



**Restriction Map of pTet-Off Vector.** Unique restriction sites are in bold.

### Description

pTet-Off expresses the tet-responsive transcriptional activator (tTA) 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-Off was originally described as pUHD15-1neo by Resnitzky *et al.* (1994) and was created by insertion of a neomycin resistance gene into pUHD15-1 (Gossen & Bujard, 1992). pTet-Off can be distinguished from pTet-On by digestion with *Hind* III.

### Use

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



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### Location of Features

- Fragment containing  $P_{CMV}$  : 86–673
- Tetracycline-responsive transcriptional activator (tTA): 774–1781
- Col E1 origin of replication: 2604–3247
- Ampicillin resistance gene:  
     $\beta$ -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: DH5 $\alpha$  and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50  $\mu$ g/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.

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