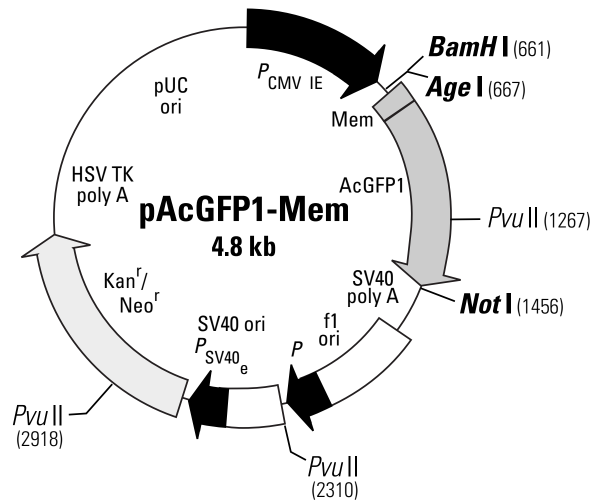
pAcGFP1-Mem Vector Information

PT3841-5

Catalog No. 632491



Restriction Map of pAcGFP1-Mem. Unique restriction sites are in bold.

Description:

pAcGFP1-Mem 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.

Expression of AcGFP1-Mem is driven by the immediate early promoter of CMV ($P_{CMV\ IE}$). The vector contains an SV40 origin of replication and a neomycin resistance (Neo^r) gene for selection in mammalian cells. A bacterial promoter upstream of this cassette (*P*) expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use:

pAcGFP1-Mem can be transfected into mammalian cells using any standard method. If required, stable transformants can be selected using G418 (3). Expression of AcGFP1-Mem 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. pAcGFP1-Mem cannot be used as an exclusive plasma membrane marker because it also partially labels intracellular membranes.

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(PR56802; published 22 June 2005)

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
- 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-Mem.)
- Bacterial promoter for expression of Kan^r gene:
    - –35 region: 2220–2225; –10 region: 2243–2248
    - Transcription start point: 2255
- SV40 origin of replication: 2499–2634
- 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
    - Major transcription start points: 2551, 2589, 2595 & 2600
- Kanamycin/neomycin resistance gene
    - Neomycin phosphotransferase coding sequences:
    - Start codon (ATG): 2683–2685; stop codon: 3475–3477
    - G→A mutation to remove *Pst* I site: 2865
    - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3211
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
    - Polyadenylation signals: 3713–3718 & 3726–3731
- pUC plasmid replication origin: 4062–4705

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 kanamycin (50 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. Gorman, C. (1985) In *DNA cloning: a practical approach, vol. II.* Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.
4. Moriyoshi, K., *et al.* (1996) *Neuron* **16**:255–260.

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.

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