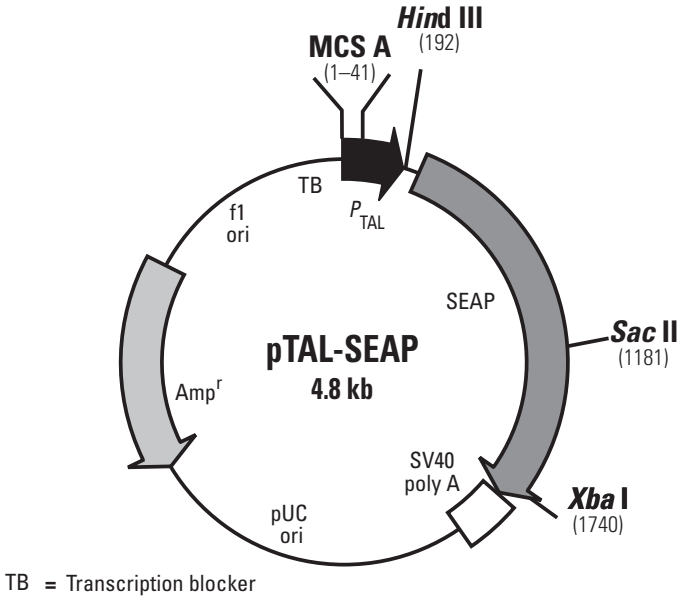
Vector Information

pTAL-SEAP Vector Information PT3271-5

Catalog No. 631908



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

Description

pTAL-SEAP is designed for analyzing enhancer sequences by assaying for expression of the secreted alkaline phosphatase (SEAP) gene. 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 the MCS. The SEAP coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the SEAP 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 (1). 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-SEAP is ideal for use as a negative control or for studying putative enhancers that are inserted upstream of the SEAP reporter gene. The secreted SEAP enzyme is assayed directly from the culture medium using one of Clontech's Great EscAPe™ SEAP Detection Kits (Cat. Nos. 631701, 631704). In addition, the SEAP assay permits time-course studies not possible with assays dependent on cell lysates. The pTAL-SEAP 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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Location of features

- Multiple Cloning Site: 1–41
- TATA-like promoter (P_{TAL}): 42–190
- Secreted alkaline phosphatase (SEAP) gene:
  SEAP coding sequences:
  start codon (ATG): 218–220; stop codon: 1775–1777
  signal peptide: 218–268
  mature protein: 269–1774
  C-terminal extension to SEAP: 1736–1774
- SV40 late mRNA polyadenylation signal: 1888–1893
  mRNA 3' end: 1906
- pUC plasmid replication origin: 2286–2929
- Ampicillin resistance gene:
  Promoter: –35 region: 4012–4007; –10 region: 3984–3979
  Transcription start point: 3972
  Ribosome binding site: 3949–3945
  β-lactamase coding sequences:
  start codon (ATG): 3937–3935; stop codon: 3079–3077
  β-lactamase signal peptide: 3937–3869
  β-lactamase mature protein: 3868–3080
- f1 single-strand DNA origin (packages the noncoding strand of SEAP): 4069–4524
- Transcription blocker (TB): 4655–4808
  Synthetic polyadenylation site (2): 4655–4751
  Transcription pause site from human α2 globin gene (3): 4717–4808

Recommended sequencing primer

5′ of MCS: 4632–4649 (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. Eggermont, J. & Proudfoot, N. (1993) *EMBO J.* **12**:2539–2548.
2. Levitt, N., *et al.* (1989) *Genes Dev.* **3**:1019–1025.
3. Enriquez-Harris, P. *et al.* (1991) *EMBO J.* **10**:1833–1842.