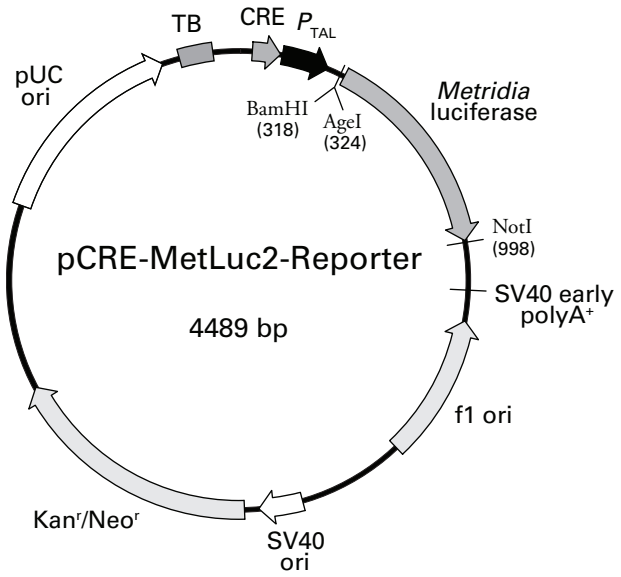
Vector Information

pCRE-MetLuc2-Reporter Vector Information PT4070-5

Catalog No. 631745



pCRE-MetLuc2-Reporter Vector Map. All restriction sites shown are unique.

Description

pCRE-MetLuc2-Reporter allows you to monitor cAMP-mediated signal transduction directly from the cell culture medium, without cell lysis. The vector contains two copies of the cAMP response element (CRE; 1) fused to a TATA-like promoter (P_{TAL}) region from the herpes simplex virus thymidine kinase (HSV-TK) gene. Located downstream of P_{TAL} is a human codon optimized secreted-luciferase gene from the marine copepod *Metridia longa*. This gene encodes a 219 amino acid (24 kDa) polypeptide that includes a 17 amino acid N-terminal signal peptide necessary for secretion. Binding of transcription factors, such as the cAMP response element binding protein (CREB), to the CRE sequence allows *Metridia* luciferase (MetLuc) to be expressed and secreted into the surrounding medium (2).

To prevent read-through transcription of the *Metridia* luciferase gene, a synthetic transcription blocker (TB; 3, 4), composed of adjacent polyadenylation and transcription pause sites, is located upstream of the CRE. To ensure efficient processing of the luciferase transcript in eukaryotic cells, an SV40 early polyadenylation signal is located downstream of the *Metridia* luciferase coding sequence. The vector backbone also contains an f1 origin for single-stranded DNA production, a pUC origin of replication, and a kanamycin resistance gene for propagation and selection in *E. coli*.

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Use

pCRE-MetLuc2-Reporter is available as part of the Ready-To-Glow™ CRE Secreted Luciferase Reporter System (Cat. No. 631745). In mammalian cells containing pCRE-MetLuc2-Reporter, the addition of cAMP, or forskolin to the culture medium induces transcription factors, such as CREB or activating transcription factor (ATF) proteins, to bind to the CRE, thereby initiating transcription of the secreted luciferase reporter gene. Activation of cAMP-mediated signal transduction can be monitored simply by sampling the culture medium. To monitor the effect of a specific protein on the system, pCRE-MetLuc2-Reporter can be cotransfected with an expression vector containing the gene of interest. pCRE-MetLuc2-Reporter can be transfected into mammalian cells using any standard transfection method. Stable transfectants can be selected using G418 when required.

Location of features:

- CRE (cAMP response element; 1): 37–123
- P_{TAL} (TATA-like promoter): 130–278
- *Metridia longa* secreted luciferase (human codon optimized):
  Start codon (ATG): 336–338; stop codon: 993–995
- SV40 early mRNA polyadenylation signal: 1148–1153
  mRNA 3' end: 1186
- f1 origin of replication: 1245-1700 (complimentary)
- SV40 origin of replication: 2041-2179
- Kan^r/Neo^r (kanamycin/neomycin resistance gene):
  Neomycin phosphotransferase coding sequence
  Start codon (ATG): 2225–2227; stop codon: 3017–3019
- pUC origin of replication: 3604–4247
- TB (transcription blocker; 3, 4): 4339–4430

Propagation in *E. coli*:

- Suitable host strains: DH5α™, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F' episome, such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Plasmid incompatibility group: pMB1/Col E1

References:

1. Himmler, A., *et al.* (1993) *J. Recep. Res.* **13**(1–4):79–94.
2. Markova, S. V., *et al.* (2004) *J. Biol. Chem.* **279**(5):3212–3217.
3. Eggermont, J. & Proudfoot, N. (1993) *EMBO J.* **12**(6):2539–2548.
4. Enriquez-Harris, P., *et al.* (1991) *EMBO J.* **10**(7):1833–1842.

Note:
The vector 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.

Markova, S. V., Golz, S., Frank, L. A., Kalthof, B. & Vysotski, E. S. (2004) Cloning and expression of cDNA for a luciferase from the marine copepod *Metridia longa*. A novel secreted bioluminescent reporter enzyme. *J. Biol. Chem.* **279**(5):3212–3117.