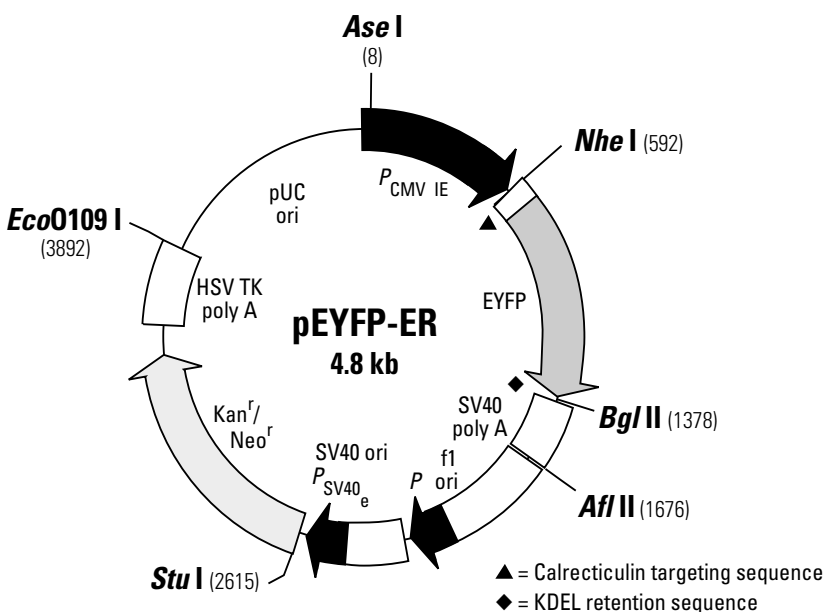




Restriction Map of pEYFP-ER. All restriction sites are unique.

Description

pEYFP-ER encodes a fusion protein consisting of enhanced yellow fluorescent protein (EYFP); the endoplasmic reticulum (ER) targeting sequence of calreticulin (1), which is cloned at the 5' end; and the sequence encoding the ER retrieval sequence, KDEL (2, 3), which is cloned at the 3' end. The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (4, 5): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and Thr-203 to Tyr. 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 $84,500 \text{ cm}^{-1}\text{M}^{-1}$ and the fluorescence quantum yield is 0.61 (5), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.

In addition to the chromophore mutations, pEYFP-ER contains more than 190 silent base changes that correspond to human codon-usage preferences (6). SV40 polyadenylation signals downstream of the EYFP-ER 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 allows 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-ER backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

pEYFP-ER is designed for fluorescent labeling of the ER in mammalian cells (8, 9). 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 epifluorescence microscopy (10). Please refer to the Living Colors User Manual for additional information.

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
- Calreticulin signal sequence: 597–647
- Enhanced yellow fluorescent protein (EYFP) gene: 648–1361
Insertion of Val at position 2: 648–650
GFP-10C mutations:
Ser-65 to Gly: 840–842; Val-68 to Leu: 849–851; Ser-72 to Ala: 861–863; Thr-203 to Tyr: 1254–1256
His-231 to Leu mutation (A→T): 1339
Last amino acid in EYFP: 1359–1361
KDEL coding sequence (in frame with EYFP): 1362–1373
Stop codon: 1374–1376
- SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1588–1593 & 1617–1622; mRNA 3' ends: 1626 & 1638
- f1 single-strand DNA origin: 1685–2140 (packages the noncoding strand of EYFP-ER)
- Bacterial promoter for expression of Kan^r gene
–35 region: 2202–2207; –10 region: 2225–2230
Transcription start point: 2237
- SV40 origin of replication: 2481–2616
- SV40 early promoter
Enhancer (72-bp tandem repeats): 2314–2385 & 2386–2457
21-bp repeats: 2461–2481, 2482–2502 & 2504–2524
Early promoter element: 2537–2543
Major transcription start points: 2533, 2571, 2577 & 2582
- Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2665–2667; stop codon: 3457–3459
G→A mutation to remove *Pst* I site: 2847
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3193
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3695–3700 & 3708–3713
- pUC plasmid replication origin: 4044–4687

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 711–690; EGFP-C Sequencing Primer (#6478-1): 1298–1319

Propagation in *E. coli*

- Suitable host strains: DH5 α , 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 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC; copy number: $\approx$ 500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Fliegel, L., *et al.* (1989) *J. Biol. Chem.* **264**:21522–21528.
2. Munro, S. & Pelham, H. R. (1987) *Cell* **48**:899–907.
3. Pelham, H. R. (1996) *Cell Struct. Funct.* **21**:413–419.
4. Ormö, M., *et al.* (1996) *Science* **273**:1392–1395.
5. Piston, D., pers. comm.
6. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.
8. Roderick, H. L., *et al.* (1997) *FEBS Letters* **405**:181–185.
9. Kendall, J. M., *et al.* (1994) *Anal. Biochem.* **221**:173–181.
10. Living Colors Subcellular Localization Vectors (October 1998) *CLONTECHniques XIII*(4):8–9.

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