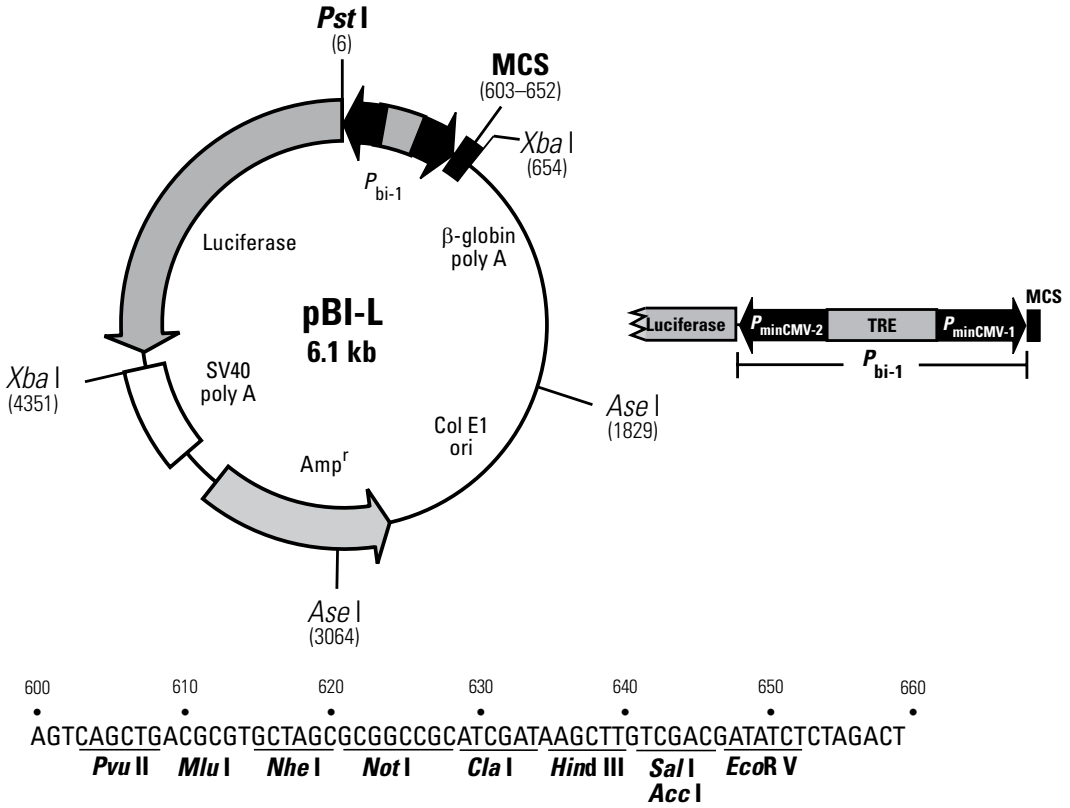
Vector Information

pBI-L Vector Information PT3069-5

GenBank Accession No.: U89934 Catalog No. 631005



Restriction Map and Multiple Cloning Site (MCS) of pBI-L Tet Vector. Unique restriction sites are shown in bold.

Description

The pBI-L Tet Vector is a response plasmid that can be used to express a gene of interest and luciferase from a 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-L 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. $P_{minCMV-1}$ controls the expression of the gene of interest; and $P_{minCMV-2}$ controls the expression of luciferase. Therefore, the expression of a gene of interest for which there is no convenient assay may be monitored indirectly via the luciferase reporter function. Note that the cloned insert 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-L allows the simultaneous regulation of both a gene of interest and luciferase 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-L is cotransfected with pTK-Hyg (Cat. No. 631750) to permit selection of a double-stable cell line which expresses both the gene of interest and the luciferase reporter gene. Alternatively, pPUR (Cat. No. 631601) or another selection plasmid can be used. If this plasmid contains an enhancer element, as does pPUR, cointegration of pBI-L and the selection plasmid may lead to higher background expression. Double-stable, tet-responsive cell lines with the pBI-L response constructs can be developed using the protocols described for pTRE response plasmids in the Tet Systems User Manual (PT3001-1). After the double-stable cell line is established, expression of luciferase can be monitored using any standard assay.

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Location of Features

- 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): 603–652
- Fragment containing the β-Globin poly A signal: 659–1826
- Col E1 origin of replication: 2027–2670
- Ampicillin resistance gene
  β-lactamase coding sequences: 3678–2818
- Fragment containing the SV40 poly A signal: 4343–3892
- Luciferase gene: 6075–4423

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.