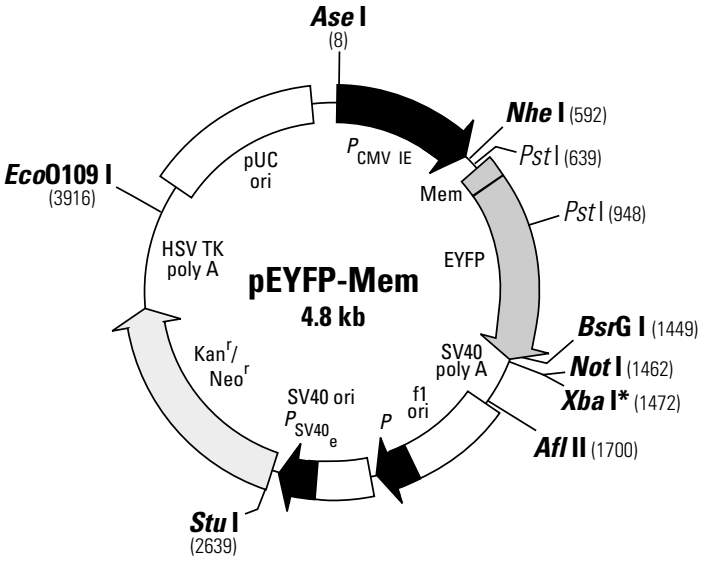


**Restriction Map of pEYFP-Mem.** Unique restriction sites are in bold. The *Xba* I site (*) is methylated in the DNA provided by CLONTECH. If you wish to digest this vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

## Description:

pEYFP-Mem encodes a fusion protein consisting of the N-terminal 20 amino acids of neuromodulin, also called GAP-43 (1), and a yellow-green fluorescent variant of the enhanced green fluorescent protein (EGFP). The neuromodulin fragment contains a signal for posttranslational palmitoylation of cysteines 3 and 4 that targets EYFP to membranes. The EYFP gene contains four amino acid substitutions previously published as GFP-10C (2). The fluorescence excitation maximum of EYFP is 513 nm, and the emission maximum is 527 nm (in the yellow-green region).

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (3). Furthermore, upstream sequences flanking the EYFP-Mem fusion protein have been converted to a Kozak consensus translation initiation site (4). These changes increase the translational efficiency of the fusion protein and consequently its expression in mammalian cells.

Expression of EYFP-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:

pEYFP-Mem can be transfected into mammalian cells using any standard method. If required, stable transformants can be selected using G418 (5). Expression of EYFP-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 study of fine cellular processes such as neuronal axons (6), leading edges of migrating cells, filopodia, or microvilli on cell surfaces. pEYFP-Mem cannot be used as an exclusive plasma membrane marker because it also partially labels intracellular membranes.

(PR08305; published 30 August 2000)

**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
- EYFP-Mem fusion gene
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681
  - Neuromodulin N-terminal sequence: 679–738
  - Enhanced yellow fluorescent protein (EYFP) gene: 739–1458
    - Insertion of Val at position 2: 742–744
    - GFP-10C mutations:
      - Ser-65 to Gly: 934–936
      - Val-68 to Leu: 943–945
      - Ser-72 to Ala: 955–957
      - Thr-203 to Tyr: 1348–1350
    - His-231 to Leu mutation (A→T): 1433
  - Stop codon: 1456–1458
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1612–1617 & 1641–1646
  - mRNA 3' ends: 1650 & 1662
- f1 single-strand DNA origin: 1709–2164
  - (Packages the noncoding strand of EYFP-Mem.)
- Bacterial promoter for expression of Kan$^r$ gene:
  - –35 region: 2226–2231; –10 region: 2249–2254
  - Transcription start point: 2261
- SV40 origin of replication: 2505–2640
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2338–2409 & 2410–2481
  - 21-bp repeats: 2485–2505, 2506–2526 & 2528–2548
  - Early promoter element: 2561–2567
  - Major transcription start points: 2557, 2595, 2601 & 2606
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2689–2691; stop codon: 3481–3483
  - G→A mutation to remove *Pst* I site: 2871
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3217
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3719–3724 & 3732–3737
- pUC plasmid replication origin: 4068–4711

**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: ~500
- Plasmid incompatibility group: pMB1/ColE1

**References:**

1. Skene, J. H. P. & Virag, I. (1989) *J. Cell. Biol.* **108**:613–625.
2. Ormö, M. *et al.* (1996) *Science* **273**:1392–1395.
3. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
4. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
5. Gorman, C. (1985) In *DNA cloning: a practical approach, vol. II.* Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.
6. Moriyoshi, K., *et al.* (1996) *Neuron* **16**:255–260.

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