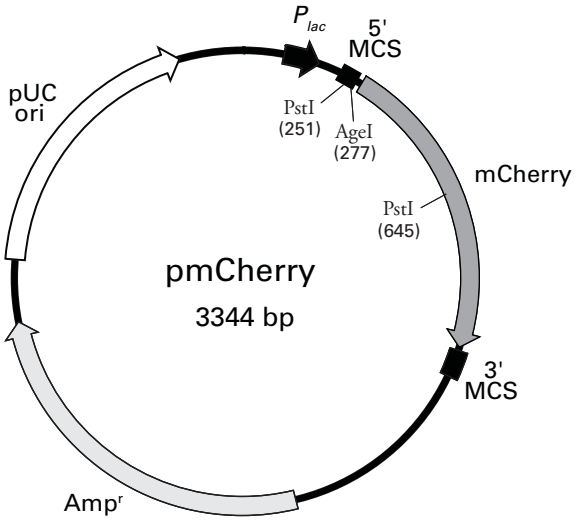
Vector Information

pmCherry Vector Information PT3973-5

Cat. No. 632522



5' MCS

Asp718 KpnI
SmaI XmaI
SphI SalI
HindIII AccI BamHI AgeI Start mCherry

```
231 GCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGGATCCC CGGGTACCGG TCGCCACCAT
    CGGTTCGAAC GTACGGACGT CCAGCTGAGA TCTCCTAGGG GCCCATGGCC AGCGGTGGTA
```

Kozak Sequence

3' MCS

NotI EcoRI

```
991 TACAAGTAGC GGCCGCGACT CTAGAATTCC AACTGAGCGC CGGTCGCTAC CATTACCAAC
    ATGTTCATCG CCGGCGCTGA GATCTTAAGG TTGACTCGCG GCCAGCGATG GTAATGGTTG
```

End mCherry

BsiWI SpeI ApaI

```
1051 TTGTCTGGTG TCAAAAATAA TAGGCCTACT AGTCGGCCGT ACGGGCCCTT
     AACAGACCAC AGTTTTTATT ATCCGGATGA TCAGCCGGCA TGCCCGGGAA
```

pmCherry Restriction Map and Multiple Cloning Sites (MCS).

Description

pmCherry is a prokaryotic expression vector that encodes mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma* sp. red fluorescent protein, DsRed (1). The excitation and emission maxima are 587 nm and 610 nm, respectively.

In pmCherry, the human codon-optimized, mCherry 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 mCherry coding sequence can be amplified by PCR. In *E. coli*, mCherry 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 mCherry coding sequence is excised using a restriction site in the 5' MCS, the resulting DNA fragment will encode only the mCherry protein (without the additional amino acids that are expressed using the *lac* promoter). A Kozak consensus sequence is located immediately upstream of the mCherry gene to enhance translational efficiency

(PR882570; published 2 September 2008)

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in eukaryotic systems (3). In the pmCherry vector, the entire mCherry 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

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

For Western analysis, either the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) or DsRed Monoclonal Antibody (Cat. Nos. 632392 and 632393) can be used to detect the mCherry 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-mCherry fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon 996–999
- 5' MCS (5' multiple cloning site): 234–281
- mCherry (human codon-optimized)
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; Stop codon: 996–999
- 3' MCS (3' multiple cloning site): 999–1098
- Amp^r (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 origin of replication: 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 mCherry

- Excitation maximum = 587nm
- Emission maximum = 610 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.

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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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