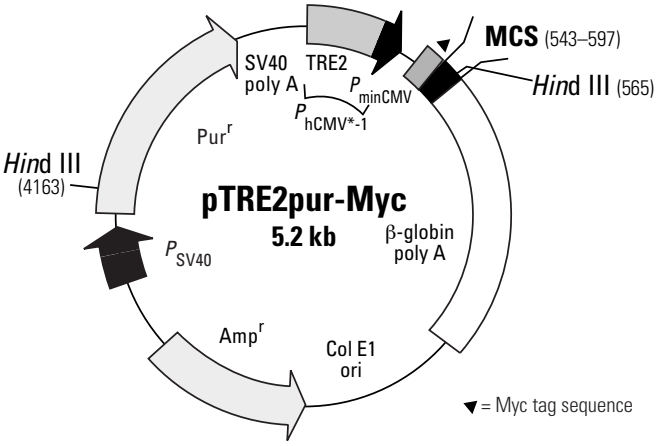
Vector Information

pTRE2pur-Myc Vector Information PT3688-5

Catalog No. 6261-1



500 510 520 530

ATG GCA TCA ATG CAG AAG CTG ATC TCA GAG GAG GAC CTG CTT

Myc tag

540 550 560 570 580 590

ATG GCC ACT GAC GCG TTG CTA GCG CAG CTG GAA GCT TAT CGA TGC GGC CGC GTC GAC GAT ATC

***Mlu* I** ***Nhe* I** ***Pvu* II** ***Cla* I** ***Not* I** ***Eco*R V**

Map and Multiple Cloning Site (MCS) of pTRE2pur-Myc Vector. Unique restriction sites are in bold.

Description

pTRE2pur-Myc is a Tet-responsive vector that expresses a gene of interest bearing a Myc tag for use with Tet-On™ and Tet-Off™ Gene Expression Systems and Tet-On and Tet-Off Cell Lines (1–3). The Tet Expression Systems and Cell Lines give researchers ready access to the tetracycline-regulated expression systems described by Gossen & Bujard (2; Tet-Off) and Gossen *et al.* (3; Tet-On). pTRE2pur-Myc contains an MCS immediately downstream of the Tet-responsive promoter $P_{hCMV*-1}$. cDNAs or genes inserted into the MCS will be responsive to the tTA and rtTA regulatory proteins in the Tet systems. $P_{hCMV*-1}$ contains the Tet response element (TRE), which consists of seven copies of the 19-bp tet operator sequence (*tetO*). The TRE element is just upstream of the minimal CMV promoter (P_{minCMV}), which lacks the enhancer that is part of the complete CMV promoter. Consequently, $P_{hCMV*-1}$ is silent in the absence of binding of tTA or rtTA to the *tetO* sequences. pTRE2pur-Myc also contains the puromycin resistance gene for direct selection of stable transformants. The parental vector pTRE2 was originally described as pUHD10-3 in reference 4.

The pTRE2pur-Myc-Luc Control Vector, packaged with the pTRE2pur-Myc Vector, contains an additional 1653 bp encoding firefly luciferase inserted into the MCS. This vector can be used as a reporter of induction efficiency using standard luciferase detection reagents. It is not intended as a cloning vector.

Clontech

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

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

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Location of features

- Tet-responsive promoter $P_{hCMV^{*}-1}$: 7–439
  - Tet response element (TRE)
    - Location of seven *tetO* 19-mers: 7–319
  - Fragment containing $P_{min\ CMV}$: 320–439
    - TATAA box: 342–349
- Myc tag sequence: 491–532
- Multiple cloning site (MCS): 540–597
- Fragment containing β-globin poly-A signal: 603–1769
- Fragment containing Col E1 origin of replication: 1973–2616
- Ampicillin resistance gene (β-lactamase):
  - Start codon (ATG): 3424–3422; stop codon: 2764–2762
- Puromycin resistance gene: 3847–5159
  - P_{SV40} promoter: 3847–4113
  - Puromycin coding sequence: 4254–4853
  - SV40 poly-A signal: 5113–5159

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1

References

1. New Tet Vectors: pTRE2pur & pTRE2hyg (October 2000) *Clontechniques* **XV**(4):20.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci USA* **89**:5547–5551.
3. Gossen, M., *et al.* (1995) *Science* **268**:1766–1769.
4. Resnitzky, D., *et al.* (1994) *Mol. Cell. Biol.* **14**:1669–1679.

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The attached sequence file has been complied 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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Hans Peter Kneubuehl

TET Systems Holding GmbH & Co. KG

Im Neuenheimer Feld 582

69120 Heidelberg

Germany

Tel +49 6221 588 04 00

Fax +49 6221 588 04 04

eMail: kneubuehl@tet-systems.de

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