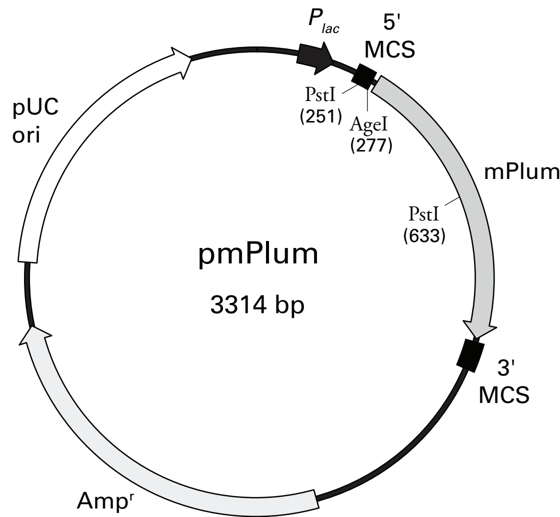
pmPlum Vector Information

PT3978-5

Cat. No. 632527



5' MCS

```
                                                               SmaI
                                                               XmaI
                                                               Asp718
                SphI           SalI                            KpnI            Start
     HindIII                   AccI          BamHI                  AgeI       mPlum →
231  GCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGGATCCC CGGGTACCGG TCGCCACCAT GG
     CGGTTCGAAC GTACGGACGT CCAGCTGAGA TCTCCTAGGG GCCCATGGCC AGCGGTGGTA CC
                                                                 Kozak Sequence
```

3' MCS

```
     End
     mPlum        NotI          EcoRI
961  GGCGCCTAGC GGCCGCGACT CTAGAATTCC AACTGAGCGC CGGTCGCTAC CATTACCAAC
     CCGCGGATCG CCGGCGCTGA GATCTTAAGG TTGACTCGCG GCCAGCGATG GTAATGGTTG

                                             BsiWI
                                  SpeI            ApaI
1021 TTGTCTGGTG TCAAAAATAA TAGGCCTACT AGTCGGCCGT ACGGGCCCTT
     AACAGACCAC AGTTTTTATT ATCCGGATGA TCAGCCGGCA TGCCCGGGAA
```

pmPlum Restriction Map and Multiple Cloning Sites (MCS).

Description

pmPlum is a prokaryotic expression vector that encodes mPlum, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The excitation and emission maxima are 590 nm and 649 nm, respectively.

In pmPlum, the human codon-optimized, mPlum coding sequence (2) is flanked by separate and distinct multiple cloning sites (MCS) that make it easy to excise the gene for use in other cloning applications. Alternatively, the mPlum coding sequence can be amplified by PCR. In *E. coli*, mPlum 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 the mPlum coding sequence is excised using a restriction site in the 5' MCS, the resulting DNA fragment will encode only the mPlum protein (without the additional amino acids that are expressed using the *lac* promoter). A Kozak consensus sequence is located immediately upstream of the mPlum gene to enhance translational efficiency in eukaryotic systems (3). In the pmPlum vector, the entire mPlum 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*.

(111111)

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Use

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

For Western analylsis, 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 mPlum protein.

Location of features

- P_{lac} (*lac* Promoter): 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - *lac* operator: 179–199
- lacZ-mPlum fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon 964–966
- 5' MCS (5' multiple cloning site): 234–281
- mPlum (human codon-optimized)
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 967–969
- 3' MCS (multiple cloning site): 969–1068
- Amp^r (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 origin of replication: 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 mPlum

- Excitation maximum = 590 nm
- Emission maximum = 649 nm

References

1. Wang, L., *et al.* (2004) *PNAS.* **101**(48):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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