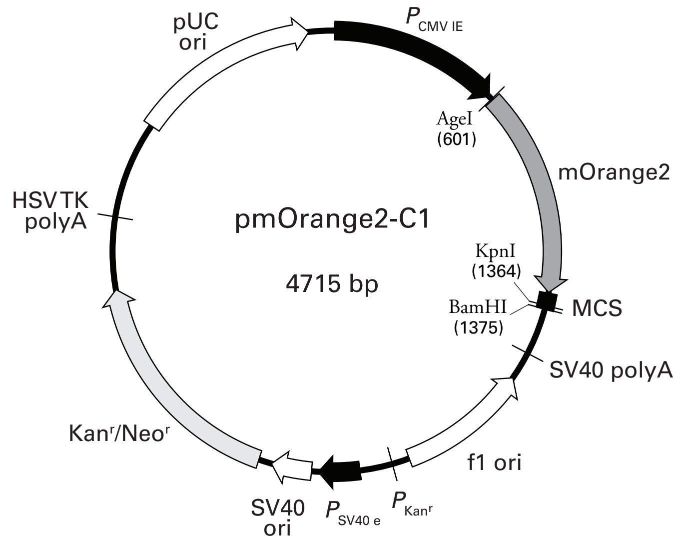
pmOrange2-C1 Vector Information

PT5054-5

Cat. No. 632550

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                                              BglII          SacI
      End of mOrange2   BspEI                        XhoI          HindIII       EcoRI
1305  CTG TAC AAG TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA
      GAC ATG TTC AGG CCT GAG TCT AGA GCT CGA GTT CGA AGC TTA AGA CGT

                 KpnI               XmaI
                        SacII       SmaI
                 Acc65I      ApaI         BamHI
1353  GTC GAC GGT ACC GCG GGC CCG GGA TCC
      CAG CTG CCA TGG CGC CCG GGC CCT AGG
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pmOrange2-C1 Vector Map and Multiple Cloning Site (MCS).

Description

pmOrange2-C1 is a mammalian expression vector designed to express a protein of interest fused to the C-terminus of mOrange2. mOrange2 is a mutant fluorescent protein derived from mOrange (1) that has been optimized for photostability. The excitation and emission maxima of the native mOrange2 protein are 549 nm and 565 nm, respectively. Expression of fusion proteins that retain the fluorescent properties of the unmodified mOrange2 protein can be monitored by flow cytometry and their localization *in vivo* can be determined by fluorescence microscopy.

The multiple cloning site (MCS) in pmOrange2-C1 is positioned downstream of the mOrange2 coding sequence. SV40 polyadenylation signals downstream of the mOrange2 gene and the MCS direct proper processing of the 3' end of the mOrange2 (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. coli*.

(PR963267; published 6 July 2009)

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Use

The gene of interest must be cloned into pmOrange2-C1 so that it is in-frame with the mOrange2 coding sequence, and should contain a stop codon at the 3' end of its coding region.

The pmOrange2-C1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (2). pmOrange2-C1 can also be used as a cotransfection marker, as the unmodified vector will express mOrange2 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 mOrange2 protein.

Location of features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- mOrange2: 606–1313
- MCS (multiple cloning site): 1314–1379
- SV40 polyA signal: 1534–1568
- f1 origin of replication: 1631–2086 (complementary)
- P_{Kan^r} (bacterial promoter for Kan^r gene expression): 2148–2176
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 2260–2403
- SV40 origin of replication: 2427–2565
- Kan^r/Neo^r (kanamycin/neomycin resistance gene; neomycin phosphotransferase): 2611–3405
- HSV TK polyA (herpes simplex virus thymidine kinase polyadenylation signals): 3641–3646 & 3654–3659
- pUC origin of replication: 3990–4633

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 mOrange2

- Excitation maximum = 549 nm
- Emission maximum = 565 nm

References

1. Shaner, N. C., *et al.* (2004) *Nature Biotech.* **22**(12):1567-72.
2. 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 vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech.This vector has not been completely sequenced.

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