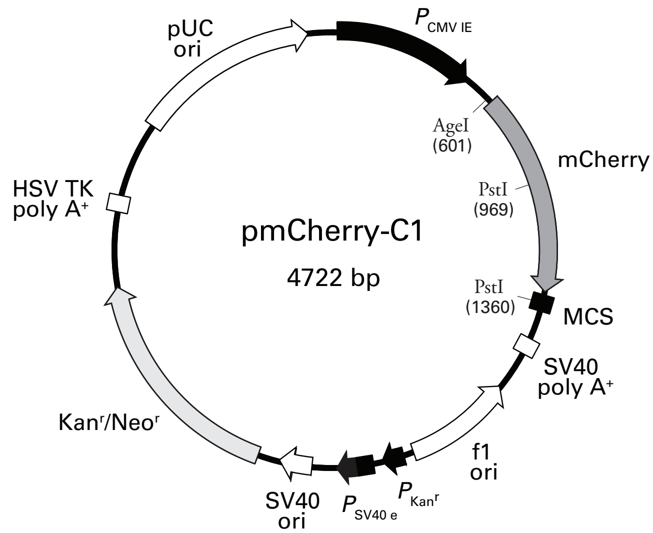
pmCherry-C1 Vector Information

PT3975-5

Cat. No. 632524




```
                                               SacI
                                        XhoI
      End mCherry  BspEI       BglII              HindIII   EcoRI          SalI
                                                                           AccI
1315  TAC AAG  TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA GTC

            KpnI           XmaI
      SalI        SacII    SmaI
      AccI  Asp718    ApaI    BamHI            XbaI*
1363  GAC GGT ACC GCG GGC CCG GGA TCC ACC GGA TCT AGA TAA CTG ATC ATA
                                                   STOP   STOP  STOP
```

* The XbaI site is methylated in the DNA provided by Clontech. If you wish to digest the vector with XbaI enzyme, you will need to transform the vector into a dam - host and make fresh DNA.

pmCherry-C1 Restriction Map and Multiple Cloning Site (MCS).

Description

pmCherry-C1 is a mammalian expression vector designed to express a protein of interest fused to the C-terminus of mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively. Expression of fusion proteins that retain the fluorescent properties of the unmodified mCherry protein can be monitored by flow cytometry and their localization *in vivo* can be determined by fluorescence microscopy.

The multiple cloning site (MCS) in pmCherry-C1 is positioned downstream of the mCherry coding sequence. A Kozak consensus sequence is located immediately upstream of the mCherry gene to enhance translational efficiency in eukaryotic systems (2). SV40 polyadenylation signals downstream of the mCherry gene and the MCS direct proper processing of the 3' end of the mCherry (or fusion gene) 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 Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter (P_{Kan^r}) upstream of the cassette confers kanamycin resistance in *E.*

(060412)

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

coli.

Use

The gene of interest must be cloned into pmCherry-C1 so that it is in-frame with the mCherry coding sequence, with no intervening stop codons.

The pmCherry-C1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (3). pmCherry-C1 can also be used as a cotransfection marker, as the unmodified vector will express mCherry in mammalian cells.

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

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove the SacI site: 569
- mCherry (human codon-optimized)
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; Stop codon: 1395–1397, 1399-1401 & 1403-1405
  Last amino acid: 1318-1320
- MCS (multiple cloning site): 1321–1408
- SV40 early polyA$^+$ signals
  Polyadenylation signals: 1541–1546 & 1570–1575; mRNA 3' ends: 1579 & 1591
- f1 origin of replication: 1638–2093 (complementary)
- P_{Kan^r} (bacterial promoter for Kan^r gene expression)
  –35 region: 2155–2164; –10 region: 2178–2183
  Transcription start point: 2190
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences)
  Enhancer (72-bp tandem repeats): 2267–2338 & 2339–2410
  21-bp repeats: 2414–2434, 2435–2455 & 2457–2477
  Early promoter element: 2490–2496
  Major transcription start points: 2486, 2524, 2530 & 2535
- SV40 origin of replication: 2434–2569
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2618–2620; stop codon: 3410–3412
  G→A mutation to remove the PstI site: 2800
  C→A (Arg to Ser) mutation to remove BssHII site: 3146
- HSV TK polyA$^+$ (herpes simplex virus thymidine kinase polyadenylation signals): 3648–3653 & 3661–3666
- pUC origin of replication: 3997–4640

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
- Plasmid incompatibility group: pMB1/Col E1

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. Kozak, M. (1987) *Nucleic Acids Res.* **15**(20):8125–8148.
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.