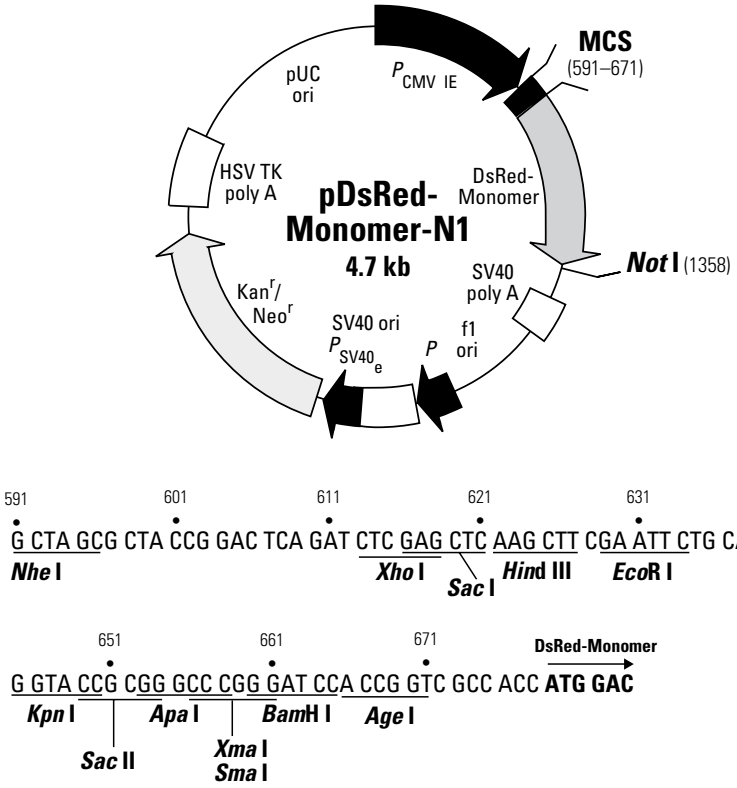

Restriction Map and Multiple Cloning Site (MCS) of pDsRed-Monomer-N1 Vector. Unique restriction sites are in bold. The *Not* I site follows the DsRed-Monomer stop codon. NOTE: The *Xba* I and *BclI* I sites are methylated in the DNA provided by Clontech Laboratories, Inc. If you wish to digest the vector with these enzymes, you will need to transform the vector into a dam– host and make fresh DNA.

Description

pDsRed-Monomer-N1 is a mammalian expression vector that encodes DsRed-Monomer (DsRed.M1), a monomeric mutant derived from the tetrameric *Discosoma* sp. red fluorescent protein DsRed (1). DsRed-Monomer contains forty-five amino acid substitutions (listed on page 2). When DsRed-Monomer is expressed in mammalian cell cultures, red fluorescent cells can be detected by either fluorescence microscopy or flow cytometry 12–16 hr after transfection (DsRed-Monomer excitation and emission maxima = 557 nm and 585 nm, respectively). The DsRed-Monomer coding sequence is human codon-optimized for high expression in mammalian cells (2).

DsRed-Monomer is well suited for use as a fusion tag. The multiple cloning site (MCS) in pDsRed-Monomer-N1 is positioned between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the DsRed-Monomer coding sequence. Genes cloned into the MCS are expressed as fusions to the N-terminus of DsRed-Monomer if they are in the same reading frame as DsRed-Monomer and there are no intervening stop codons. A Kozak consensus sequence is located immediately upstream of the DsRed-Monomer gene to enhance translational efficiency in eukaryotic systems (3). SV40 polyadenylation signals downstream of the DsRed-Monomer gene direct proper processing of the 3' end of the DsRed-Monomer mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 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, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSVTK) gene. A bacterial promoter upstream of the cassette confers kanamycin resistance in *E. coli*.

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Use

pDsRed-Monomer-N1 can be used to construct fusions to the N-terminus of DsRed-Monomer. If a fusion construct retains the fluorescent properties of the native DsRed-Monomer protein, its expression can be monitored by flow cytometry and its localization *in vivo* can be determined by fluorescence microscopy. The target gene must be cloned into pDsRed-Monomer-N1 so that it is in frame with the DsRed-Monomer coding sequence, with no intervening in-frame stop codons. The inserted gene must include an initiating ATG codon. Recombinant pDsRed-Monomer-N1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4). pDsRed-Monomer-N1 can also be used as a cotransfection marker; the unmodified vector will express DsRed-Monomer.

The DsRed1-N Sequencing Primer (Cat. No. 632387) can be used to sequence genes cloned adjacent to the 5' end of the DsRed-Monomer coding region.

For Western blotting, the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) can be used to recognize the DsRed-Monomer protein. However, to generate optimal results it may be necessary to use a higher concentration of antibody than recommended on the DsRed Polyclonal Antibody Certificate of Analysis.

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  - Enhancer region: 59–465; TATA box: 554–560
  - Transcription start point: 583
  - C→G mutation to remove *Sac* I site: 569
- Multiple Cloning Site: 591–671
- Human codon-optimized DsRed-Monomer gene
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 1354–1356
  - Amino acid substitutions (DsRed→DsRed-Monomer)
  - GCC→GAC (Ala-2 to Asp) mutation: 682–684
  - TCC→AAC (Ser-3 to Asn) mutation: 685–687
  - TCC→ACC (Ser-4 to Thr) mutation: 688–690
  - AAG→GAG (Lys-5 to Glu) mutation: 691–693
  - AAC→GAC (Asn-6 to Asp) mutation: 694–696
  - CGC→CAG (Arg-13 to Gln) mutation: 715–717
  - ACC→TCC (Thr-21 to Ser) mutation: 739–741
  - GAG→TAC (Glu-26 to Tyr) mutation: 754–756
  - CGC→AAG (Arg-36 to Lys) mutation: 784–786
  - CAC→ACC (His-41 to Thr) mutation: 799–801
  - AAC→CAG (Asn-42 to Gln) mutation: 802–804
  - GTG→GCC (Val-44 to Ala) mutation: 808–810
  - AAG→CAG (Lys-47 to Gln) mutation: 817–819
  - GTG→GCC (Val-71to Ala) mutation: 889–891
  - AAG→ATG (Lys-83 to Met) mutation: 925–927
  - AAG→ACC (Lys-92 to Thr) mutation: 952–954
  - GTG→TCC (Val-96 to Ser) mutation: 964–966
  - ACC→GAG (Thr-106 to Glu) mutation: 994–996
  - ACC→CAG (Thr-108 to Gln) mutation: 1000–1002
  - TCC→ACC (Ser-117 to Thr) mutation: 1027–1029
  - ATC→AAG (Ile-125 to Lys) mutation: 1051–1053
  - TCC→GCC (Ser-131 to Ala) mutation: 1069–1071
  - ATG→GCC (Met-141 to Ala) mutation: 1099–1101
  - GCC→CCC (Ala-145 to Pro) mutation: 1111–1113
  - CGC→AAG (Arg-149 to Lys) mutation: 1123–1125
  - CGC→CAG (Arg-153 to Gln) mutation: 1135–1137
  - CAC→TCC (His-162 to Ser) mutation: 1162–1164
  - AAG→CAC (Lys-163 to His) mutation: 1165–1167
  - CTG→ACC (Leu-174 to Thr) mutation: 1198–1200
  - GTG→TGC (Val-175 to Cys) mutation: 1201–1203
  - GAG→GAC (Glu-176 to Asp) mutation: 1204–1206
  - TCC→ACC (Ser-179 to Thr) mutation: 1213–1215
  - ATC→GTG (Ile-180 to Val) mutation: 1216–1218
  - ATG→AAG (Met-182 to Lys) mutation: 1222–1224
  - TAC→AAC (Tyr-192 to Asn) mutation: 1252–1254

TAC→CAC (Tyr-193 to His) mutation: 1255–1257
TCC→AAC (Ser-203 to Asn) mutation: 1285–1287
ATC→GTG (Ile-210 to Val) mutation: 1306–1308
CGC→CAC (Arg-216 to His) mutation: 1324–1326
ACC→GCC (Thr-217 to Ala) mutation: 1327–1329
GGC→GCC (Gly-219 to Ala) mutation: 1333–1335
CAC→TCC (His-222 to Ser) mutation: 1342–1344
CTG→GGC (Leu-223 to Gly) mutation: 1345–1347
TTC→TCC (Phe-224 to Ser) mutation: 1348–1350
CTG→CAG (Leu-225 to Gln) mutation: 1351–1353

- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1510–1515 & 1539–1544; mRNA 3' ends: 1548 & 1560
- f1 single-strand DNA origin: 1607–2062 (Packages the noncoding strand of DsRed-Monomer)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2124–2129; –10 region: 2147–2152
  Transcription start point: 2159
- SV40 origin of replication: 2403–2538
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2236–2307 & 2308–2379
  21-bp repeats: 2383–2403, 2404–2424 & 2426–2446
  Early promoter element: 2459–2465
  Major transcription start points: 2455, 2493, 2499 & 2504
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: Start codon (ATG): 2587–2589; Stop codon: 3379–3381
  G→A mutation to remove *Pst* I site: 2769
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3115
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3617–3622 & 3630–3635
- pUC plasmid replication origin: 3966–4609

Sequencing primer location

- DsRed1-N Sequencing Primer (Cat. No. 632387; 5'-GTACTGGAACTGGGGGGACAG-3'): 879–859

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 DsRed-Monomer

- Excitation maximum = 557 nm
- Emission maximum = 585 nm

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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.

This product is covered under U.S. Patent No. 7,250,298.