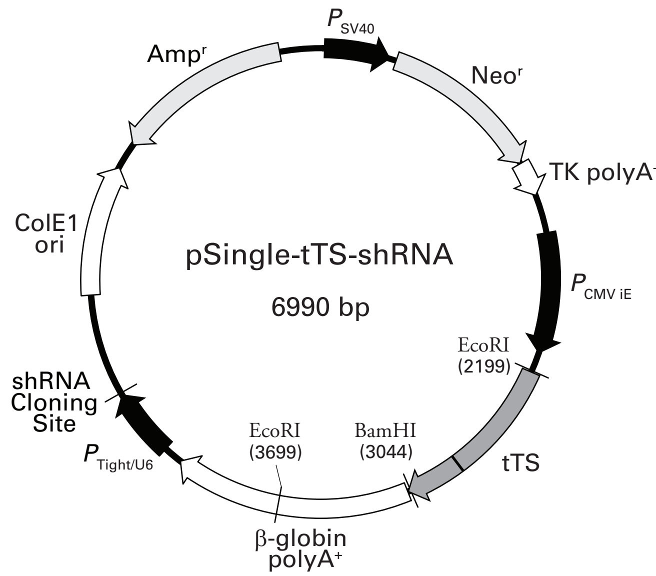
Vector Information

pSingle-tTS-shRNA Vector Information

PT3923-5

Sold as part of Cat. No. 630933

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 4641                 XhoI       HindIII                   XhoI       HindIII               4730
5′-GAAAGGACGA CTCGAGGGAC AAGCTTATCT - 30 bp - ACTCGAGGGA CAAGCTTATC TATGTCGGGT-3′
3′-CTTTCCTGCT GAGCTCCCTG TTCGAATAGA --------- TGAGCTCCCT GTTCGAATAG ATACAGCCCA-5′
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pSingle-tTS-shRNA vector map and shRNA cloning site. Complete digestion of the vector with XhoI and HindIII results in the removal of the nucleotides indicated in gray in the above shRNA cloning site. The XhoI/HindIII digested vector will accept annealed ds shRNA oligonucleotides with the corresponding XhoI and HindIII overhangs.

Description

The pSingle-tTS-shRNA vector expresses the tetracyline-controlled transcriptional suppressor (tTS), which controls expression of an shRNA sequence inserted into the shRNA cloning site. The tTS protein 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 the inducer doxycycline (Dox, a tetracycline derivative), tTS binds to the *tet*O sequences in the modified Tet-responsive Pol III hybrid promoter ($P_{Tight/U6}$) and blocks expression of the shRNA. As Dox is added to the culture medium, tTS dissociates from $P_{Tight/U6}$ to allow Pol III-mediated transcription of the shRNA, resulting in suppression of the target gene in a highly dose-dependent manner. pSingle-tTS-shRNA also contains a ColE1 origin of replication and an ampicillin resistance gene (Amp^r) for propagation and selection in bacteria, as well as a neomycin resistance gene (Neo^r) for selection of stable transformants in mammalian cells.

Use

After digesting pSingle-tTS-shRNA with HindIII and XhoI, clone an annealed ds oligonucleotide encoding your desired shRNA sequence into the vector. Transfect the recombinant vector into cells and select stable, clonal tranformants with G418 to obtain a cell line capable of induced suppression of the shRNA's target gene.

(PR943208; published 27 April 2009)

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Location of Features

- P_{SV40} (DNA fragment containing the SV40 promoter): 8–326
- Neo^r (neomycin resistance gene): 361–1155
- TK polyA$^+$ (DNA fragment containing the thymidine kinase polyA signal): 1158–1328
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1518–2105
- tTS (tetracyline-controlled transcriptional suppressor): 2207–3055
- β-globin polyA$^+$ (DNA fragment containing the β-globin polyA signal): 3058–4221
- $P_{Tight/U6}$ (modified Tet-responsive Pol III hybrid promoter): 4313–4650
- ColE1 ori (ColE1 origin of replication): 5139–5782
- Amp^r (Ampicillin resistance gene; β-lactamase): 6789–5930

Sequencing Primer Locations

- Sing-Tet-U6-R: (4999–4967): 5'-GAAGC GGAAG AGCGC CCAAT ACGCA AACCG CCT-3'

Selection of Stable Transfectants

- The neomycin resistance gene (Neo^r) 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: high

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 vector sequence was 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.