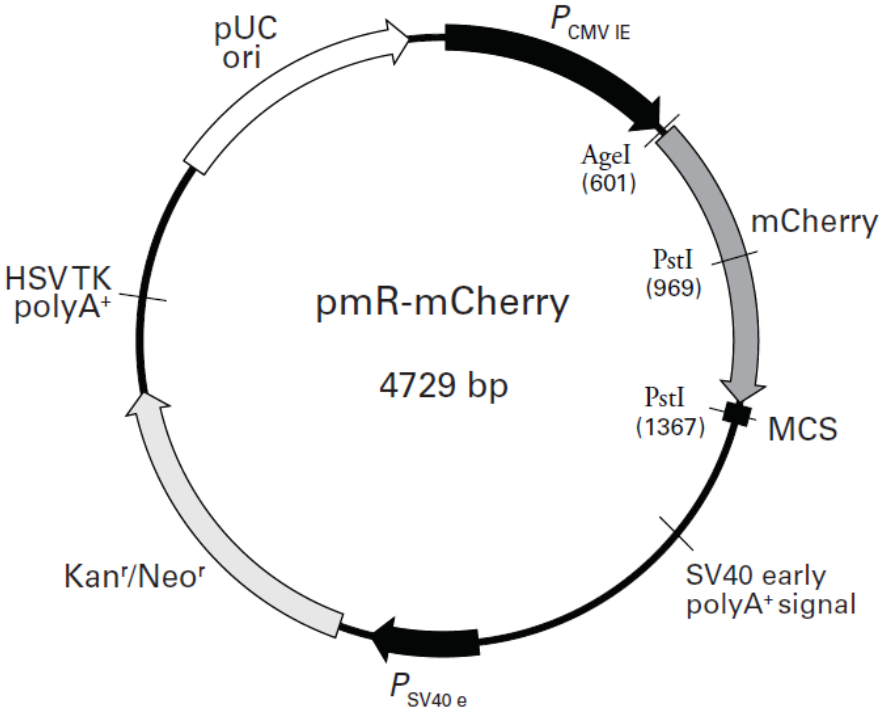
pmR-mCherry Vector Map

Catalog No.
632542

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                                                    SacI                                                KpnI
                                                    XhoI                                    SalI
                mCherry                                                                     AccI
                Stop
                Codon                BglII                  HindIII      EcoRI
1321  TCCGGAAACT AGTCTCAGAT CTCGAGCTCA AGCTTCGAAT TCTGCAGTCG ACGGTACCGC
      AGGCCTTTGA TCAGAGTCTA GAGCTCGAGT TCGAAGCTTA AGACGTCAGC TGCCATGGCG

          SmaI
          XmaI
      ApaI      BamHI              XbaI
1381  GGGCCCGGGA TCCACCGGAT CTAGATAACT
      CCCGGGCCCT AGGTGGCCTA GATCTATTGA
```

pmR-mCherry Vector Map and Multiple Cloning Site (MCS).

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pmR-mCherry

Description

pmR-mCherry is a mammalian expression vector designed to constitutively express a microRNA of interest. Transfected cells can be identified by the coexpression of mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (Shaner et al. 2004). Coexpression of mCherry and your microRNA of interest allows easy monitoring and/or selection of microRNA-expressing cells by fluorescence microscopy or flow cytometry. The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively .

The pmR-mCherry multiple cloning site (MCS) is positioned in the 3'UTR, downstream of the mCherry coding sequence. Expression of mCherry and microRNA precursors cloned into the MCS is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located just upstream of the mCherry sequence. Both the fluorescent protein and the microRNA are expressed from a single mRNA transcript, which is cleaved by Drosha and Dicer to generate the mature microRNA.

Use

A small genomic fragment containing the precursor of the microRNA of interest must be isolated and cloned into pmR-mCherry. This is most easily accomplished by PCR amplification from genomic DNA. We recommend including 100–300 bp of genomic DNA flanking the actual microRNA precursor to ensure efficient processing by Drosha. The orientation of the cloned microRNA precursor should be the same as that of the mCherry transcript. The sequence of the microRNA precursor and flanking genomic DNA can be obtained from a number of public databases including GenBank (http://www.ncbi.nlm.nih.gov/) and EMBL-Bank (http://www. ebi.ac.uk/embl/). The UCSC Genome Bioinformatics Site (http://genome.ucsc.edu/) hosts an easy-to-navigate genomic database with tracks for microRNAs. The Sanger Institute hosts miRBase, a compilation of known microRNA sequences (http://microrna.sanger.ac.uk/).

The pmR-mCherry vector can be transfected into mammalian cells using any standard transfection method. If desired, stable transfectants can be selected using G418. Overexpressed microRNA can be detected using our Mir-X™ miRNA qRT-PCR TB Green® Kit (Cat. Nos. 638314 and 638316). For Western analysis, the mCherry protein can be detected using either the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) or the Monoclonal Antibody (Cat. Nos. 632392 and 632393).

Location of Features

- $P_{CMV\ IE}$ (human cytomegalovirus immediately early promoter): 1–589
- mCherry (human codon optimized; (Haas, Park, and Seed 1996): 613–1332CS (multiple cloning site): 1338–1401
- SV40 early polyA$^+$ signals: 1548–1582
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 2274–2542
- Kanamycin/neomycin resistance gene: 2625–3419
- HSV TK polyA$^+$ (herpes simplex virus thymidine kinase polyadenylation signals): 3655–3673
- pUC origin of replication: 4004–4647

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Excitation and emission maxima of mCherry

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

References

Haas, J., Park, E. C. & Seed, B. Codon usage limitation in the expression of HIV-1 envelope glycoprotein. *Curr. Biol.* **6,** 315–24 (1996).

Gorman, C. In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190 (1985).

Shaner, N. C. *et al.* Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.* **22,** 1567–1572 (2004).

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Takara Bio USA, Inc. This vector has not been completely sequenced.

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This document has been reviewed and approved by the Quality Department.