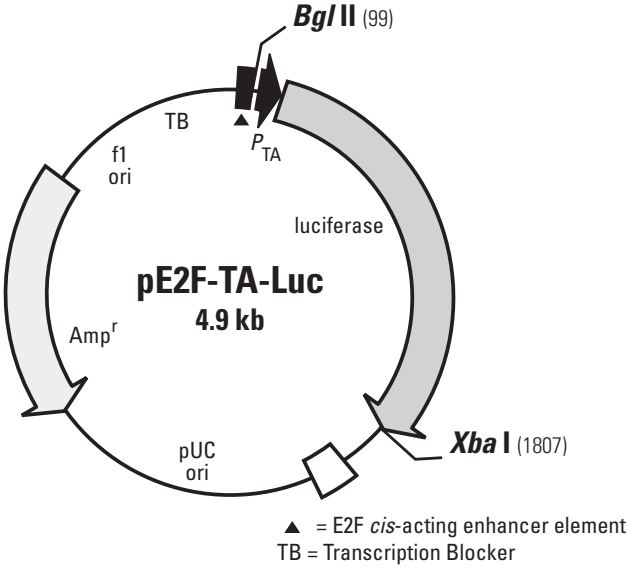
Vector Information

pE2F-TA-Luc Vector Information PT3509-5W

Sold as part of Catalog No. 631914



Restriction Map of pE2F-TA-Luc. All restriction sites shown are unique.

Description

pE2F-TA-Luc is designed to monitor the induction of E2F-mediated signal transduction pathways. E2F, a major target of the retinoblastoma gene (Rb), is a key regulator of cell-cycle checkpoints in mammalian cells (1–2). E2F plays a critical role in stimulating expression of genes encoding growth-promoting proteins (3), and is involved in regulating the expression of important genes during cell proliferation (4–5). The E2F protein forms a heterodimer complex with the DP1 protein, which binds to E2F response elements and initiates transcription of genes necessary for DNA replication. Studies have shown that deregulation of E2F results in a loss of cell-cycle checkpoints—thereby predisposing cells to uncontrolled growth (1–5). pE2F-TA-Luc contains four copies of the E2F enhancer element (6), located upstream of the minimalTA promoter, the TATA box from the herpes simplex virus thymidine kinase promoter (P_{TA}). Located downstream of P_{TA} is the firefly luciferase reporter gene (*luc*). Upon binding of the E2F/DP1 complex to the *cis*-acting E2F enhancer element, transcription is induced and the reporter gene is activated.

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 the *cis*-acting enhancer element. It is composed of adjacent polyadenylation and transcription pause sites for blocking nonspecific transcription (7). 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

pE2F-TA-Luc is designed for monitoring cell-cycle signaling in mammalian cells by assaying for luciferase activity. For example, induction of E2F-mediated signal transduction pathways may be compared across different cell types or cell states by transiently transfecting this vector into appropriate cell lines. After transfection, treat each culture individually with a drug candidate or stimulus of interest, then compare the activation of the E2F response element by assaying for the luciferase reporter gene. Additionally, you can monitor pathway activation by cotransfecting this vector with an expression vector containing a gene of interest. Luciferase is a highly sensitive enzymatic reporter that can be assayed by any standard luciferase-detection method, providing quantitative data on induction levels. pE2F-TA-Luc 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, and selecting resistant clones.

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Location of features

- E2F DNA binding sequence (E2F; 6): 27–97
- TA minimal promoter (P_{TA}): 107–113
- Firefly luciferase gene:
  - Luciferase coding sequences:
    - start codon (ATG): 152–154; stop codon: 1802–1804
- SV40 late mRNA polyadenylation signal: 1955–1960
  - mRNA 3' end: 1974
- pUC plasmid replication origin: 2353–2996
- Ampicillin resistance gene:
  - Promoter: –35 region: 4074–4069; –10 region: 4051–4046
  - Transcription start point: 4039
  - Ribosome binding site: 4016–4011
  - β-lactamase coding sequences:
    - start codon (ATG): 4004–4002; stop codon: 3146–3144
  - β-lactamase signal peptide: 4004–3936
  - β-lactamase mature protein: 3935–3147
- f1 single-strand DNA origin (packages the noncoding strand of *luc*): 4136–4573
- Transcription blocker (TB): 4722–4875
  - Synthetic polyadenylation site (8): 4722–4770
  - Transcription pause site from human α2 globin gene (9): 4784–4875

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

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