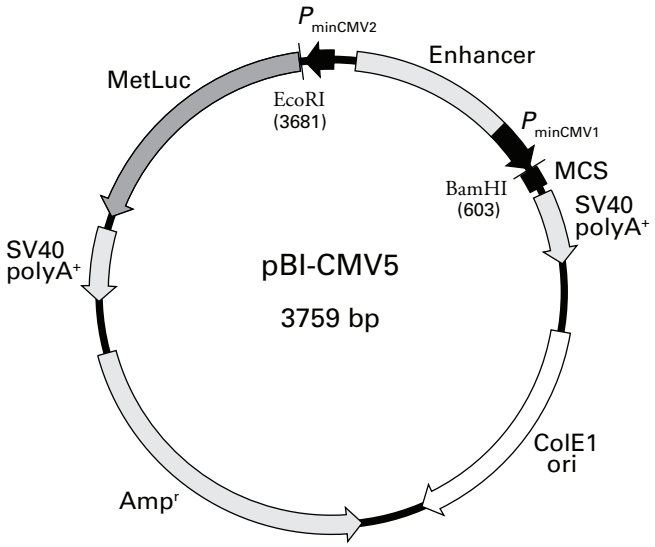
Vector Information

pBI-CMV5 Vector Information PT4444-5

Catalog No. 631757



NheI HindIII
NotI SalI
BamHI MluI EagI ClaI EcoRV

```
601  GGGATCCTCT AGTCAGCTGA CGCGTGCTAG CGCGGCCGCA TCGATAAGCT TGTCGACGAT
     CCCTAGGAGA TCAGTCGACT GCGCACGATC GCGCCGGCGT AGCTATTCGA ACAGCTGCTA
```

EcoRV

```
661  ATCTCCAGAG
     TAGAGGTCTC
```

pBI-CMV5 Vector Map and Multiple Cloning Site.

Description

pBI-CMV5 is a mammalian bidirectional expression vector designed to constitutively express a protein of interest and *Metridia* luciferase (MetLuc), a secreted reporter protein that can be easily detected in the medium surrounding the cells.

Metridia luciferase is a naturally secreted luciferase from the marine copepod *Metridia longa*. The human codon-optimized *Metridia* luciferase gene encodes a 219 amino acid (24 kDa) polypeptide that includes a 17 amino acid N-terminal signal peptide necessary for secretion (1). *Metridia* luciferase is expressed and secreted into the surrounding medium, where it is easily detected by the addition of a chemiluminescent substrate.

Protein expression is driven by one of two constitutively active, minimal human cytomegalovirus promoters: $P_{minCMV1}$ (located upstream of the multiple cloning site [MCS]), drives the expression of the protein of interest, and $P_{minCMV2}$ drives the expression of MetLuc. To allow propagation and selection in *E. coli*, the vector contains a ColE1 origin of replication and an ampicillin resistance gene (Amp^r).

(PR963275; published 17 June 2009)

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

Use

The pBI-CMV5 vector, available as part of the Bidirectional Secreted Luciferase System (Cat. No. 631757), is designed to constitutively express a protein of interest and *Metridia* luciferase. The gene of interest must contain an initiation codon and a stop codon.

pBI-CMV5 can be transfected into mammalian cells using any standard transfection method. Cells expressing the secreted *Metridia* luciferase can be detected by adding chemiluminescent substrate (provided) to a sample of the cell medium.

Location of features

- Enhancer: 64–473
- $P_{minCMV1}$ (minimal human cytomegalovirus promoter 1): 474–599
- MCS (multiple cloning site): 602–663
- SV40 polyA signals: 675–862
- ColE1 origin of replication: 1038–1637
- Amp^r (ampicillin resistance gene): 1799–2659 (complementary)
- SV40 polyA signals: 2795–2982 (complementary)
- MetLuc (*Metridia* luciferase): 3017–3676
- $P_{minCMV2}$ (minimal human cytomegalovirus promoter 2): 3691–3759

Propagation in *E. coli*

- Recommended host strain: DH5α™ and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: ColE1
- Copy number: low
- Plasmid incompatibility group: pMB1/ColE1

References

1. Markova, S.V. *et al.* (2004) *J. Bio. Chem.* **279**(5):3212-3217.

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

Metridia luciferase
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.