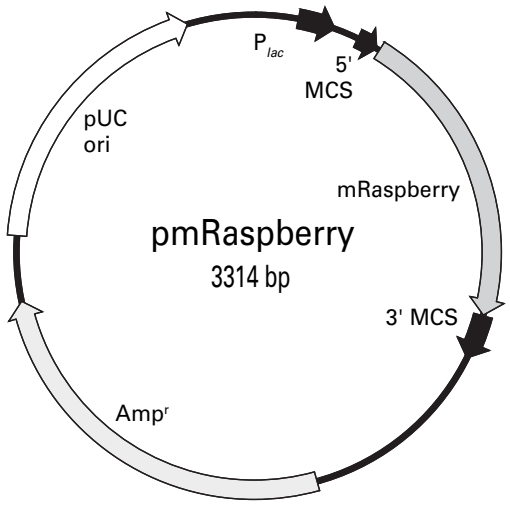
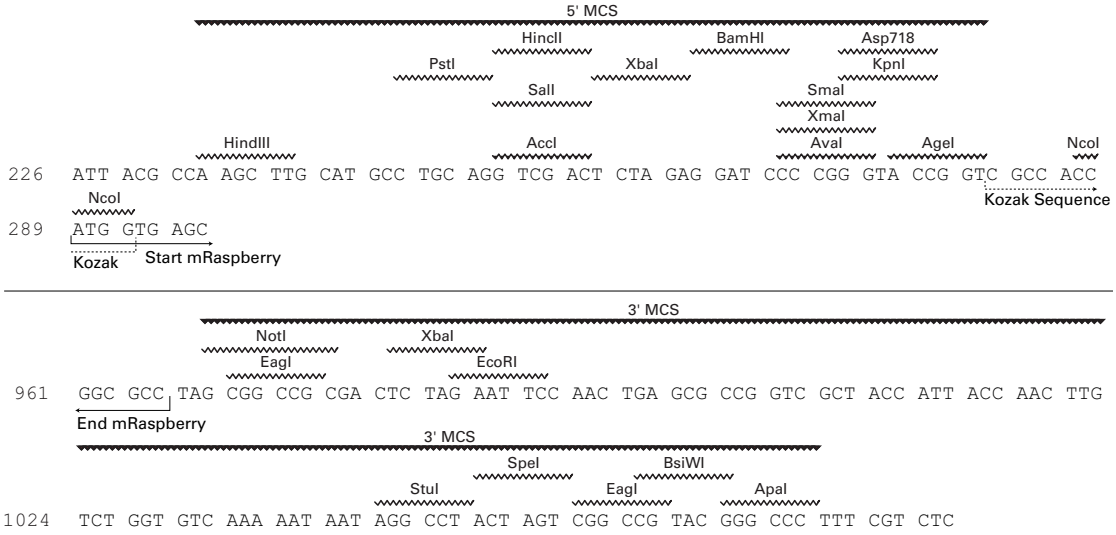
Vector Information

pmRaspberry Vector Information PT3977-5

Cat. No. 632526





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

Description

pmRaspberry is a prokaryotic expression vector that encodes mRaspberry, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The excitation and emission maxima are 598 nm and 625 nm, respectively. The mRaspberry coding sequence has been human codon-optimized for high-level expression in mammalian cells (2).

In pmRaspberry, the mRaspberry 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 mRaspberry coding sequence can be amplified by PCR. In *E. coli*, mRaspberry 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 mRaspberry coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode only the mRaspberry protein (i.e., without the additional amino acids that are expressed using the *lac* promoter). A Kozak consensus sequence

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is located immediately upstream of the mRaspberry gene to enhance translational efficiency in eukaryotic systems (3). In the pmRaspberry vector, the entire mRaspberry 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
pmRaspberry is primarily intended to serve as a source of mRaspberry cDNA. The flanking MCS regions make it possible to excise the mRaspberry coding sequence and insert it into other vector systems. The vector can also be used to express mRaspberry 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 mRaspberry 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*-mRaspberry fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon 964–966
- 5' Multiple Cloning Site: 234–281
- Human codon-optimized mRaspberry gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 964–966
- 3' Multiple cloning site: 969–1068
- Ampicillin resistance gene
  - Promoter
    - –35 region: 1442–1447; –10 region: 1465–1470
  - Ribosome binding site: 1500-1504
  - β-lactamase coding sequences
    - Start codon (ATG): 1514–1516; Stop codon: 2372–2374
    - β-lactamase signal peptide: 1514–1582
    - β-lactamase mature protein: 1583–2371
- pUC plasmid replication origin: 2522–3164

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 mRaspberry
- Excitation maximum = 598 nm
- Emission maximum = 625 nm

References
1. Wang, L., *et al.* (2004) *PNAS.* **101**(45):16745-16749.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**(3):315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**(25):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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