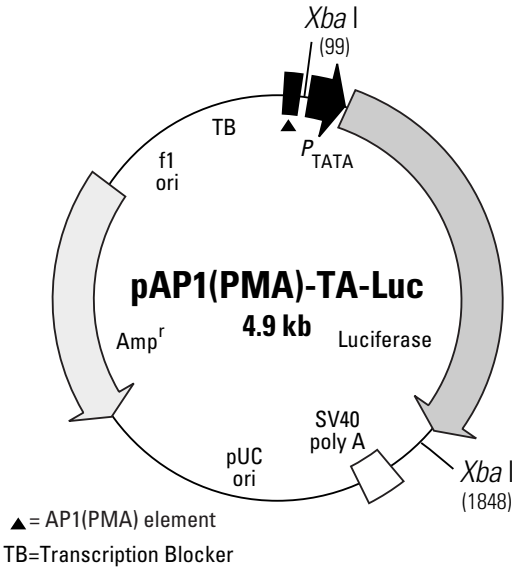
Vector Information

pAP1(PMA)-TA-Luc† Vector Information PT3350-5

GenBank Accession No.: Submission in progress. Cat. No. 631906



Restriction Map of pAP1(PMA)-TA-Luc.

Description:

pAP1(PMA)-TA-Luc is a member of the signal transduction reporter vectors. It is designed for monitoring the induction of the protein kinase C (PKC) signal transduction pathway, as well as related pathways such as the MAPK pathway. pAP1(PMA)-TA-Luc contains the firefly luciferase gene (*luc*) as the reporter. The vector contains multiple copies of the activator protein 1 (AP1) enhancer, located upstream of *luc*, that responds specifically to phorbol ester treatment (1). AP1(PMA) is fused to a minimal TA promoter, the TATA box from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. The luciferase coding sequence is followed by the SV40 late polyadenylation signal to ensure proper, efficient processing of the luciferase transcript in eukaryotic cells. A synthetic transcription blocker (TB) is located upstream of AP1(PMA), which is composed of adjacent polyadenylation and transcription pause sites for blocking nonspecific 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:

Activating the protein kinase C pathway by adding phorbol esters such as PMA results in transcription factors binding the AP1 element on the vector and initiating transcription of the luciferase reporter gene. Firefly luciferase is a highly sensitive enzymatic reporter that can provide quantitative data on induction levels using any standard luciferase assay. The pAP1(PMA)-TA-Luc Vector can be transfected into mammalian cells by any standard method. Stable cell lines expressing this construct can be developed by cotransfecting with a vector containing an antibiotic resistance gene, such as neomycin, hygromycin, or puromycin, and selecting resistant clones.

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(PR661872; published 14 June 2006)

Location of features:

- Activator protein 1 (AP1) element (1): 48–89
- TA minimal promoter: 107–113
- Firefly luciferase gene:
  Luciferase coding sequences:
  start codon (ATG): 193–195; stop codon: 1843–1845
- SV40 late mRNA polyadenylation signal: 1996–2001
  mRNA 3' end: 2015
- pUC plasmid replication origin: 2394–3037
- Ampicillin resistance gene:
  Promoter: –35 region: 4115–4110; –10 region: 4092–4087
  Transcription start point: 4080
  Ribosome binding site: 4057–4053
  β-lactamase coding sequences:
  start codon (ATG): 4045–4043; stop codon: 3187–3185
  β-lactamase signal peptide: 4045–3977
  β-lactamase mature protein: 3976–3188
- f1 single-strand DNA origin (packages the noncoding strand of Luc): 4177–4632
- Transcription blocker (TB): 4763–4916
  Synthetic polyadenylation site (3): 4763–4811
  Transcription pause site from human α2 globin gene (4): 4825–4916

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. Bohmann, D. & Tjian, R. (1989) *Cell* **59**:709–717.
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.

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.