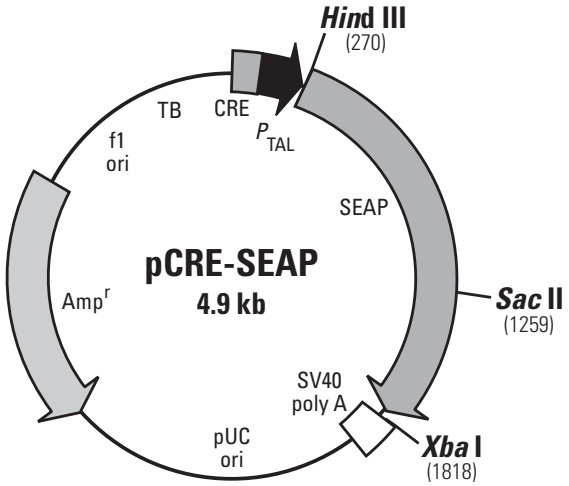
Vector Information

pCRE-SEAP Vector Information PT3288-5

GenBank Accession No.: Submission in progress. Sold as part of Cat. No. 631910



TB=Transcription Blocker

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

Description:

pCRE-SEAP is designed to monitor the activation of cAMP binding protein (CREB) and cAMP-mediated signal transduction pathways. Several signal transduction pathways are associated with the cAMP response element (CRE; 1), including Jun N-terminal kinase (JNK), p38, and protein kinase A (PKA; 2–3). Induction of these pathways enables endogenous transcription factors such as CREB or ATF to bind CRE. pCRE-SEAP contains the secreted alkaline phosphatase (SEAP) reporter gene (4–6). This vector also contains three copies of the CRE-binding sequence fused to a TATA-like promoter (P_{TAL}) region from the Herpes simplex virus thymidine kinase (HSV-TK) promoter. After transcription factors bind CRE, 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 CRE is a synthetic transcription blocker (TB), which is composed of adjacent polyadenylation and transcription pause sites for reducing background 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:

pCRE-SEAP is designed to measure the binding of transcription factors to CRE, providing a direct measurement of activation for this pathway. For example, treatment of cells with cAMP or forskolin activates CREB or ATF to bind CRE, thus initiating 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 pCRE-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.

(PR93952; published 05 May 1999)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Location of features:

- cAMP response element (CRE; 1): 27–113
- TATA-like promoter (P_{TAL}): 120–268
- Secreted alkaline phosphatase (SEAP) gene:
  - SEAP coding sequences:
    - start codon (ATG): 296–298; stop codon: 1853–1855
    - signal peptide: 296–346
    - mature protein: 347–1852
    - C-terminal extension to SEAP: 1814–1852
- SV40 late mRNA polyadenylation signal: 1966–1971
  - mRNA 3' end: 1985
- pUC plasmid replication origin: 2364–3007
- Ampicillin resistance gene:
  - Promoter: –35 region: 4085–4080; –10 region: 4062–4057
  - Transcription start point: 4050
  - Ribosome binding site: 4027–4023
  - β-lactamase coding sequences:
    - start codon (ATG): 4015–4013; stop codon: 3157–3155
  - β-lactamase signal peptide: 4015–3947
  - β-lactamase mature protein: 3946–3158
- f1 single-strand DNA origin (packages the noncoding strand of SEAP): 4147–4602
- Transcription blocker (TB): 4733–4886
  - Synthetic polyadenylation site (8): 4733–4781
  - Transcription pause site from human α2 globin gene (9): 4795–4886

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. Himmler, A., *et al.* (1993) *J. Recep. Research* **13**:79–94.
2. Roesler, W. J., *et al.* (1988) *J. Biol. Chem.* **263**:9063–9066.
3. Montminy, M. R. & Bilezikjian, L. M. (1987) *Nature* **328**:175–178.
4. Yang, T. T., *et al.* (July 1994) *Clontechniques* **IX**(3):1–5.
5. Berger, J., *et al.* (1988) *Gene* **66**:1–10.
6. Cullen, B. R. & Malim, M. H. (1992) *Methods Enzymol.* **216**:362–368.
7. Eggermont, J. & Proudfoot, N. (1993) *EMBO J.* **12**:2539–2548.
8. Levitt, N., *et al.* (1989) *Genes Dev.* **3**:1019–1025.
9. Enriquez-Harris, P., *et al.* (1991) *EMBO J.* **10**:1833–1842.