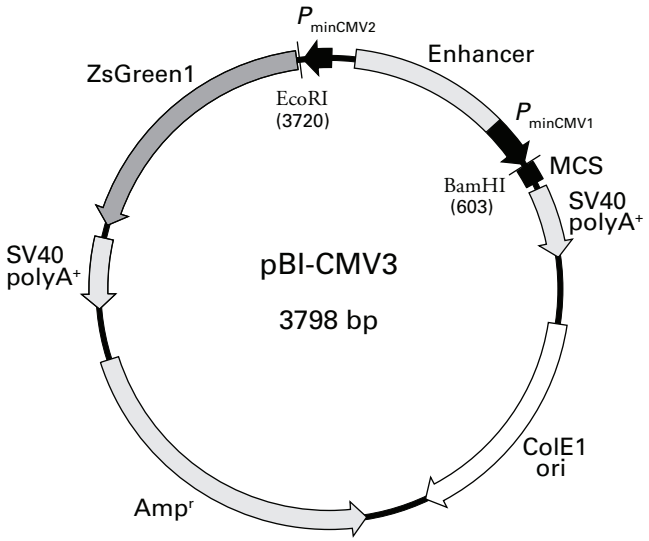
Vector Information

pBI-CMV3 Vector Information

PT4442-5

Catalog No. 631632



NheI
PvuII
NotI
HindIII
BamHI
MluI
EagI
ClaI
EcoRV

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

     EcoRV
661  ATCTCCAGAG
     TAGAGGTCTC
```

pBI-CMV3 Vector Map and Multiple Cloning Site.

Description

pBI-CMV3 is a mammalian bidirectional expression vector designed to constitutively express a protein of interest and ZsGreen1, a human codon-optimized variant of the reef coral *Zoanthus sp.* green fluorescent protein (ZsGreen) that has been engineered for brighter fluorescence (1, 2). The vector allows straightforward detection of transfected mammalian cells by flow cytometry or fluorescence microscopy, as cells expressing the protein of interest can be quickly identified by screening for ZsGreen1 fluorescence.

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 ZsGreen1. To allow propagation and selection in *E. coli*, the vector contains a ColE1 origin of replication and an ampicillin resistance gene (Amp^r).

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

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Use

pBI-CMV3 is designed to constitutively express a protein of interest and the green fluorescent protein ZsGreen1. The gene of interest must contain an initiation codon and a stop codon.

pBI-CMV3 can be transfected into mammalian cells using any standard transfection method. Cells expressing ZsGreen1 (excitation and emission maxima: 493 and 505, respectively) can be detected by flow cytometry or fluorescence microscopy 8–12 hr after transfection. ZsGreen1 can be detected with standard FITC filter sets.

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)
- ZsGreen1 (human codon optimized): 3017–3712
- $P_{minCMV2}$ (minimal human cytomegalovirus promoter 2): 3730–3798

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
- Plasmid incompatibility group: pMB1/ColE1

References

1. Matz, M. V. *et al.* (1999) *Nature Biotech.* **17**(10):969–973.
2. Haas, J. *et al.* (1996) *Curr. Biol.* **6**(3):315-324

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.

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DH5α™ is a trademark of Invitrogen Corporation.

The RCFPs (including DsRed-Express and DsRed-Express2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.

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