Vector Information

pBI-GL Tet Control Vector Information

PT3071-5

GenBank Accession No. U89935

Cat. No. 631004

Cat. No. 631005

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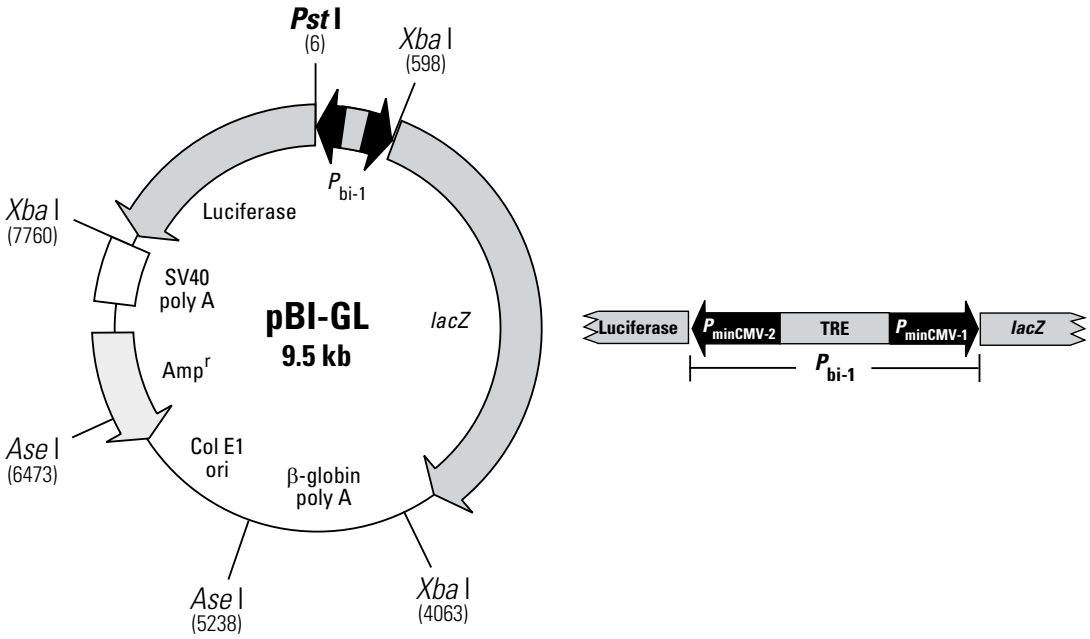

Restriction Map of pBI-GL Tet Control Vector. (Unique restriction sites are in bold).

Description:

The pBI-GL Tet Control Vector is a response plasmid that can be used to express luciferase and β-galactosidase from one bidirectional tet-responsive promoter (P_{bi-1}; 1) in 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-GL Tet Control 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 β-galactosidase; and $P_{minCMV-2}$ controls the expression of luciferase.

Use:

pBI-GL allows the simultaneous regulation of both luciferase and β-galactosidase 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-GL is cotransfected with pTK-Hyg (Cat. No. 631750) to permit selection of a double-stable cell line which expresses both luciferase and β-galactosidase reporter genes. 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 and the selection plasmid may lead to higher background expression. Double-stable, tet-responsive cell lines with pBI-GL 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 and β-galactosidase can be monitored using standard assays.

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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
- *LacZ* gene
  β-galactosidase coding sequence: 650–3804
- Fragment containing the β-Globin poly A signal: 4068–5235
- Col E1 origin of replication: 5436–6079
- Ampicillin resistance gene
  β-lactamase coding sequences: 7087–6227
- Fragment containing the SV40 poly A signal: 7752–7301
- Luciferase gene: 9484–7832

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

Note: We have found errors in the attached sequence; therefore, we are currently sequencing parts of this vector to correct the errors.

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.

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.