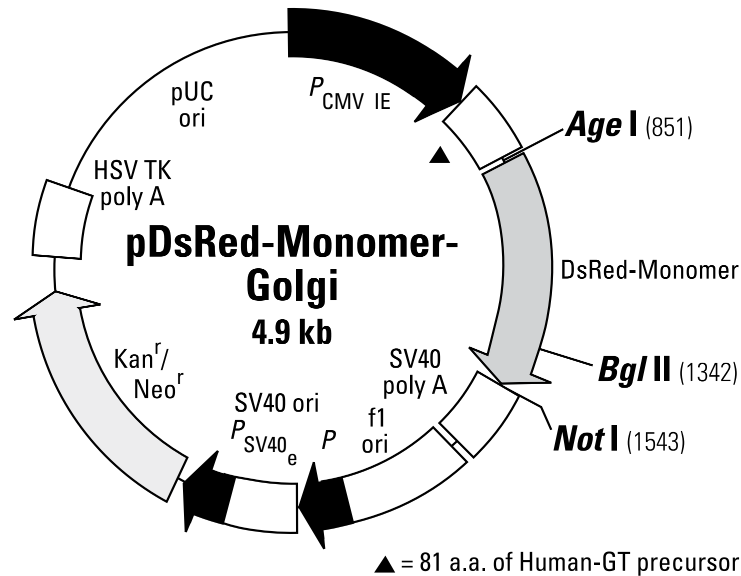
pDsRed-Monomer-Golgi Vector Information

PT3828-5

Catalog No. 632480



Restriction Map of pDsRed-Monomer-Golgi. All restriction sites shown are unique.

Description

pDsRed-Monomer-Golgi encodes a fusion protein consisting of DsRed-Monomer, a monomeric mutant derived from the tetrameric *Discosoma* sp. red fluorescent protein DsRed (1), and a sequence encoding the N-terminal 81 amino acids of human beta 1,4-galactosyltransferase (GT; 2). This region of human beta 1,4-GT contains the membrane-anchoring signal peptide that targets the fusion protein to the trans-medial region of the Golgi apparatus (3–5).

The DsRed-Monomer coding sequence is human codon-optimized for high expression in mammalian cells (6). 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).

SV40 polyadenylation signals downstream of the DsRed-Monomer-Golgi fusion direct proper processing of the 3' end of the 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 allow stably transfected eukaryotic cells to be selected using G418 (7). A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*. The pDsRed-Monomer-Golgi backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

The pDsRed-Monomer-Golgi Vector is designed for fluorescent labeling of the trans-medial region of the Golgi apparatus in mammalian cells. Fluorescence can be observed in living cells by microscopy or flow cytometry. If required, stable cell lines can be selected using G418 (7). Filter sets are available for detection of DsRed-Monomer using conventional epifluoresence microscopy (8).

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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
- Human beta 1,4-GT-DsRed-Monomer fusion protein:
  - Start codon: 597–599; stop codon: 1539–1541
  - N-terminal 81 a.a. of human beta 1,4-GT (1): 597–842
  - DsRed-Monomer fluorescent protein gene: 864–1538
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1695–1700 & 1724–1729; mRNA 3' ends: 1733 & 1745
- f1 single-strand DNA origin: 1792–2247 (packages the noncoding strand of DsRed-Monomer-Golgi)
- Bacterial promoter for expression of Kan^r gene
  - –35 region: 2309–2314; –10 region: 2332–2337
  - Transcription start point: 2344
- SV40 origin of replication: 2588–2723
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2421–2492 & 2493–2564
  - 21-bp repeats: 2568–2588, 2589–2609 & 2611–2631
  - Early promoter element: 2644–2650
  - Major transcription start points: 2640, 2678, 2684 & 2689
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2772–2774; stop codon: 3564–3566
  - G→A mutation to remove *Pst* I site: 2954
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3300
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3802–3807 & 3815–3820
- pUC plasmid replication origin: 4151–4794

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

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3. Llopis, J., *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**:6803–6808.
4. Yamaguchi, N. & Fukuda, M. N. (1995) *J. Biol. Chem.* **270**:12170–12176.
5. Gleeson, P. A., *et al.* (1994) *Glycoconjugate J.* **11**:381–394.
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7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.
8. 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. This vector has not been completely sequenced.

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