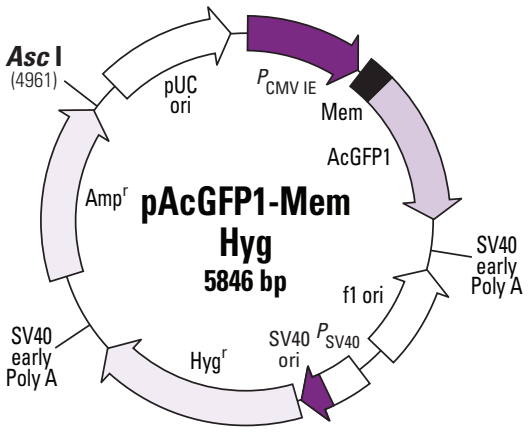
Vector Information

pAcGFP1-Mem Hyg Vector Information PT3888-5

Catalog No. 632509



Description

pAcGFP1-Mem Hyg encodes a fusion protein consisting of the N-terminal 20 amino acids of neuromodulin, also called GAP-43 (1), and the *Aequorea coerulescens* green fluorescent protein AcGFP1. (excitation maximum = 475 nm; emission maximum = 505 nm.) The coding sequence of the AcGFP1 gene contains silent base changes, which correspond to human-codon-usage preferences (2). The neuromodulin fragment contains a signal for posttranslational palmitoylation of cysteines 3 and 4 that targets AcGFP1 to cellular membranes, and the plasma membrane in particular (3).

Expression of AcGFP1-Mem Hyg is driven by the immediate early promoter of CMV ($P_{CMV\ IE}$). The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A hygromycin resistance cassette (Hyg^r), consisting of the SV40 early promoter, the hygromycin resistance gene, and SV40 polyadenylation signals, allows stably transfected eukaryotic cells to be selected using hygromycin B. A bacterial promoter upstream of the ampicillin gene expresses ampicillin resistance in *E. coli.* The pAcGFP1-Mem Hyg backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

pAcGFP1-Mem Hyg can be transfected into mammalian cells using any standard method. If required, stable transformants can be selected using hygromycin B. Expression of AcGFP1-Mem Hyg in mammalian cells results in strong labeling of the plasma membrane and allows easy tracking of individual cells in a population. This membrane labeling also permits the study of fine cellular processes such as neuronal axons (4), leading edges of migrating cells, filopodia, or microvilli on cell surfaces. The added feature of a hygromycin resistance cassette complements neomycin resistance marker in currently available Living Colors vectors. pAcGFP1-Mem Hyg cannot be used as an exclusive plasma membrane marker because it also partially labels intracellular membranes.

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

(PR651763; published 3 May 2006)

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
- AcGFP1-Mem fusion gene
  Start codon (ATG): 679–681
  Neuromodulin N-terminal sequence: 679–738
  Aequorea coerulescens green fluorescent protein (AcGFP1) gene: 739–1449
  Stop codon: 1450–1452
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1606–1611 & 1635–1640; mRNA 3' ends: 1644 & 1656
- f1 single-strand DNA origin: 1703–2158 (packages the noncoding strand of AcGFP1)
- SV40 origin of replication: 2499–2637
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2332–2403 & 2404–2475
  21-bp repeats: 2479–2499, 2500–2520 & 2522–2542
  Early promoter element: 2555–2561
- Hygromycin resistance gene:
  Start codon (ATG): 2656–2658; stop codon: 3679–3681
- SV40 early mRNA polyadenylation signal: 3829–3834 & 3858–3863; mRNA 3' ends: 3867 & 3879
- Bacterial promoter for expression of Amp^r gene:
  –35 region: 4028–4033; –10 region: 4051–4056
- Ampicillin resistance gene:
  Start codon (ATG): 4098–4100; stop codon: 4956–4958
- pUC plasmid replication origin: 5121–5764

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 JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to ampicilin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

References

1. Skene, J. H. P. & Virag, I. (1989) *J. Cell. Biol.* **108:**613–625.
2. Haas, J., et al. (1996) *Curr. Biol.* **6:**315–324.
3. Moriyoshi, K., et al. (1996) *Neuron* **16:**255–260.
4. Jiang, W. & Hunter, T. (1998) *BioTechniques* **24:**348–354.

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.