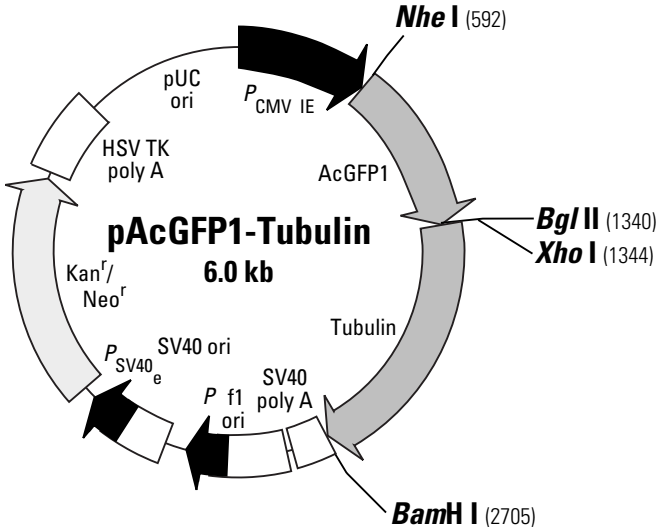
Vector Information

pAcGFP1-Tubulin Vector Information PT3836-5

Catalog No. 632488



Restriction Map of pAcGFP1-Tubulin. All sites shown are unique.

Description

pAcGFP1-Tubulin encodes a fusion protein consisting of the monomeric green fluorescent protein AcGFP1 and the gene encoding human α-tubulin (1, 2). AcGFP1, a derivative of AcGFP from *Aequorea coerulescens*, has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 475 nm; emission maximum = 505 nm.) AcGFP1 contains silent mutations that create an open reading frame composed almost entirely of preferred human codons (3). SV40 polyadenylation signals downstream of the AcGFP1-Tubulin 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. A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*. The pAcGFP1-Tubulin backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

The pAcGFP1-Tubulin Vector is designed for expressing the AcGFP1-Tubulin fusion protein in mammalian cells. The protein incorporates directly into microtubules and thereby allows the observation of microtubules in living or fixed cells by fluorescence microscopy. pAcGFP1-Tubulin can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4).

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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
- *Aequorea coerulescens* green fluorescent protein (AcGFP1) gene
  Start codon (ATG): 613–615
  Insertion of Val at position 2: 616–618
  Last amino acid in AcGFP1: 1327–1329
- Human α-tubulin sequence (in frame with AcGFP1): 1351–2700; 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 AcGFP1-Tubulin)
- 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

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

References

1. Kimble, M., *et al.* (2000) *Cell Motil. Cytoskeleton* **47**(1):48–62.
2. Living Colors™ Subcellular Localization Vectors (October 1998) *Clontechniques* **XIII**(4):8–9.
3. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc. This vector has not been completely sequenced.