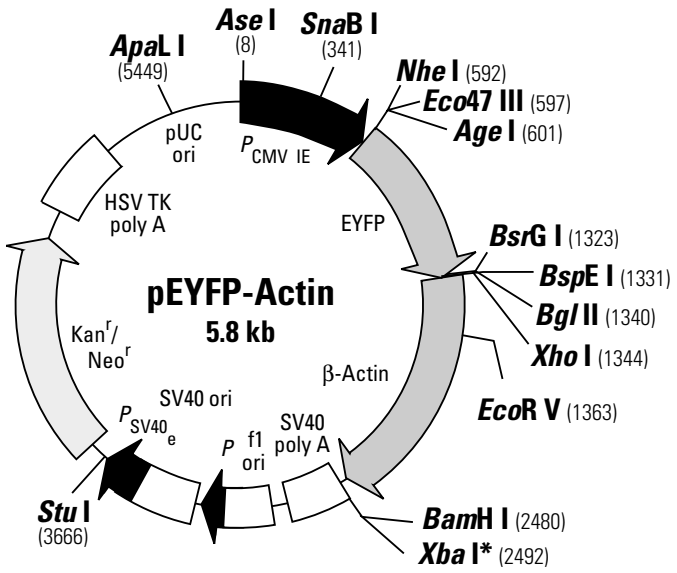

Restriction map of pEYFP-Actin. All sites shown are unique. The *Xba* I* and *Bcl* I sites (positions 2492 & 2502) are methylated in the DNA provided by CLONTECH. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pEYFP-Actin encodes a fusion of the enhanced yellow fluorescent protein (EYFP) and human cytoplasmic β-actin (1). EYFP is an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP; 2–4). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (5): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and Thr-203 to Tyr. It also contains the Phe-64 to Leu mutation of GFPmut1 (6). 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 513 nm, the E_m of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescence quantum yield is 0.63 (5), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (7). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (8). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells. The vector contains an SV40 origin for replication and a neomycin resistance (Neor) gene for selection (using G418) in eukaryotic cells (9). A bacterial promoter (*P*) upstream of Neor 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 EYFP-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 (10, 11). With the appropriate filter sets, pEYFP-Actin can be used for dual labeling experiments with any of CLONTECH's vectors encoding enhanced cyan fluorescent protein (ECFP). pEYFP-Actin is not intended as a cloning vector; however, there are unique restriction sites at the 5' end of EYFP, at the EYFP-Actin fusion junction, and at the 3' end of the β-actin sequences for excision of the sequences encoding the EYFP-Actin protein fusion. Note that the sites at the 3' end of the β-actin sequences are downstream of the stop codon. pEYFP-Actin can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (9).

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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
- EYFP-Actin fusion:
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615
  Insertion of Val at position 2 of GFP: 616–618
  GFP-10C mutations:
  Ser-65 to Gly: 808–810; Val-68 to Leu: 817–819; Ser-72 to Ala: 829–831; Thr-203 to Tyr: 1222–1224
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in EYFP coding region: 1327–1329
  β-actin sequences: 1351–2478
  Stop codon: 2476–2478
- SV40 early mRNA polyadenylation signal:
  Polyadenylation signals: 2639–2644 & 2668–2673; mRNA 3' ends: 2677 & 2689
- f1 single-strand DNA origin: 2736–3191 (Packages the noncoding strand of EYFP-Actin.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 3253–3258; –10 region: 3276–3281
  Transcription start point: 3288
- SV40 origin of replication: 3532–3667
- SV40 early promoter:
  Enhancer (72-bp tandem repeats): 3365–3436 & 3437–3508
  21-bp repeats: 3512–3532, 3533–3553 & 3555–3575
  Early promoter element: 3588–3594
  Major transcription start points: 3584, 3622, 3628, 3633
- Kanamycin/neomycin resistance gene:
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 3716–3718; stop codon: 4508–4510
  G→A mutation to remove *Pst* I site: 3898
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 4244
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4746–4751 & 4759–4764
- pUC plasmid replication origin: 5095–5738

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

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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11. Time-lapse studies of EGFP-Actin expression in mammalian cells are available at **http://www.stanford.edu/~angelab/**

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