



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 coerulea*. 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).



Clontech

United States/Canada
800.662.2566

Asia Pacific
+1.650.919.7300

Europe
+33.(0)1.3904.6880

Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR651859; published 5 June 2006)

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: DH5 α , 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 μ g/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.

Notice to Purchaser

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. Clontech products may not be resold, modified for resale, or used to manufacture commercial products without written approval of Clontech Laboratories, Inc.

This product is the subject of pending U.S. and foreign patents.

This product contains a Living Colors™ fluorescent protein. Not-For-Profit entities may place orders in the normal manner by contacting your local representative or Clontech Customer Service at 800.662.2566, extension 1. Clontech Laboratories, Inc. grants not-for-profit research entities a worldwide, non-exclusive, royalty-free, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product or its components to third parties. Any other use of this product will require a license from Clontech Laboratories, Inc. Please contact the licensing hot line by phone at 800.662.2566, or by e-mail at licensing@clontech.com.

For-Profit entities that wish to use this product in non-commercial or commercial applications are required to obtain a license from Clontech Laboratories, Inc. For license information, please contact the licensing hot line by phone at 800.662.2566, or by e-mail at licensing@clontech.com.

Clontech, Clontech Logo and all other trademarks are the property of Clontech Laboratories, Inc.
Clontech is a Takara Bio Company. ©2006

