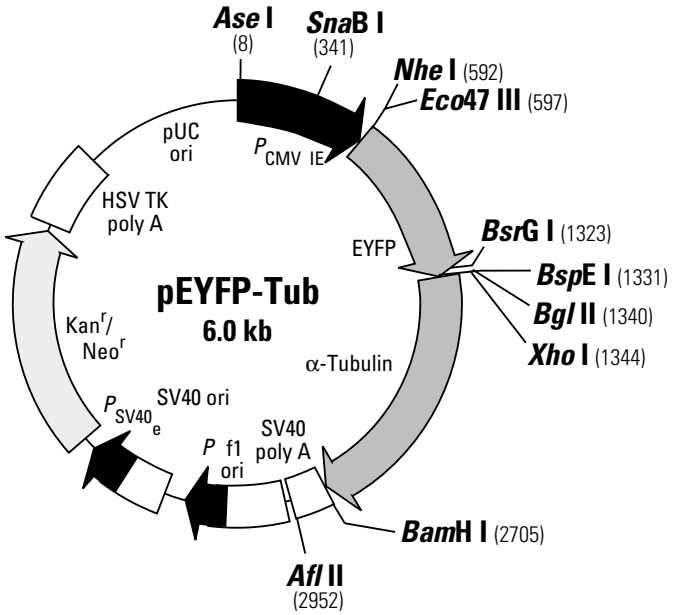

Restriction Map of pEYFP-Tub. All sites shown are unique. The *Xba*I and *Bcl*I sites at positions 2717 & 2727 are methylated in the DNA provided by CLONTECH. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and isolate fresh DNA.

Description

pEYFP-Tub encodes a fusion protein consisting of enhanced yellow fluorescent protein (EYFP) and human α-tubulin (1, 2). EYFP is an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP; 3–5). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (6): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and and Thr-203 to Tyr. It also contains the Phe-64 to Leu mutation of GFPmut1 (7). 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 (6), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.

In addition to the chromophore mutations, pEYFP-Tub contains more than 190 silent base changes that correspond to human codon-usage preferences (8). SV40 polyadenylation signals downstream of the EYFP-Tub 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 (9). A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*. The pEYFP-Tub 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-Tub Vector is designed for expressing the EYFP-tubulin fusion protein in mammalian cells. pEYFP-Tub can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (9). The protein incorporates directly into microtubules and thereby allows the observation of microtubules in living or fixed cells by fluorescence microscopy (10). pEYFP-Tub is not sold as a cloning vector; however, there are unique restriction sites at the 5' end of EFYP, at the EYFP-Tub fusion junction, and at the 3' end of the α-tubulin sequences. Note that the sites at the 3' end of the α-tubulin sequences are downstream of the stop codon.

(PR08336; published 30 August 2000)

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-Tubulin fusion: 613–2703
  Start codon (ATG): 613–615
  Insertion of Val at position 2: 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
  GFPmut1 chromophore mutation (Phe-64 to Leu): 805–808
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in EYFP: 1327–1329
  Human α-tubulin sequence (in frame with EYFP): 1348–2703
  Stop codon: 2701–2703
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2864–2869 & 2893–2898; mRNA 3' ends: 2902–2914
- f1 single-strand DNA origin: 2961–3416 (packages the noncoding strand of EYFP-Tub)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 3478–3483; –10 region: 3501–3506
  Transcription start point: 3513
- SV40 origin of replication: 3757–3892
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 3590–3661 & 3662–3733
  21-bp repeats: 3737–3757, 3758–3778 & 3780–3800
  Early promoter element: 3813–3819
  Major transcription start points: 3809, 3847, 3853 & 3858
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 3941–3943; stop codon: 4733–4735
  G→A mutation to remove *Pst* I site: 4123
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 4469
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4971–4976 & 4984–4989
- pUC plasmid replication origin: 5320–5963

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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8. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
9. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.
10. Go to **http://www.stanford.edu/~angelab//** to view time-lapse photos of the microtubule network using EGFP fluorescence.

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