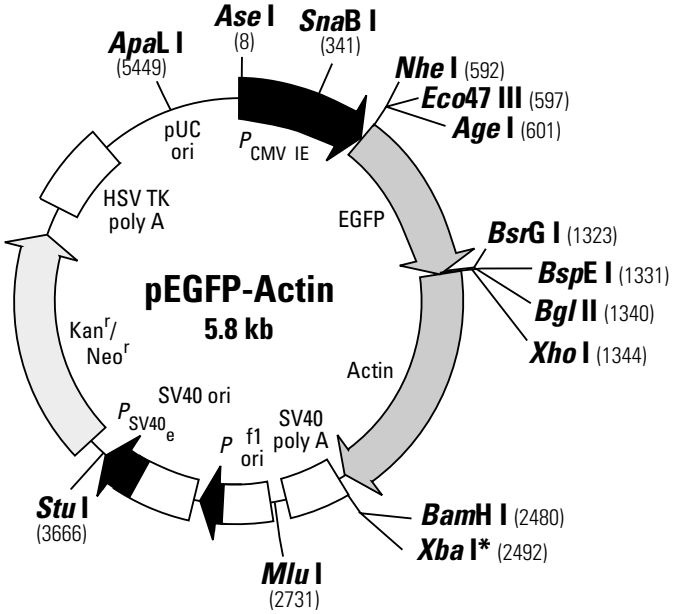

Restriction Map of pEGFP-Actin. All sites shown are unique. The *Xba* I site (*) is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and isolate fresh DNA.

Description

pEGFP-Actin encodes a fusion protein consisting of the red-shifted, human codon-optimized variant of green fluorescent protein (EGFP; 1–3) and the gene encoding human cytoplasmic β-actin (4). EGFP, a derivative of the GFPmut1 variant (5), has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) This variant contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr, and also contains more than 190 silent base changes which correspond to human codon-usage preferences (6). SV40 polyadenylation signals downstream of the EGFP-Actin fusion direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in any mammalian cell line that expresses 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. A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*. The pEGFP-Actin backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

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Use

The pEGFP-Actin Vector expresses the EGFP-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. (7, 8). pEGFP-Actin can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (9).

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
- Enhanced green fluorescent protein (EGFP) gene
  Start codon (ATG): 613–615
  Insertion of Val at position 2: 616–618
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in EGFP sequence: 1327–1329
- Human cytoplasmic β-actin sequence: 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 EGFP-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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