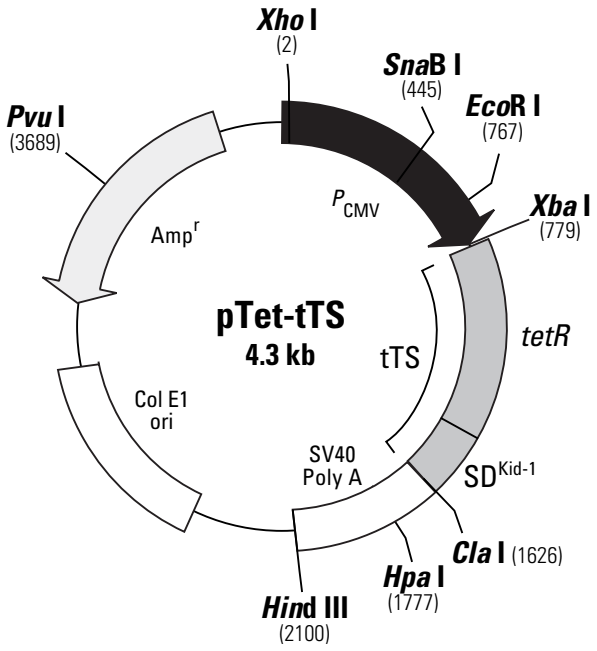
Vector Information

pTet-tTS Vector Information PT3334-5

GenBank Accession No.: Submission in progress. Cat. No. 631011



Restriction map of pTet-tTS Vector. All restriction sites shown are unique.

Description:

The pTet-tTS Vector, designed for use with the Tet-On™ Gene Expression System, prevents unregulated gene expression in the absence of the inducing agent Doxycycline (Dox).

pTet-tTS helps overcome one of the major limitations of regulated gene expression in mammalian systems: low-level background expression. In transient transfections, background expression can result from the high copy number of the introduced plasmid and lack of chromatin repression. In stable cell lines, the level of background expression is dependent on where the gene of interest integrates into the genome. If the expression construct integrates too close to an enhancer element, for instance, unregulated expression may occur.

pTet-tTS encodes the tetracycline-controlled transcriptional silencer (tTS), which 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 repressor (1, 2). In the *absence* of Dox, tTS binds to the *tetO* sequence in the tet-response element (TRE) region of the Tet response plasmid (pTRE2 or pRevTRE) and blocks expression of the gene of interest. As Dox is added to the culture medium, the tTS dissociates from the TRE, relieving transcriptional suppression. At sufficient Dox concentrations, the rtTA transactivator encoded by the pTet-On™ Vector binds to the TRE, thus activating expression of the gene of interest. By silencing unregulated transcription in the *absence* of Dox, the tTS provides complete on/off control of gene expression in either stable or transient systems—regardless of integration site or copy number of the response plasmid.

Use:

tTS will bind to the TRE in the presence of 0–10 ng/ml of Dox. It begins to dissociate from the TRE at concentrations >10 ng/ml. rtTA will bind to the TRE at >100 ng/ml of Dox. We recommend a working Dox concentration of 1 µg/ml for inducing gene expression.

pTet-tTS can be introduced in one of three ways: during the establishment of a Tet-On cell line; after establishing a Tet-On cell line and before introducing the response plasmid; or during introduction of the response plasmid. The last option is recommended if you are introducing a potentially toxic gene. pTet-tTS should only be used with the Tet-On Gene Expression System. It is not compatible for use with the Tet-Off™ System.

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For introduction during establishment of Tet-On cell lines

pTet-tTS does not contain a drug-resistance marker for clonal selection in mammalian cell culture. Therefore, we recommend cotransfecting pTet-tTS with the pTet-On Vector, which contains the rtTA and the neomycin-resistance gene. Transfections should be performed at a 1:10 molar ratio (i.e., 1 µg pTet-On to 10 µg pTet-tTS).

If you have already established a Tet-On cell line

First, cotransfect pTet-tTS with a plasmid containing a selectable marker (such as pTK-Hyg, Cat. No. 631750) at a 1:10 molar ratio (i.e., 1 µg selection marker to 10 µg pTet-tTS). After selecting stable cell lines, transfect the pTRE response plasmid.

If you are infecting cells with a potentially toxic gene

Create a Tet-On cell line first, then cotransfect these vectors in the following ratios:

Selection marker (pTK-Hyg)	:	pTRE-toxic gene	:	pTet-tTS
1 (50–100 ng)		10 (0.5–1.0 µg)		50–100 (5–10 µg)

Location of features:

- Fragment containing P_{CMV} promoter: 86–673
- tTS transcriptional silencer:
  Start codon: 775–777; stop codon: 1621–1623
  - Tet Repressor (TetR): 775–1407
  - SV40 nuclear localization sequence: 1408–1425
  - KRAB-AB Silencing Domain (SD^{Kid-1}): 1426–1623
- Fragment containing SV40 poly-A sequence: 1647–2104
- Col E1 origin of replication: 2454–3097
- Ampicillin resistance (β-lactamase) gene:
  Start codon: 4105–4103; stop codon: 3247–3245

Propagation in *E. coli*:

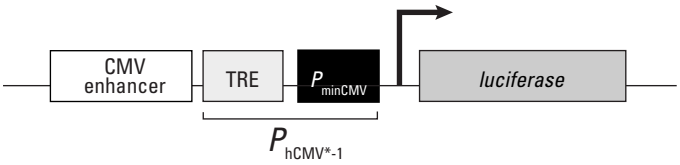
- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

ptTS-Control Vector:

The ptTS-Control Vector is provided to simplify functional testing of clones expressing tTS. ptTS-Control contains the firefly luciferase gene as the reporter, which is controlled by the tet-response element (TRE). Unlike pTRE2 or other TRE-based vectors available from Clontech, ptTS-Control contains the CMV enhancer located upstream of P_{hCMV^*-1} to drive high level background expression. Essentially, this vector construct mimics chromosomal integration of pTRE Vector near an endogenous enhancer sequence. In the absence of doxcycline, functional tTS binds the TRE, thus blocking enhancer activity. This action results in low basal activity and high inducibility of reporter gene expression.

Use of control vector:

To test the function of tTS in stable cell lines, transfect ptTS-Control Vector into the cell line by any standard method. Prepare parallel transfections to test tTS in the presence and absence of Dox. In the absence of Dox, tTS will bind to the TRE of ptTS-Control Vector, resulting in low-level gene expression. Conversely, in the presence of Dox (i.e., 1 µg/ml), tTS will dissociate from TRE, allowing the CMV enhancer to drive expression of the reporter gene. Luciferase is a highly sensitive enzymatic reporter that can be assayed by any standard luciferase-detection method.



ptTS-Control Vector

Schematic map of ptTS-Control Vector.

References:

1. Freundlieb, S., *et al.* (1999) *J. Gene Med.* **1**:4–12.
2. Witzgall, R., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**:4514–4518.

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