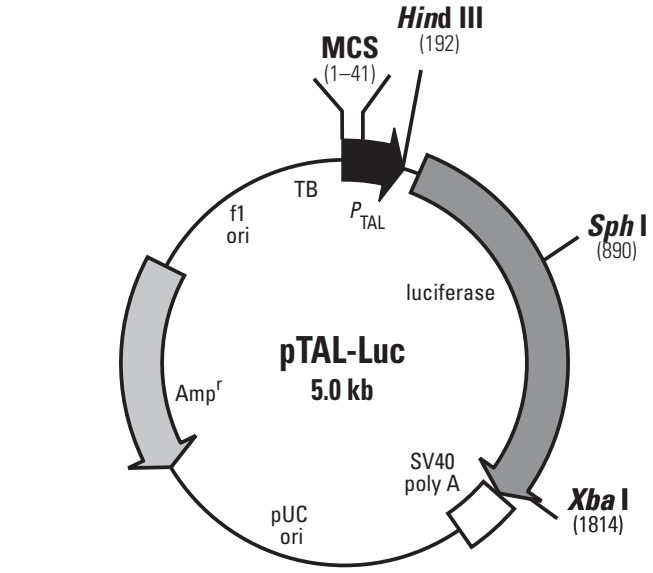
Vector Information

pTAL-Luc Vector Information PT3272-5

GenBank Accession No.: Submission in progress. Catalog No. 631909



Restriction Map and Multiple Cloning Site (MCS) of pTAL-Luc. All sites shown are unique.

Description:
pTAL-Luc is designed for analyzing enhancer sequences by assaying for expression of the firefly luciferase (*luc*) gene from *Photinus pyralis* (1). This vector contains a TATA-like promoter (P_{TAL}) region from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. Putative enhancers can be inserted into one of the MCSs. The luciferase coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the *luc* transcript in eukaryotic cells. Located upstream of the MCS is a synthetic transcription blocker (TB), which is composed of adjacent polyadenylation and transcription pause sites for reducing background transcription (2). The vector backbone also contains an f1 origin for single-stranded DNA production, a pUC origin of replication, and an ampicillin resistance gene for propagation and selection in *E. coli*.

Use:
pTAL-Luc is ideal for use as a negative control or for studying putative enhancers that are inserted upstream of the luciferase reporter gene. Luciferase is a highly sensitive enzymatic reporter that can be assayed by any standard luciferase-detection method, providing quantitative data on induction levels. The pTAL-Luc Vector can be transfected into mammalian cells by any standard method. For selecting stable clones, cotransfect with a vector containing an antibiotic resistance gene, such as neomycin, hygromycin or puromycin.

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(PR94999; published 05 May 1999)

Location of features:

- Multiple Cloning Sites: 1–41
- TATA-like promoter (P_{TAL}): 42–190
- Firefly luciferase (*luc*) gene:
  Start codon (ATG): 226–228; stop codon: 1876–1878
- SV40 late mRNA polyadenylation signal: 2029–2034
  mRNA 3' end: 2048
- pUC plasmid replication origin: 2427–3070
- Ampicillin resistance gene:
  Promoter: –35 region: 4148–4143; –10 region: 4125–4120
  Transcription start point: 4113
  Ribosome binding site: 4090–4086
  β-lactamase coding sequences:
  start codon (ATG): 4078–4076; stop codon: 3220–3218
  β-lactamase signal peptide: 4078–4010
  β-lactamase mature protein: 4009–3221
- f1 single-strand DNA origin (packages the noncoding strand of *luc*): 4210–4665
- Transcription blocker (TB): 4796–4949
  Synthetic polyadenylation site (3): 4796–4844
  Transcription pause site from human α2 globin gene (4): 4858–4949

Recommended sequencing primer:
5' of MCS: 4773–4790 (5'-CGGGAGGTACTTGGAGCG-3')

Propagation in *E. coli*:

- Suitable host strains: DH5α and other general purpose strains. Single-stranded DNA production requires a host containing an F' episome, such as JM109.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

References:

1. De Wet, J. R., *et al.* (1987) *Mol. Cell Biol.* **7**:725–737.
2. Eggermont, J. & Proudfoot, N. (1993) *EMBO J.* **12**:2539–2548.
3. Levitt, N., *et al.* (1989) *Genes Dev.* **3**:1019–1025.
4. Enriquez-Harris, P., *et al.* (1991) *EMBO J.* **10**:1833–1842.