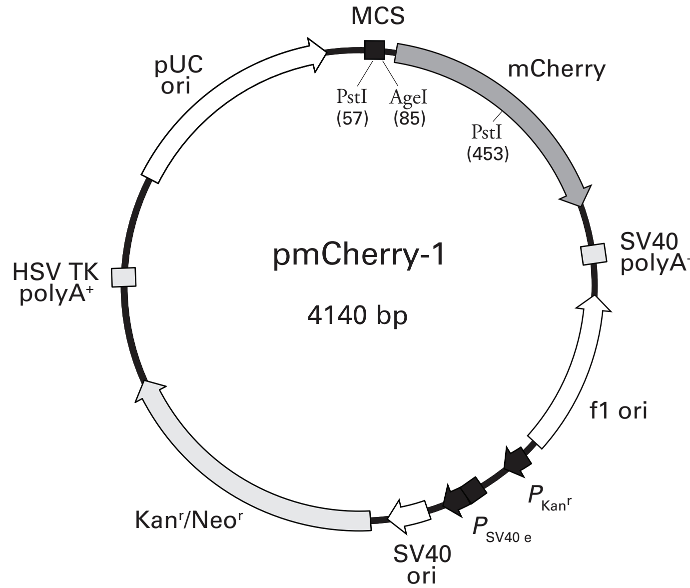
Cat. No. 632525




```
                                      SacI                                              Asp718
                                 XhoI                                          SalI     KpnI
     Eco47III            BglII            HindIII     EcoRI            AccI            SacII
11   TAGCGCTACC GGACTCAGAT CTCGAGCTCA AGCTTCGAAT TCTGCAGTCG ACGGTACCGC
     ATCGCGATGG CCTGAGTCTA GAGCTCGAGT TCGAAGCTTA AGACGTCAGC TGCCATGGCG

          SmaI
   SacII  XmaI
     ApaI       BamHI   AgeI
71   GGGCCCGGGA TCCACCGGTC
     CCCGGGCCCT AGGTGGCCAG
```

pmCherry-1 Restriction Map and Multiple Cloning Site (MCS).

Description

pmCherry-1 is a reporter vector that allows the functional analysis of promoters and promoter/enhancer combinations cloned into its multiple cloning site (MCS). The vector encodes mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (excitation and emission maxima: 587 nm and 610 nm, respectively; 1). The mCherry coding sequence has been human codon-optimized to allow optimal expression after the insertion of a functional promoter into the MCS (2).

SV40 polyadenylation signals downstream of the mCherry gene direct proper processing of the 3' end of the mCherry mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists of the SV40 early promoter ($P_{SV40\ e}$), the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter (P_{Kan^r}) upstream of the cassette allows kanamycin resistance in *E. coli*.

(PR882569; published 2 September 2008)

Use

pmCherry can be used as an *in vivo* reporter of gene expression. Promoters should be cloned into the pmCherry-1 MCS upstream from the mCherry coding sequence. Without the addition of a functional promoter, this vector will not express mCherry. The mCherry vector can be transfected into mammalian cells using any standard method. If required, stable transfectants can be selected using G418 (3).

For Western analysis, 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 mCherry protein.

Location of features

- MCS (multiple cloning site): 12–83
- mCherry (human codon optimized)
  Kozak consensus translation initiation site: 90–100
  Start codon (ATG): 97–99; Stop codon: 805-807
- SV40 early polyA$^+$ signals
  Polyadenylation signals: 959–964 & 988–993; mRNA 3' ends: 997 & 1009
- f1 origin of replication: 1056–1511 (complementary)
- P_{Kan^r} (bacterial promoter for Kan^r gene expression)
  –35 region: 1573–1578; –10 region: 1596–1601
  Transcription start point: 1608
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences)
  Enhancer (72-bp tandem repeats): 1683–1756 & 1757–1828
  21-bp repeats: 1832–1852, 1853–1873 & 1875–1895
  Early promoter element: 1918–1924
  Major transcription start points: 1904, 1942, 1948 & 1953
- SV40 origin of replication: 1852–1987
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2036–2038; stop codon: 2828–2830
  G→A mutation to remove PstI site: 2218
  C→A (Arg→Ser) mutation to remove BssHII site: 2564
- HSVTK polyA$^+$ (herpes simplex virus thymidine kinase polyadenylation signals): 3066–3071 & 3079–3084
- pUC plasmid replication origin: 3415–4058

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: ~500

Excitation and emission maxima of mCherry

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

References

1. Shaner, N. C., *et al.* (2004) *Nature Biotech.* **22**(12):1567-72.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**(3):315–324.
3. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D. M. Glover (IRL Press, Oxford, U.K.) pp. 143–190.

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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