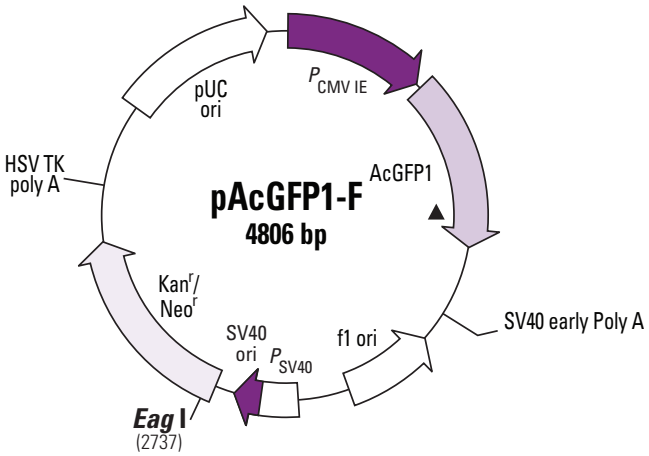
Vector Information

pAcGFP1-F Vector Information PT3887-5

Catalog No. 632511



Description

pAcGFP1-F encodes a fusion protein consisting of a 20-amino-acid farnesylation signal from c-Ha-Ras (1, 2) fused to the C-terminus of AcGFP1. Post-translation of this farnesylation signal targets AcGFP1-F to the inner leaflet of the plasma membrane.

AcGFP1 is derived from *Aequorea coerulescens.* When AcGFP1-F is expressed in mammalian cell cultures, green fluorescent cells can be detected by either fluorescence microscopy or flow cytometry 12–16 hr after transfection (excitation maximum = 475 nm; emission maximum = 505 nm, respectively). The AcGFP1 coding sequence is human-codon-optimized for increased translation efficiency in mammalian cells (3). SV40 polyadenylation signals downstream of the AcGFP1-F gene direct proper processing of the 3' end of the AcGFP1-F 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. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli.* The pAcGFP1-F backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

pAcGFP1-F is designed for use as a plasma membrane marker, as well as a cotransfection marker. Because it remains attached to the plasma membrane, it can be detected by fluorescence microscopy in permeabilized cells after ethanol fixation (4). The vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (5).

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Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
- Farnesylated monomeric green fluorescent protein (AcGFP1-F) gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; Stop codon: 1405–1407
  Last amino acid in AcGFP1: 1327–1329
  c-Ha-Ras farnesylation signal: 1345–1407
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1625–1630 & 1654–1659; mRNA 3' ends: 1633 & 1675
- f1 single-strand DNA origin: 1722–2177 (packages the noncoding strand of AcGFP1)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2239-2244; –10 region: 2262–2267
- SV40 origin of replication: 2518–2656
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2351–2422 & 2423–2494
  21-bp repeats: 2498–2518, 2519–2539 & 2541–2561
  Early promoter element: 2574–2580
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2702–2704; stop codon: 3494–3496
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3732–3737 & 3745–3750
- pUC plasmid replication origin: 4081–4724

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: high

References

1. Aronheim, A., et al. (1994) *Cell* **78:**949–961.
2. Hancock, J. F., et al. (1991) *EMBO J.* **10:**4033–4039.
3. Haas, J., et al. (1996) *Curr. Biol.* **6:**315–324.
4. Jiang, W. & Hunter, T. (1998) *BioTechniques* **24:**348–354.
5. 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. This vector has not been completely sequenced.