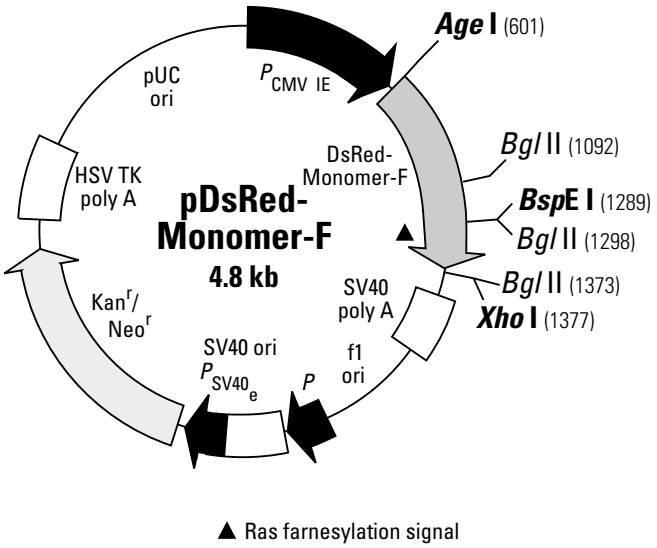
Vector Information

pDsRed-Monomer-F Vector Information PT3845-5

Catalog No. 632493



Restriction Map of pDsRed-Monomer-F. Restriction sites shown in bold are unique.

Description:

pDsRed-Monomer-F encodes farnesylated monomeric red fluorescent protein, a modified form of DsRed-Monomer that remains bound to the plasma membrane in both living and fixed cells. The vector encodes the 20-amino-acid farnesylation signal from c-Ha-Ras (1, 2) fused to the C-terminus of DsRed-Monomer. Post-translation of this farnesylation signal targets DsRed-Monomer-F to the inner leaflet of the plasma membrane.

DsRed-Monomer (DsRed.M1) is a monomeric mutant derived from the tetrameric *Discosoma* sp. red fluorescent protein DsRed (3). DsRed-Monomer contains a total of forty-five amino acid substitutions. 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 592 nm, respectively). The DsRed-Monomer coding sequence is human codon-optimized for increased translation efficiency in mammalian cells (4). SV40 polyadenylation signals downstream of the DsRed-Monomer-F gene direct proper processing of the 3′ end of the DsRed-Monomer-F mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pDsRed-Monomer-F backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use:

DsRed-Monomer-F is designed for use as a plasma membrane marker, as well as a cotransfection marker. Because it remains attached to the plasma membrane, it can be detected by fluorescence microscopy in permeabilized cells after ethanol fixation (5). The vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (6).

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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
- Farnesylated monomeric red fluorescent protein (DsRed-Monomer-F) gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; Stop codon: 1363–1365
  Last amino acid in DsRed-Monomer: 1285–1287
  c-Ha-Ras farnesylation signal: 1303–1365
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1583–1588 & 1612–1617; mRNA 3′ ends: 1621 & 1633
- f1 single-strand DNA origin: 1680–2135 (packages the noncoding strand of DsRed-Monomer)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2197–2202; –10 region: 2220–2225
  Transcription start point: 2232
- SV40 origin of replication: 2476–2611
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2309–2380 & 2382–2452
  21-bp repeats: 2456–2476, 2477–2497 & 2499–2519
  Early promoter element: 2532–2538
  Major transcription start points: 2528, 2566, 2572 & 2577
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2660–2662; stop codon: 3452–3454
  G→A mutation to remove *Pst* I site: 2842
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3188
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3690–3695 & 3703–3708
- pUC plasmid replication origin: 4039–4682

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

References:

1. Aronheim, A., *et al.* (1994) *Cell* **78**:949–961.
2. Hancock, J. F., *et al.* (1991) *EMBO J.* **10**:4033–4039.
3. Matz, M. V., et al. (1999) Nature Biotech. 17(10):969–973.
4. Haas, J., et al. (1996) Curr. Biol. 6:315–324.
5. Jiang, W. & Hunter, T. (1998) *BioTechniques* **24**:348–354.
6. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) 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.