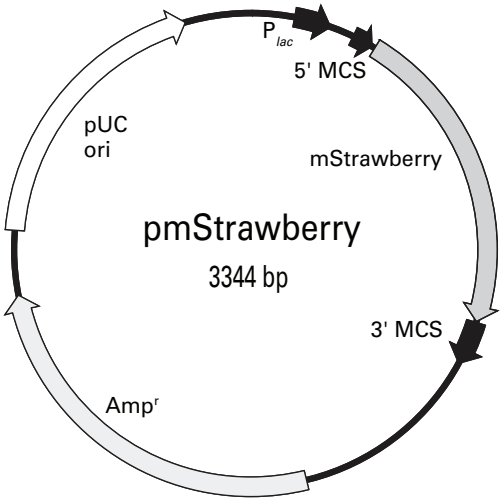
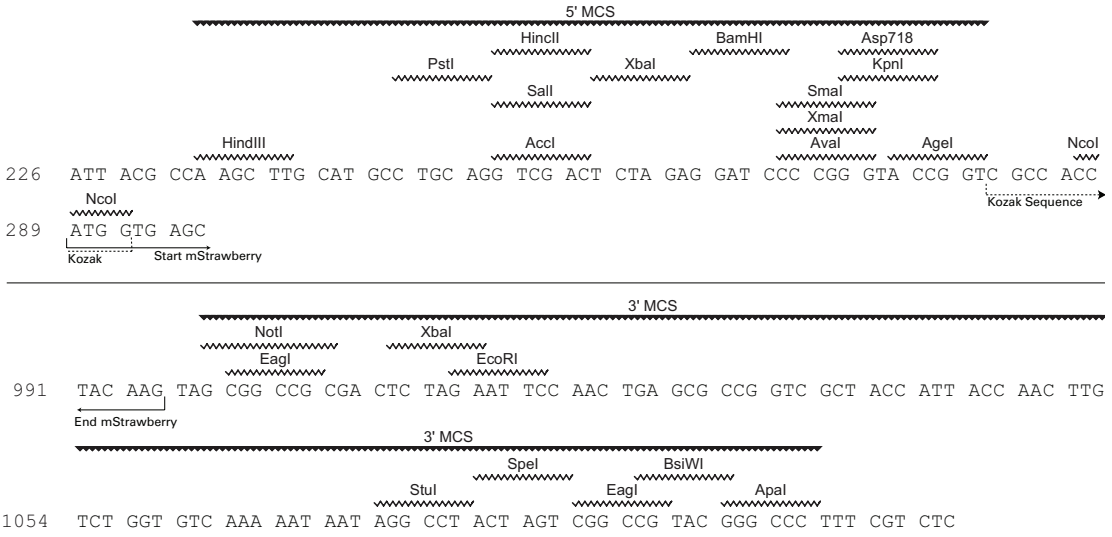
Vector Information

pmStrawberry Vector Information PT3971-5

Cat. No. 632530





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

Description

pmStrawberry is a prokaryotic expression vector that encodes mStrawberry, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The excitation and emission maxima are 574 nm and 596 nm, respectively. The mStrawberry coding sequence has been human codon-optimized for high-level expression in mammalian cells (2).

In pmStrawberry, the mStrawberry 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 mStrawberry coding sequence can be amplified by PCR. In *E. coli*, mStrawberry 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 mStrawberry coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode only the mStrawberry 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 mStrawberry gene to enhance translational efficiency

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in eukaryotic systems (3). In the pmStrawberry vector, the entire mStrawberry 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*.

Use
pmStrawberry is primarily intended to serve as a source of mStrawberry cDNA. The flanking MCS regions make it possible to excise the mStrawberry coding sequence and insert it into other vector systems. The vector can also be used to express the mStrawberry 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 mStrawberry 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*-mStrawberry 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 mStrawberry 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 mStrawberry
- Excitation maximum = 574 nm
- Emission maximum = 596 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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The CMV promoter is covered under U.S. Patent Nos. 5,168,062, and 5,385,839 assigned to the University of Iowa Research Foundation.

DsRed-Express: Patent Pending.

Fruit Fluorescent Protein Products pmCherry, pmRaspberry, pmPlum, pmBanana, pmOrange, pmStrawberry and their variants:

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