



pmCherry-N1 Restriction Map and Multiple Cloning Site (MCS).

Description

pmCherry-N1 is a mammalian expression vector designed to express a protein of interest fused to the N-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 is positioned between the cytomegalovirus immediate early promoter ($P_{CMV IE}$) and 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 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 Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter (P_{Kanr}) upstream of the cassette confers kanamycin resistance in *E. coli*.

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Use

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

The pmCherry-N1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (3). pmCherry-N1 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_{CMVIE} (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
- MCS (multiple cloning site): 591–671
- mCherry (human codon-optimized)
Kozak consensus translation initiation site: 672–682
Start codon (ATG): 679–681; Stop codon: 1387–1389
Last amino acid: 1384–1386
- SV40 early polyA⁺ signals
Polyadenylation signals: 1541–1546 & 1570–1575; mRNA 3' ends: 1579 & 1591
- f1 origin of replication: 1638–2073 (complementary)
- P_{Kan^r} (bacterial promoter for Kan^r gene expression):
–35 region: 2155–2160; –10 region: 2178–2183
Transcription start point: 2150
- P_{SV40e} (SV40 early promoter and enhancer sequences)
Enhancer (72-bp tandem repeats): 2267–2338 & 2339–2410
21-bp repeats: 2383–2403, 2404–2424 & 2426–2446
Early promoter element: 2490–2496
Major transcription start points: 2486, 2524, 2530 & 2534
- 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 the BssHII site: 3146
- HSVTK 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: DH5 α , 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 μ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

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.

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