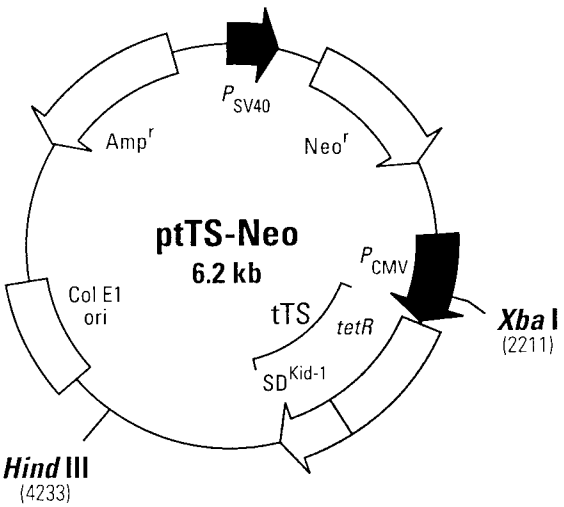
ptTS-Neo Vector Information

PT3813-5

Sold as part of Cat. Nos. 630925 & 630926



Restriction Map of ptTS-Neo Vector. Unique restriction sites are in bold.

Description

The ptTS-Neo Vector is an expression vector designed to express the tetracyline-controlled transcriptional suppressor (tTS). The tTS is a fusion of the Tet repressor protein (TetR) and the KRAB-AB silencing domain of the Kid-1 protein (SD^{Kid-1}), a powerful transcriptional suppressor (1, 2). In the *absence* of Dox, tTS binds to the *tetO* sequences in the modified Tet-responsive promoter (P_{TREmod}) of a Tet response plasmid (pSIREN-RetroQ-TetH or pSIREN-RetroQ-TetP) and blocks expression of the shRNA. As Dox is added to the culture medium, the tTS dissociates from the P_{TREmod}, relieving transcriptional suppression. ptTS-Neo also contains a bacterial origin of replication and *E. coli* Amp^r gene for propagation and selection in bacteria, as well as a neomycin^r gene for the selection of stable transformants.

Use

The ptTS-Neo Vector is used to develop stable Tet tTS cell lines. ptTS-Neo is designed to deliver and express the regulatory protein tTS. After a pSIREN-RetroQ-Tet vector—containing an inserted shRNA under the control of $P_{TREmod/U6}$—is transfected into a Tet tTS cell line, the tTS binds to the P_{TREmod}, repressing transcription in the absence of tetracycline (Tc) or its derivative doxycycline (Dox). As Tc or Dox is added to the culture medium, the tTS dissociates from the P_{TREmod} and transcription of the shRNA is turned on in a highly dose-dependent manner. More information on pSIREN-RetroQ-Tet vectors and protocols describing the construction of Tet tTS cell lines can be found in the BD™ Knockout Inducible RNAi Systems User Manual (PT3810-1).

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Location of Features

- Fragment containing SV40 promoter: 8–326
- Neomycin resistance gene (Neo^r): 361–1155
- Fragment containing thymidine kinase poly A: 1158–1328
- Cytomegalovirus (CMV) immediate early promoter (P_{CMV}): 1516–2189
- Tetracyline-controlled transcriptional suppressor (tTS): 2217–3057
- Fragment containing ß-globin poly A: 2852–4014
- Col E1ori (Site of replication initiation): 4378-5021
- Ampicillin resistance gene (β-lactamase): 6029–5169
  Start codon (ATG): 6029–6027 stop codon (TAA): 5171–5169

Sequencing Primer Locations

- tTS Seq/PCR Primers:
  tTS-1 (6368–6386): 5'-TGACGTCTAAGAAACCA-3'
  tTS-3 (2133–2152): 5'-GACGCCATCCACGCTGTT-3'

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to G418 (400 μg/ml).

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10B, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: low

References

1. Freundlieb S., Schirra-Muller, C. & Bujard. H. (1999) *J. Gene Med.* **1**(1):4–12.
2. Witzgall R., O'Leary, E., Leaf, A., Onaldi, D. & Bonventre, J. (1994) *Proc. Natl. Acad. Sci. USA* **91**(10):4514–4518.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with sequences obtained by BD Biosciences – Clontech. This vector has not been completely sequenced.