Tet-On® 3G Vector Set (Bicistronic Version)

Table of Contents

Catalog No.
631161 (Not sold separately)

Lot Number
Specified on product label.

Product Information

The Tet-On 3G Vector Set (Bicistronic Version) 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 Bicistronic Version allows the simultaneous expression of two genes of interest.

Package Contents

- 20 µl pCMV-Tet3G Vector (500 ng/µl)
- 20 µl pTRE3G-IRES 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

- Dry ice (–70°C)

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

United States/Canada 800.662.2566
Asia Pacific +1.650.919.7300
Europe +33.(0)1.3904.6880
Japan +81.(0)77.543.6116

(PA164517)

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

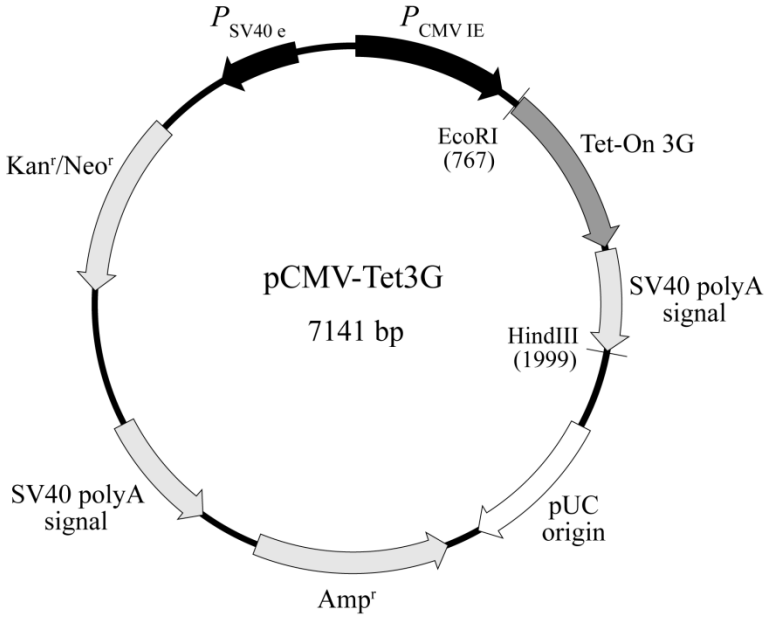

Figure 1. pCMV-Tet3G Vector Map.

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 ($P_{CMV\ IE}$).

Location of Features in pCMV-Tet3G

- $P_{CMV\ IE}$ (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)

- $P_{SV40\ e}$ (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-IRES Vector and pTRE3G-Luc Control Vector Information

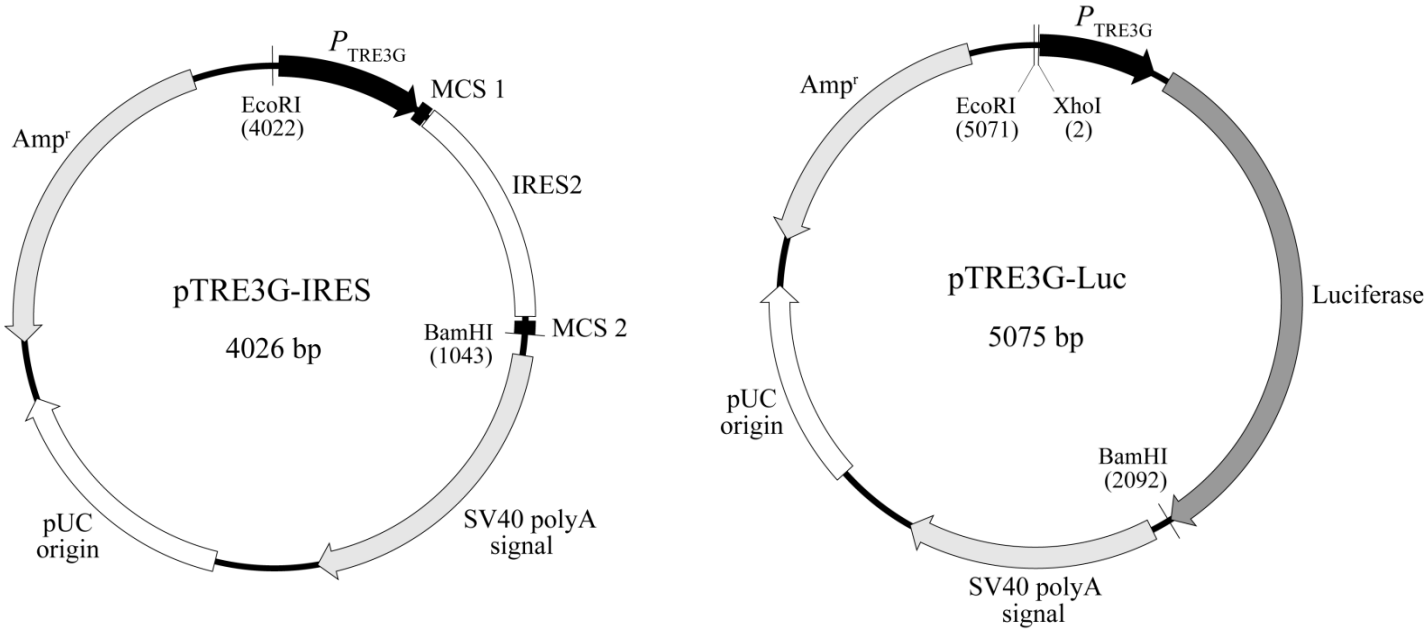

Figure 2. pTRE3G-IRES Vector and pTRE3G-Luc Control Vector Maps.

MCS 1

```
                                BglII
        SalI                          ClaI*     EagI
381  AAGTCGACAC CGGGGCCCAG ATCTATCGAT CGGCCGGCCC
     TTCAGCTGTG GCCCCGGGTC TAGATAGCTA GCCGGCCGGG
```

MCS 2

```
     End of IRES2                EcoRV   MluI    NdeI    NheI    PstI    BamHI
994  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-IRES Vector Multiple Cloning Sites (MCS 1 & MCS 2). The internal start site (ATG) at the IRES2/MCS2 junction is indicated in bold. For optimal expression of the downstream gene, the gene placed upstream of the IRES should not exceed 2.5 kb. *The ClaI restriction site is blocked by dam methylation as a result of overlapping with the dam methylase site. Therefore, if you want to perform a restriction with ClaI, you have to propagate the pTRE3G-IRES vector in a dam- bacterial strain such as Clontech's Stellar Competent Cells (dam-/dcm-) Cat. No. 636764.

Description

pTRE3G-IRES is a Tet-inducible, mammalian expression vector designed to coexpress two genes of interest 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. 631166).

pTRE3G-IRES allows Dox-inducible coexpression of two genes of interest from a bicistronic mRNA transcript. An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES2) positioned between the two multiple cloning sites (MCS1 and MCS 2) facilitates cap-independent translation of the gene cloned into MCS2, from an internal start site at the IRES2/MCS2 junction (6). 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*.

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-IRES

- P_{TRE3G} (3^{rd} generation Tet-responsive promoter): 7–382
- MCS 1 (multiple cloning site 1): 383–416
- IRES2 (encephalomyocarditis virus internal ribosome entry site): 417–999
- MCS 2 (multiple cloning site 2): 1012–1047
- SV40 polyA signal: 1102–1899
- pUC origin of replication: 2164–2807
- Amp^r (ampicillin resistance gene; β-lactamase): 2955–3815 (complementary)

Location of Features in pTRE3G-Luc

- P_{TRE3G} (3^{rd} 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-IRES is a mammalian expression vector that allows tightly regulated, Dox-controlled coexpression of two genes of interest. 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 4; see the User Manual for details on how to use In-Fusion® to simplify your cloning). Cotransfection of pTRE3G constructs with Linear Hygromycin or Puromycin Markers allows antibiotic selection of stable 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. 631166).

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

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. 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-IRES	BamHI	4.0 kb
	EcoRI & BamHI	1.0 & 3.0 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.
- A_{260}/A_{280}: 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 puromycin (depending on the linear marker used to transfect the cells) was added to the plates. After 2–3 weeks, >20 clones were identified.

Notice to Purchaser

Tet-On® 3G Vector Set (Bicistronic Version)

CATALOG NO.

631161

NOTICE TO PURCHASER:

Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with the licensing requirements listed below and described on the product´s web page at http://www.clontech.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

STATEMENT 42

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

TRADEMARKS:

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

10/13/2015

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.543.6116

Notice to Purchaser

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

10/13/2015

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.543.6116