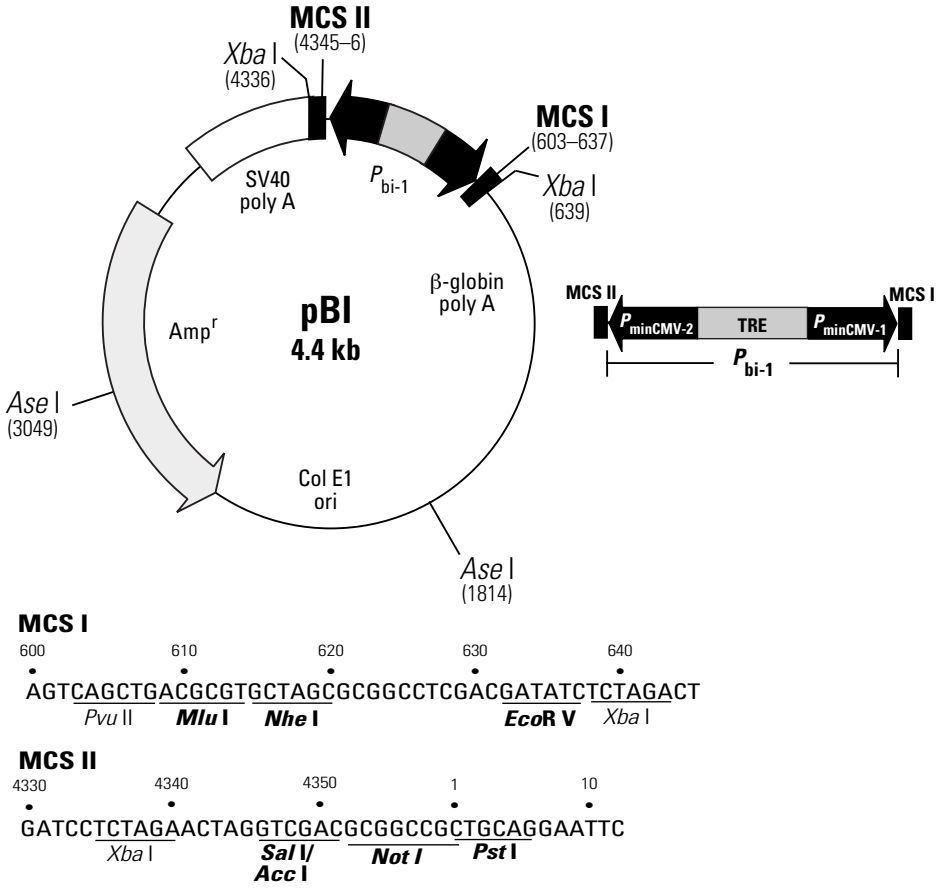
Vector Information

pBI Tet Vector Information PT3070-5

GenBank Accession No.: U89932 Cat. No. 631006



Restriction Map and Multiple Cloning Site (MCS) of pBI Tet Vector. Unique restriction sites areshown in bold.

Description

The pBI Tet Vector is a response plasmid that can be used to express two genes of interest from one bidirectional tet-responsive promoter (P_{bi-1}; 1) in Clontech's Tet-On® and Tet-Off® Gene Expression Systems and Cell Lines (2). The Tet Expression Systems and Cell Lines give researchers ready access to the tetracycline-regulated expression systems described by Gossen & Bujard (3; Tet-Off) and Gossen *et al.* (4; Tet-On). The pBI Tet Vector contains the bidirectional promoter P_{bi-1} which is responsive to the tTA and rtTA regulatory proteins in the Tet-Off and Tet-On systems, respectively. P_{bi-1} contains the Tet-responsive element (TRE), which consists of seven copies of the 42-bp tet operator sequence (*tetO*). The TRE element is between two minimal CMV promoters (P_{minCMV}), which lack the enhancer that is part of the complete CMV promoter. Consequently, P_{bi-1} is silent in the absence of binding of TetR or rTetR to the *tetO* sequences. $P_{minCMV-1}$ and $P_{minCMV-2}$ control the expression of two separate genes of interest. Note that the cloned inserts must have an initiation codon. In some cases, addition of a Kozak consensus ribosome binding site (5) may improve expression levels; however, many cDNAs have been efficiently expressed in Tet systems without the addition of a Kozak sequence.

Use

pBI allows the simultaneous regulation of two genes of interest by one central TRE. After a stable Tet-On or Tet-Off cell line has been established by transfecting with a tTA or rtTA regulator plasmid, pBI is cotransfected with pTK-Hyg (Cat No. 631750) to permit selection of a double-stable cell line which expresses both genes of interest. Alternatively, pPUR (Cat No. 631601) or another selection plasmid can be used. If this plasmid contains an enhancer element, as does pPUR, cointegration

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of pBI and the selection plasmid may lead to higher background expression. Double-stable, tet-responsive cell lines with pBI response constructs can be developed using the protocols described for pTRE response plasmids in the Tet Systems User Manual (PT3001-1).

Location of Features

- Multiple cloning site (MCS II): 4345–6
- P_{bi-1} Bidirectional Tet-responsive promoter: 12–568
  - $P_{minCMV-2}$: 122–12
  - Tet-responsive element (TRE): 128–439
  - $P_{minCMV-1}$: 440–568
- Multiple cloning site (MCS I): 603–637
- Fragment containing the β-Globin poly A signal: 644–1811
- Col E1 origin of replication: 2012–2655
- Ampicillin resistance gene
  - β-lactamase coding sequences: 3663–2803
- Fragment containing the SV40 poly A signal: 4328–3877

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) on *E. coli* hosts.
- *E. coli* replication origin: Col E1

References

1. Baron, U., *et al.* (1995) *Nucleic Acids Res.* **17**:3605–3606.
2. Tet Expression Systems and Cell Lines (July 1996) *Clontechniques* **XI**(3):2–5.
3. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**:5547–5551.
4. Gossen, M., *et al.* (1995) *Science* **268**:1766–1769.
5. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.

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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