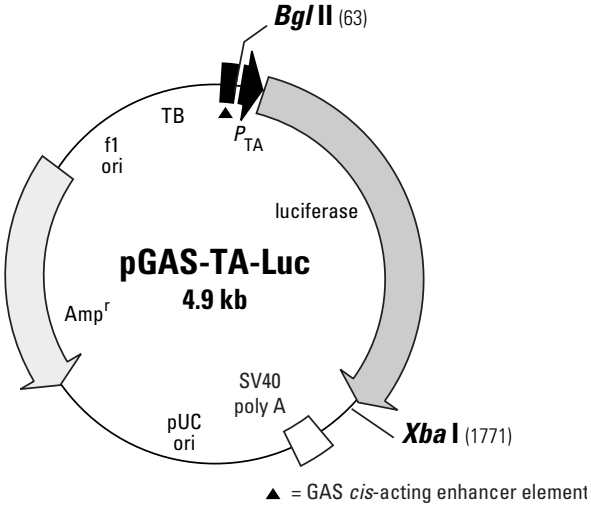

Restriction Map of pGAS-TA-Luc. All restriction sites shown are unique.

Description

pGAS-TA-Luc is designed to monitor the induction of STAT1, a component of JAK/STAT-mediated signal transduction pathways. Cytokines bind and induce receptor dimerization at the cell surface, causing the receptor itself to be phosphorylated. The phosphorylated receptor then acts as a docking site for STAT1. STAT1 is phosphorylated, dimerizes and translocates to the nucleus to regulate transcription (1). pGAS-TA-Luc (interferon-γ activation sequence) contains two copies of the STAT1 enhancer element, located upstream of the minimal TA 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 STAT1 homodimer to the *cis*-acting STAT1 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 (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

pGAS-TA-Luc is designed for monitoring cytokine signaling in certain mammalian cells by assaying for luciferase activity. For example, induction of STAT1-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 activity of the STAT1 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. pGAS-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 select resistant clones.

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Location of features

- Interferon-γ activation sequence (GAS) repeats: 7–41
- TA minimal promoter (P_{TA}): 51–67
- Firefly luciferase gene:
  start codon (ATG): 96–98; stop codon: 1756–1758
- SV40 late mRNA polyadenylation signal: 1909–1914
  mRNA 3' end: 1928
- pUC plasmid replication origin: 2307–2950
- Ampicillin resistance gene:
  Promoter: –35 region: 4028–4023; –10 region: 4005–4000
  Transcription start point: 3993
  Ribosome binding site: 3970–3965
  β-lactamase coding sequences:
  start codon (ATG): 3958–3956; stop codon: 3100–2098
  β-lactamase signal peptide: 3958–3890
  β-lactamase mature protein: 3889–3101
- f1 single-strand DNA origin (packages the noncoding strand of *luc*): 4090–4522
- Transcription blocker (TB): 4676–4829
  Synthetic polyadenylation site (8): 4676–4724
  Transcription pause site from human α2 globin gene (9): 4738–4829

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. Darnell, J.E. (1997) *Science* **277**: 1630–1635.
2. Eggermont, J. & Proudfoot, N. (1993) *EMBO J.* **12**:2539–2548.

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 Laboratories, Inc. This vector has not been completely sequenced.