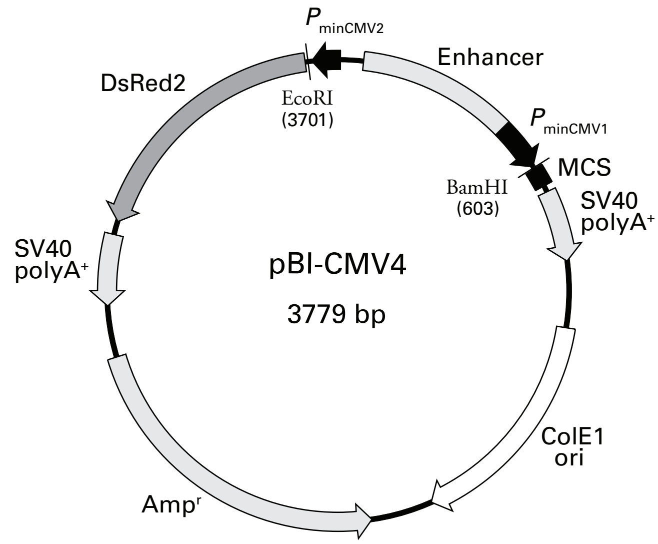
pBI-CMV4 Vector Information PT4443-5

Catalog No. 631633

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                                     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-CMV4 Vector Map and Multiple Cloning Site.

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Description

pBI-CMV4 is a mammalian bidirectional expression vector designed to constitutively express a protein of interest and DsRed2, a human codon-optimized variant of the *Discosoma sp.* red fluorescent protein, DsRed (1), that has been engineered for faster maturation and lower non-specific aggregation. 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 DsRed2 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 DsRed2. To allow propagation and selection in *E. coli*, the vector contains a ColE1 origin of replication and an ampicillin resistance gene (Amp^r).

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Use

pBI-CMV4 is designed to constitutively express a protein of interest and the red fluorescent protein DsRed2. The gene of interest must contain an initiation codon and a stop codon.

pBI-CMV4 can be transfected into mammalian cells using any standard transfection method. Cells expressing DsRed2 (excitation and emission maxima: 558 and 583, respectively) can be detected by flow cytometry or fluorescence microscopy 24 hr after transfection. However, in some cases, up to 48 hr may be required for detection of red-emitting cells.

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)
- DsRed2 (human codon optimized): 3017–3693
- $P_{minCMV2}$ (minimal human cytomegalovirus promoter 2): 3711–3779

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) *Nat. Biotechnol.* **17**(10):969-973.

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.