

			Nhe	NheI			HindIII		
					NotI			SalI	
	BamHI	_	MluI		EagI	ClaI		E	coRV
601	GGGATCCTCT	AGTCAGCTGA	CGCGTGCTAG	CGC	GGCCGCA	TCGATA	AGCT	TGTCGAC	GAT
	CCCTAGGAGA	TCAGTCGACT	GCGCACGATO	GCG	CCGGCGT	AGCTAT	TCGA	ACAGCTG	CTA
	EcoRV								
661	ATCTCCAGAG								
	TAGAGGTCTC								

pBI-CMV2 Vector Map and Multiple Cloning Site.



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### Description

pBI-CMV2 is a mammalian bidirectional expression vector designed to constitutively express a protein of interest and AcGFP1, a green fluorescent protein derived from *Aequorea coerulescens*. 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 AcGFP1 fluorescence.

Protein expression is driven by one of two constitutively active, minimal human cytomegalovirus promoters:  $P_{\text{minCMV1}}$  (located upstream of the multiple cloning site [MCS]), drives the expression of the protein of interest, and  $P_{\text{minCMV2}}$  drives the expression of AcGFP1. To allow propagation and selection in *E. coli*, the vector contains a ColE1 origin of replication and an ampicillin resistance gene (Amp<sup>r</sup>).

(PR093640; published 3 September 2010)

pBI-CMV2 Vector Information

#### Use

pBI-CMV2 is designed to constitutively express a protein of interest and the green fluorescent protein AcGFP1. The gene of interest must contain an initiation codon and a stop codon.

pBI-CMV2 can be transfected into mammalian cells using any standard transfection method. Cells expressing AcGFP1 (excitation and emission maxima: 475 and 505, respectively) can be detected by flow cytometry or fluorescence microscopy 8–12 hr after transfection. AcGFP1 can be detected with standard FITC filter sets.

### Location of features

- Enhancer: 64–473
- P<sub>minCMV1</sub> (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<sup>r</sup> (ampicillin resistance gene): 1799–2659 (complementary)
- SV40 polyA signals: 2795–2982 (complementary)
- AcGFP1 (human codon optimized): 3017–3736
- P<sub>minCMV2</sub> (minimal human cytomegalovirus promoter 2): 3754–3822

# Propagation in E. coli

- Recommended host strain: DH5 $\alpha^{TM}$  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

**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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AcGFP is covered by U.S. Patent No. 7,432,053.

Living Colors Fluorescent Protein Products:

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