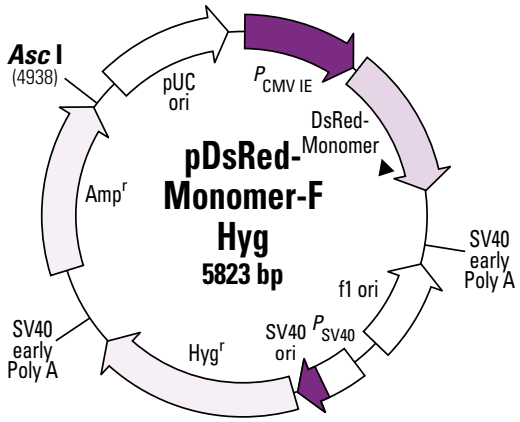
Vector Information

pDsRed-Monomer-F Hyg Vector Information PT3890-5

Catalog No. 632514



Description

pDsRed-Monomer-F Hyg encodes a fusion protein consisting of a 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 SV40T-antigen. A hygromycin resistance cassette (Hyg^r), consisting of the SV40 early promoter, the hygromycin resistance gene, and SV40 polyadenylation signals, allows stably transfected eukaryotic cells to be selected using hygromycin B. A bacterial promoter upstream of the ampicillin gene expresses ampicillin resistance in *E. coli*. The pDsRed-Monomer-F Hyg 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 Hyg is designed for use as a plasma membrane marker, as a cotransfection marker, and to complement the already available Living Colors® vectors with kanamycin/neomycin selection cassettes. 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 Hygromycin B. The added feature of a hygromycin resistance cassette complements the neomycin resistance cassette in currently available Living Colors vectors.

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Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
- 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)
- SV40 origin of replication: 2476–2614
- SV40 early promoter
  Enhancer (72 bp tandem repeats): 2309–2380 & 2381–2452
  21 bp repeats: 2456–2476, 2477–2497 & 2499–2519
  Early promoter element: 2532–2538
- Hygromycin resistance gene:
  Start codon (ATG): 2633-2635; stop codon: 3656–3658
- SV40 early mRNA polyadenylation signal: 3806–3811 & 3835-3840; mRNA 3′ ends: 3844 &3856
- Bacterial promoter for expression of Amp^r gene:
  –35 region: 4005–4010; –10 region: 4028–4033
- Ampicillin resistance gene:
  Start codon (ATG): 4075–4077; stop codon: 4933–4935
- pUC plasmid replication origin: 5098–5741

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 ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC; copy number: high

References

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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

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. This vector has not been completely sequenced.