



**Restriction Map of pAcGFP1-Actin.** All sites shown in bold are unique.

### Description

pAcGFP1-Actin encodes a green fluorescent protein (GFP) from *Aequorea coerulea* (Excitation maximum = 475 nm; emission maximum = 505 nm) and the gene encoding human cytoplasmic  $\beta$ -actin (1). SV40 polyadenylation signals downstream of the AcGFP1-Actin fusion direct proper processing of the 3' end of the AcGFP1 mRNA.

AcGFP1 contains silent mutations that create an open reading frame comprised almost entirely of optimized human codons. These changes increase the translational efficiency of the AcGFP1 mRNA and consequently the expression of AcGFP1 in mammalian and plant cells.

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<sup>r</sup>), 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 pAcGFP1-Actin 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-Actin Vector expresses the AcGFP1-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 (2, 3). This vector is not intended to be used as a cloning vector; however, unique restriction sites at the 5' end of AcGFP1, and between AcGFP1 and the  $\beta$ -actin open reading frame, allow excision or insertion of DNA. pAcGFP1-Actin can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4).

(PR3Y409; published 29 October 2003)



**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

**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 (AcGFP1) gene  
Start codon (ATG): 613–615  
Insertion of Val at position 2: 616–618  
Last amino acid in AcGFP1 sequence: 1327–1329
- Human cytoplasmic  $\beta$ -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 AcGFP1-Actin)
- Bacterial promoter for expression of Kan<sup>r</sup> 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

**Propagation in *E. coli***

- Suitable host strains: DH5 $\alpha$ , 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  $\mu$ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC; copy number:  $\approx$ 500
- Plasmid incompatibility group: pMB1/ColE1

**References**

1. Ponte, P., *et al.* (1984) *Nucleic Acids Res.* **12**:1687–1696.
2. Westphal, M., *et al.* (1997) *Curr. Biol.* **7**:176–183.
3. de Hostos, E., unpublished data.
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.

**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.

Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, royalty-free, personal, 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, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at [licensing@clontech.com](mailto:licensing@clontech.com).

For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at [licensing@clontech.com](mailto:licensing@clontech.com)

Clontech, Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc.  
Clontech is a Takara Bio Company. ©2005