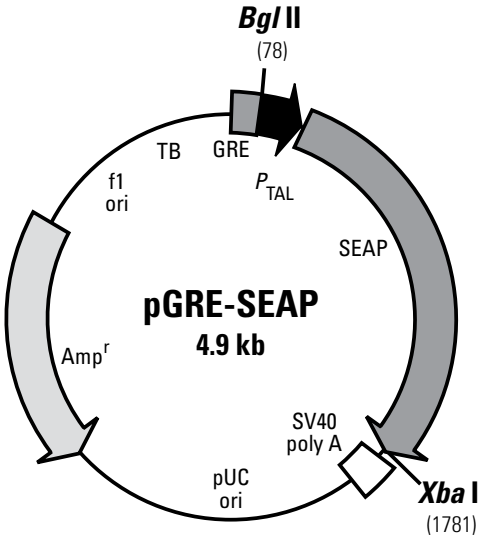
Vector Information

pGRE-SEAP Vector Information PT3278-5

GenBank Accession No.: Submission in progress.



TB=Transcription Blocker

Restriction Map of pGRE-SEAP. All restriction sites are unique.

Description:

pGRE-SEAP is designed for monitoring the induction of the glucocorticoid response element (GRE; 1–2) and the glucocorticoid-mediated signaling transduction pathway. pGRE-SEAP contains the secreted alkaline phosphatase (SEAP) reporter gene (3–5). This vector also contains multiple copies of the GRE consensus sequence fused to a TATA-like promoter (P_{TAL}) region from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. After transcription factors bind GRE, transcription is induced and the reporter gene is activated.

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 GRE is a synthetic transcription blocker (TB), which is composed of adjacent polyadenylation and transcription pause sites for reducing background transcription (6). 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:

pGRE-SEAP is designed to measure the binding of transcription factors to GRE, providing a direct measurement of activation for this pathway. For example, the addition of glucocorticoids to the cell-culture medium induces the binding of transcription factors to GRE, which initiates transcription of SEAP. Alternatively, you can cotransfect this vector with an expression vector containing your gene of interest to monitor pathway activation. The secreted SEAP enzyme can be assayed directly from the culture medium using one of Clontech's Great EscAPe Chemiluminescence Detection Kits (Cat. Nos. 631701, 631704). In addition, the SEAP assay permits time-course studies not possible with assays dependent on cell lysates. The pGRE-SEAP Vectors 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.

(PR7X2386; published 18 October 2007)

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Location of features:

- Glucocorticoid Response Element (GRE; 1): 27–71
- TATA-like promoter (P_{TAL}): 78–226
- Secreted alkaline phosphatase (SEAP) gene:
  - SEAP coding sequences:
    - start codon (ATG):254–256; stop codon: 1811–1813
    - signal peptide: 254–304
    - mature protein: 305–1810
    - C-terminal extension to SEAP: 1772–1810
- SV40 late mRNA polyadenylation signal: 1924–1929
  - mRNA 3' end: 1943
- pUC plasmid replication origin: 2322–2965
- Ampicillin resistance gene:
  - Promoter: –35 region: 4043–4048; –10 region: 4020–4015
  - Transcription start point: 4008
  - Ribosome binding site: 3985–3981
  - β-lactamase coding sequences:
    - start codon (ATG): 3973–3971; stop codon: 3115–3113
  - β-lactamase signal peptide: 3973–3905
  - β-lactamase mature protein: 3904–3116
- f1 single-strand DNA origin (packages the noncoding strand of SEAP): 4105–4560
- Transcription blocker (TB): 4691–4844
  - Synthetic polyadenylation site (7): 4691–4739
  - Transcription pause site from human α2 globin gene (8): 4753–4944

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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