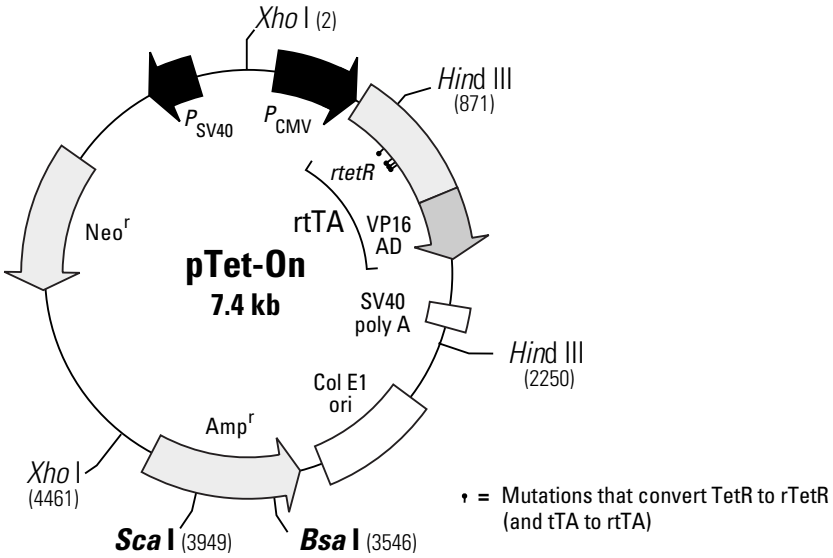
Vector Information

pTet-On® Vector Information

PT3072-5

Cat. No. 631018



Restriction Map of pTet-On® Vector. Unique restriction sites are in bold

Description

pTet-On® expresses the reverse tet-responsive transcriptional activator (rtTA) from the strong immediate early promoter of cytomegalovirus (P_{CMV}). tTA is a fusion of amino acids 1–207 of the tet repressor (TetR) and the negatively charged C-terminal activation domain (130 amino acids) of the VP16 protein of herpes simplex virus. pTet-On is similar to pTet-Off except for four amino acid changes that convert the TetR to a rTetR, and consequently, tTA to rtTA. (pTet-Off also contains several silent mutations.) pTet-On was originally described as pUHD17-1neo by Gossen *et al.* (1995). pTet-On can be distinguished from pTet-Off by digestion with *Hin*d III.

Use

The pTet-On Vector is used to develop stable Tet-On cell lines. After a vector that contains a gene under the control of a tet-responsive element (TRE) is transfected into a Tet-On cell line, the rtTA binds to the TRE, thus activating transcription in the presence of doxycycline (Dox). As Dox is removed from the culture medium, transcription from the TRE is turned off in a highly dose-dependent manner. Additional information on TRE-containing vectors and protocols describing the construction of Tet-On cell lines can be found in the Tet Systems User Manual (PT3001-1).

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

- Fragment containing P_{CMV} : 86–673
- reverse tetacycline-responsive transcriptional activator (rtTA): 774–1781
- Col E1 origin of replication: 2604–3247
- Ampicillin resistance gene:
  β-lactamase coding sequences: 4255–3395
- Fragment containing the SV40 poly A signal: 1797–2254
- Neomycin/kanamycin resistance gene: 6462–5668
- SV40 promoter (P_{SV40}) controlling expression of neomycin/kanamycin resistance gene: 7125–6782.

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) on *E. coli* hosts.
- *E. coli* replication origin: Col E1

References

1. Tet Expression Systems and Cell Lines (July 1996) *Clontechniques* **XI**(3):2–5.
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.

Note:

The attached sequence file has been 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.