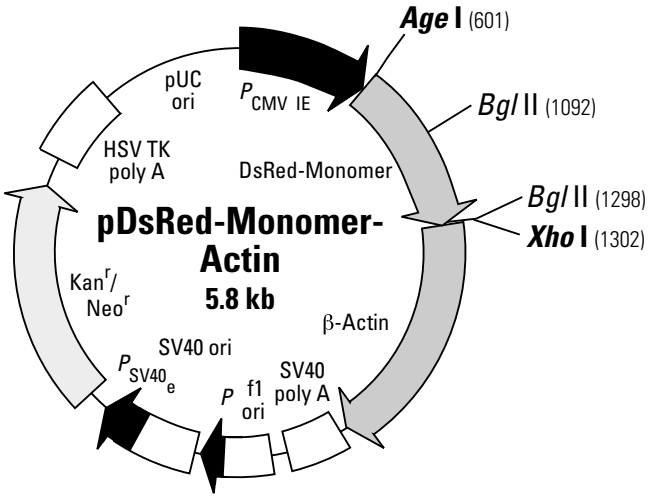
Vector Information

pDsRed-Monomer-Actin Vector Information PT3827-5

Cat. No. 632479



Restriction map of pDsRed-Monomer-Actin. All sites shown in bold are unique.

Description

pDsRed-Monomer-Actin encodes a fusion of DsRed-Monomer, a monomeric mutant derived from the tetrameric *Discosoma* sp. red fluorescent protein DsRed (1), and human cytoplasmic β-actin (2). DsRed-Monomer contains 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 high expression in mammalian cells (3).

The vector contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen and a neomycin resistance (Neo^r) gene for selection (using G418) in eukaryotic cells (4). A bacterial promoter (*P*) upstream of Neo^r expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

This vector is designed for the expression of the DsRed-Monomer-Actin fusion protein in mammalian cells. The protein is incorporated into growing actin filaments and allows for visualization of actin-containing subcellular structures in living and fixed cells (5). pDsRed-Monomer-Actin can be introduced into mammalian cells using any standard transfection method. If required, stable cell lines can be selected using G418 (4). Filter sets are available for detection of DsRed-Monomer using conventional epifluoresence microscopy (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
- DsRed-Monomer-Actin fusion:
  Start codon (ATG): 613–615
  Last amino acid in DsRed-Monomer coding region: 1285–1287
  β-actin sequence:
  Start codon:1309–1311; stop codon: 2434–2436
- SV40 early mRNA polyadenylation signal:
  Polyadenylation signals: 2597–2602 & 2626–2631; mRNA 3' ends: 2635 & 2647
- f1 single-strand DNA origin: 2694–3149 (Packages the noncoding strand of DsRed-Monomer-Actin.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 3211–3216; –10 region: 3234–3239
  Transcription start point: 3246
- SV40 origin of replication: 3490–3625
- SV40 early promoter:
  Enhancer (72-bp tandem repeats): 3323–3394 & 3395–3466
  21-bp repeats: 3470–3490, 3491–3511 & 3513–3533
  Early promoter element: 3546–3552
  Major transcription start points: 3542, 3580, 3586 & 3591
- Kanamycin/neomycin resistance gene:
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 3674–3676; stop codon: 4466–4468
  G→A mutation to remove *Pst* I site: 3856
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 4202
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4704–4709 & 4717–4722
- pUC plasmid replication origin: 5053–5696

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: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Ponte, P., *et al.* (1984) Nucleic Acids Res. **12**:1687–1696.
3. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.
5. Westphal, M., *et al.* (1997) *Curr. Biol.* **7**:176–183.
6. Living Colors™ DsRed-Monomer Fluorescent Protein (January 2005) *Clontechniques* **XX**(1):2–4.

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.