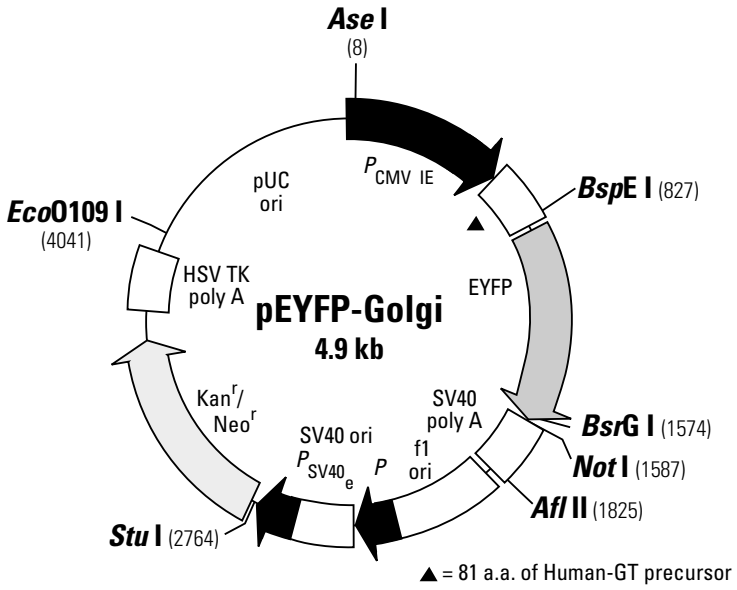

Restriction Map of pEYFP-Golgi. All restriction sites shown are unique.

Description

pEYFP-Golgi encodes a fusion protein consisting of enhanced yellow fluorescent protein (EYFP) and a sequence encoding the N-terminal 81 amino acids of human beta 1,4-galactosyltransferase (GT; 1). 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 (2–4). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (5). The fluorescence excitation maximum of EYFP is 513 nm; the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 514 nm, the E_m of EYFP is 84,500 $cm^{-1}M^{-1}$ and the fluorescence quantum yield is 0.61 (6), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that of EGFP.

In addition to the chromophore mutations, EYFP contains more than 190 silent base changes that correspond to human codon-usage preferences (6). SV40 polyadenylation signals downstream of the EYFP-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 pEYFP-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 pEYFP-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. pEYFP-ER can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). Filter sets are available for dual-color detection of EYFP and ECFP using conventional epifluoresence microscopy (8). Please refer to the Living Colors® User Manual, provided with this vector, for additional information.

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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-EYFP fusion protein:
  Start codon: 597–599; stop codon: 1581–1583
  N-terminal 81 a.a. of human beta 1,4-GT (1): 597–842
  Enhanced yellow fluorescent protein (EYFP) gene: 864–1580
  Insertion of Val at position 2: 867–869
  GFP-10C mutations:
  Ser-65 to Gly: 1059–1061; Val-68 to Leu: 1068–1070;
  Ser-72 to Ala: 1080–1082; Thr-203 to Tyr: 1473–1475
  His-231 to Leu mutation (A→T): 1558
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1737–1742 & 1766–1771; mRNA 3' ends: 1775 & 1787
- f1 single-strand DNA origin: 1834–2289 (packages the noncoding strand of EYFP-Golgi)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2351–2356; –10 region: 2374–2379
  Transcription start point: 2386
- SV40 origin of replication: 2630–2765
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2463–2534 & 2535–2606
  21-bp repeats: 2610–2630, 2631–2651 & 2653–2673
  Early promoter element: 2686–2692
  Major transcription start points: 2682, 2720, 2726 & 2731
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2814–2816; stop codon: 3606–3608
  G→A mutation to remove *Pst* I site: 2996
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3342
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3844–3849 & 3857–3862
- pUC plasmid replication origin: 4193–4836

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 (30 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC; copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

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