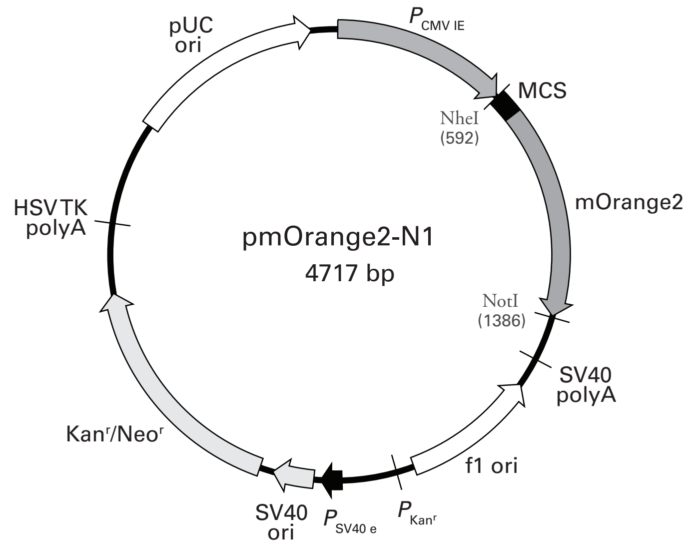
Vector Information

pmOrange2-N1 Vector Information

PT5053-5

Cat. No. 632549

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                                                SacI
                                           XhoI
           NheI                      BglII                 HindIII    EcoRI
591  CCG CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG
     GGC GAT CGC GAT GGC CTG AGT CTA GAG CTC GAG TTC GAA GCT TAA GAC

               KpnI        ApaI
                   SacII          BamHI
               Acc65I      SmaI/XmaI          AgeI    Start of mOrange2
637  CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC ATG GTG AGC
     GTC AGC TGC CAT GGC GCC CGG GCC CTA GGT GGC CAG TAC CAC TCG
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pmOrange2-N1 Vector Map and Multiple Cloning Site (MCS).

Description

pmOrange2-N1 is a mammalian expression vector designed to express a protein of interest fused to the N-terminus of mOrange2, 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 is positioned between the cytomegalovirus immediate early promoter ($P_{CMV IE}$) and the mOrange2 coding sequence. SV40 polyadenylation signals downstream of the mOrange2 gene direct proper processing of the 3' end of the mOrange2 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*.

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Use

The gene of interest must be cloned into pmOrange2-N1 so that it is in-frame with the mOrange2 coding sequence. The gene must include an initiation codon (ATG), and lack in-frame stop codons.

The pmOrange2-N1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (2). pmOrange2-N1 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
- MCS (multiple cloning site): 591–671
- mOrange2: 673-1380
- SV40 polyA signal: 1536–1570
- f1 origin of replication: 1633–2088 (complementary)
- P_{Kan^r} (bacterial promoter for Kan^r gene expression): 2150–2178
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequence): 2334–2405
- SV40 origin of replication: 2429–2567
- Kan^r/Neo^r (kanamycin/neomycin resistance gene; neomycin phosphotransferase): 2613–3407
- HSVTK polyA (herpes simplex virus thymidine kinase polyadenylation signal): 3643–3661
- pUC origin of replication: 3992–4635

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 the JM109 or XL1-Blue strains.
- 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/ColE1

Excitation and emission maxima of mOrange2

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

References

1. Shaner, N. C., *et al.* (2008) *Nature Methods.* **5**(6):545-551.
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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