TET Systems GmbH & Co. KG makes no warranties, express or implied or of any kind, and hereby disclaims any warranties, representations, or guarantees of any kind as to the Tet Technology, patents, or products. All others are invited to request a license from TET Systems GmbH & Co. KG prior to purchasing these reagents or using them for any purpose. Clontech is required by its licensing agreement to submit a report of all purchasers of the Tet-controllable expression system to TET Systems. For license information, please contact: GSF/CEO TET Systems GmbH & Co. KG, Im Neuenheimer Feld 582 69120 Heidelberg Germany Tel: +49 6221 5880400 Fax: +49 6221 5880404 email: info@tetsystems.com or use the electronic licensing request form via www.tetsystems.com/main_inquiry.htm

STATEMENT 44

The DsRed-Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,005,511; 7,157,566; 7,393,923 and 7,250,298.

STATEMENT 72

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