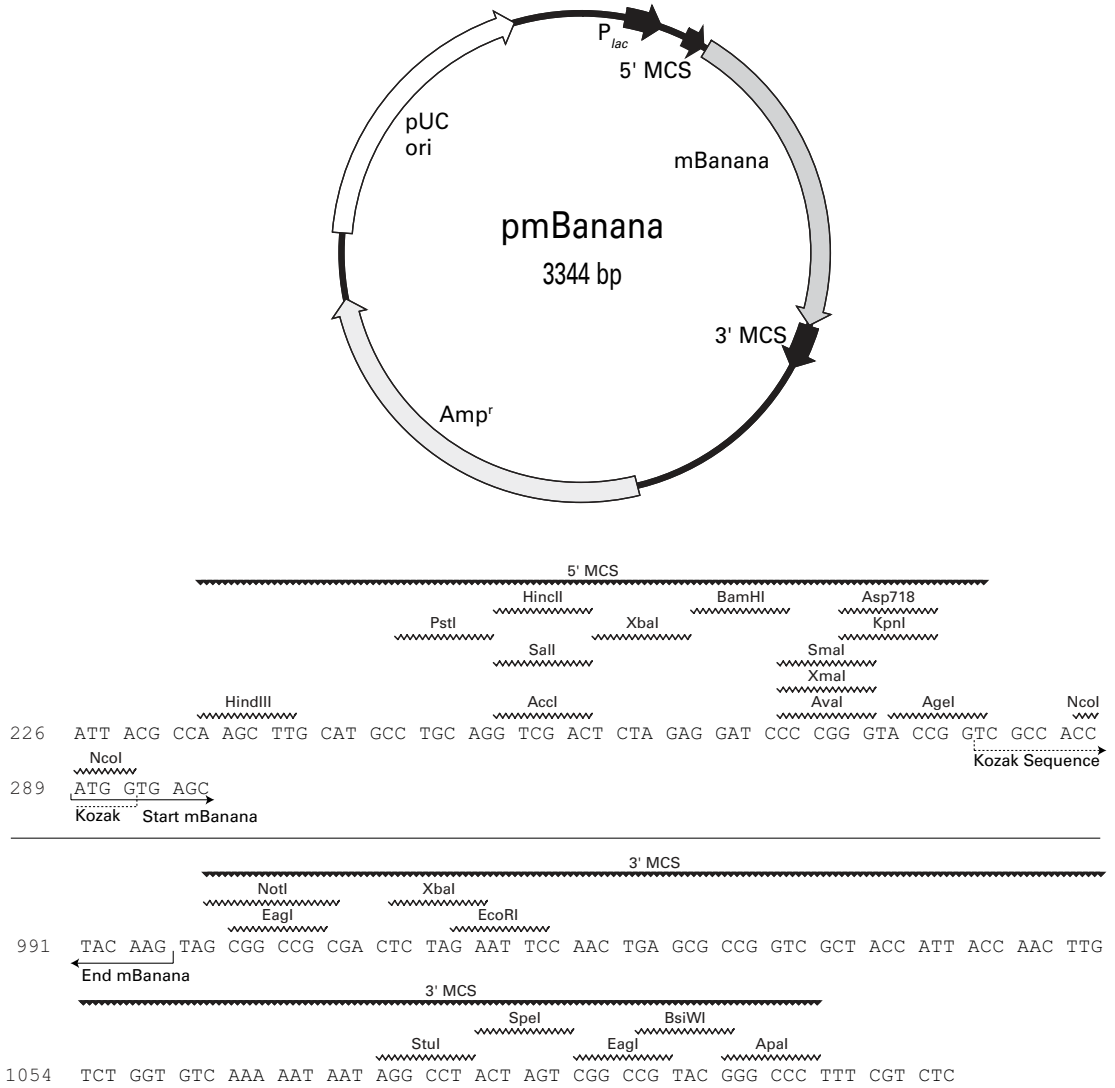
Vector Information

pmBanana Vector Information

PT3979-5

Cat. No. 632528



Restriction Map and Multiple Cloning Sites (MCS) of pmBanana.

Description

pmBanana is a prokaryotic expression vector that encodes mBanana, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The excitation and emission maxima are 540 nm and 553 nm, respectively. The mBanana coding sequence has been human codon-optimized for high-expression in mammalian cells (2).

In pmBanana, the mBanana coding sequence is flanked on each side by separate and distinct multiple cloning sites (MCS), making it easy to excise the gene for use in other cloning applications. Alternatively, the mBanana coding sequence can be amplified by PCR. In *E. coli*, mBanana is expressed from the *lac* promoter as a fusion with several amino acids, including the first five amino acids of the *LacZ* protein. Note, however, that if you excise the mBanana coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode only the mBanana protein (i.e., without the additional amino acids that are expressed using the *lac* promoter). A Kozak consensus sequence is located immediately upstream of the mBanana gene to enhance translational efficiency in eukaryotic systems (3). In the pmBanana vector, the entire mBanana expression cassette is supported by a pUC19 backbone, which contains a high copy-number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

(published September 2011)

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
pmBanana is primarily intended to serve as a source of mBanana cDNA. The flanking MCS regions make it possible to excise the mBanana coding sequence and insert it into other vector systems. The pmBanana vector can also be used to express the mBanana protein in bacteria.

For Western blotting, either the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) or the DsRed Monoclonal Antibody (Cat. Nos. 632392 and 632393) can be used to detect the mBanana protein.

Location of Features
- *lac* Promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - *lac* operator: 179–199
- *lacZ*-mBanana fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon 996–999
- 5' Multiple Cloning Site: 234–281
- Human codon-optimized mBanana gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 996–999
- 3' Multiple cloning site: 999–1098
- Ampicillin resistance gene
  - Promoter
    - –35 region: 1472–1477; –10 region: 1495–1500
  - Ribosome binding site: 1530-1534
  - β-lactamase coding sequences
    - Start codon (ATG): 1544–1546; Stop codon: 2402–2404
    - β-lactamase signal peptide: 1544–1612
    - β-lactamase mature protein: 1613–2401
- pUC plasmid replication origin: 2552–3194

Propagation in *E. coli*
- Recommended host strain: DH5α
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) in *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of mBanana
- Excitation maximum = 540 nm
- Emission maximum = 553 nm

References
1. Shaner, N. C., *et al.* (2004) *Nature Biotech.* **22**(12):1567-1572.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**(3):315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**(20):8125–8148.

Note: The attached 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.

Clontech is pleased to be able to offer researchers the Fruit Fluorescent Proteins that were developed in the laboratory of Dr. Roger Tsien at the University of California, San Diego. The Tsien group has published extensively on the characteristics and uses of these exciting products, and Clontech can provide you with a bibliography if you have any questions regarding their performance, structure, or applications. Clontech has not repeated the experiments conducted by the Tsien group. The genes, encoding the different proteins, are available in a bacterial source vector format.

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