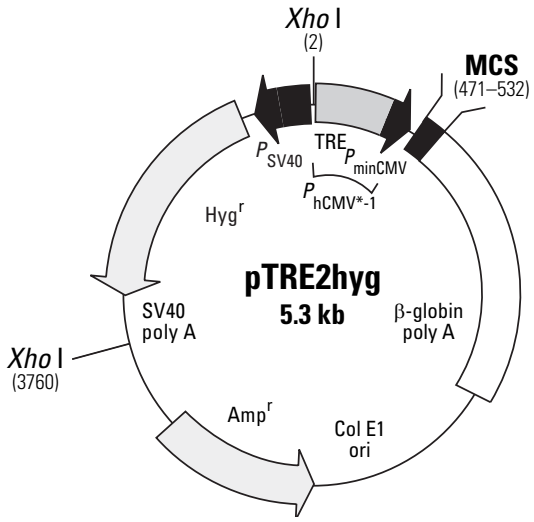

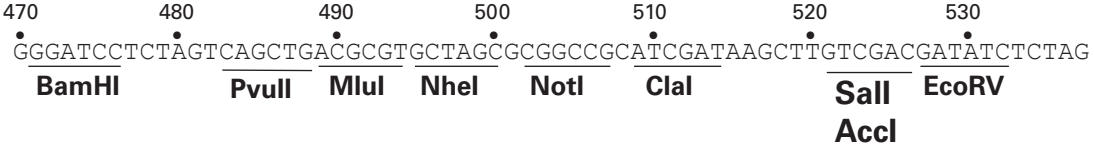

Map and Multiple Cloning Site (MCS) of pTRE2hyg Vector. Unique restriction sites are in bold.

Description

pTRE2hyg is a response plasmid that expresses a gene of interest (Gene X) in Clontech's Tet-On® and Tet-Off® Gene Expression Systems and Tet-On and Tet-Off Cell Lines (1). 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). pTRE2hyg contains an MCS immediately downstream of the Tet-responsive $P_{hCMV*-1}$ promoter. cDNAs or genes inserted into the MCS will be responsive to the tTA and rtTA regulatory proteins in the Tet-Off and Tet-On systems, respectively. $P_{hCMV*-1}$ 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 ($P_{min\ CMV}$), which lacks the enhancer that is part of the complete CMV promoter. Consequently, $P_{hCMV*-1}$ is silent in the absence of binding of TetR or rTetR to the *tetO* sequences. Note that the cloned insert must have an initiating ATG codon. In some cases, addition of a Kozak consensus ribosome binding site (4) may improve expression levels; however, many cDNAs have been efficiently expressed in Tet systems without the addition of a Kozak sequence. pTRE2hyg also contains the hygromycin resistance gene for direct selection of stable transformants. The parental vector pTRE2 was originally described as pUHD10-3 in reference 5.

The pTRE2hyg-Luc Control Vector, packaged with the pTRE2hyg Vector, contains an additional 1649 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.

(PR752241; published 7 May 2007)

Location of features

- $P_{hCMV*-1}$ Tet-responsive promoter: 7–439
  Tet response element (TRE): 7–319
  Location of seven *tetO* 19-mers: 15–33; 57–75; 99–117; 141–159; 183–201; 225–243 & 257–275
  Fragment containing $P_{min\ CMV}$: 320–439
  TATAA box: 342–349
- Multiple cloning site (MCS): 471–532
- Fragment containing β-globin poly-A signal: 539–1706
- Fragment containing Col E1 origin of replication: 1908–2551
- Ampicillin resistance gene (β-lactamase):
  Start codon (ATG): 3559-3557; stop codon: 2701-2698
- Hygromycin resistance gene: 5312–3765
  P_{SV40} promoter: 5312–5045
  Hygromycin coding sequence: 4988–3963
  SV40 poly-A signal: 3815–3765

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. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
5. Resnitzky, D., *et al.* (1994) *Mol. Cell. Biol.* **14**:1669–1679.