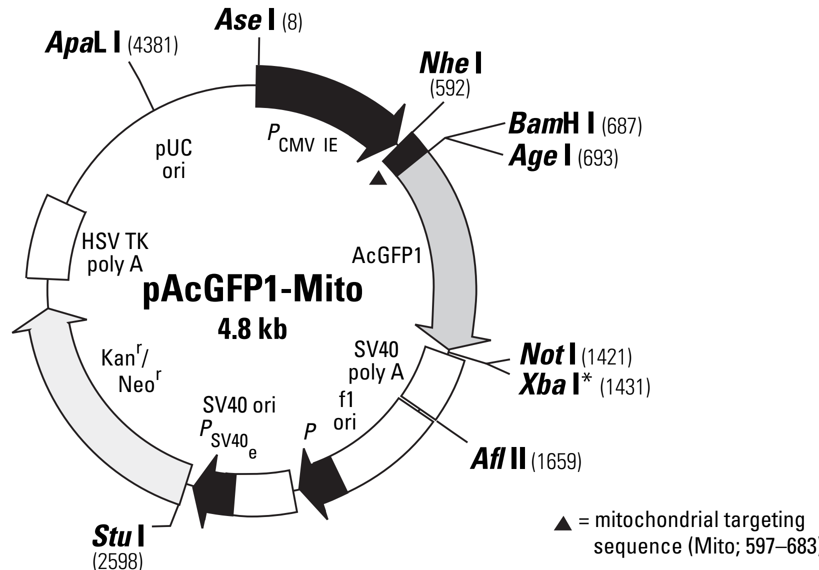
pAcGFP1-Mito Vector Information

PT3730-5

Cat. No. 632432



Restriction map of pAcGFP1-Mito. All restriction sites shown are unique. The *Xba* I site (*) is methylated in the DNA provided by Clontech Laboratories, Inc. . If you wish to digest the vectors with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pAcGFP1-Mito encodes a fusion of a mitochondrial targeting sequence derived from the precursor of subunit VIII of human cytochrome C oxidase (1, 2) and the green fluorescent protein (GFP) from *Aequorea coerulescens* (AcGFP1; excitation maximum = 475 nm; emission maximum = 505 nm). The mitochondrial targeting sequence is fused to the N-terminus of AcGFP1. 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 contains an SV40 origin for replication and a neomycin resistance (Neo^r) gene for selection (using G418) in eukaryotic cells (3). A bacterial promoter (*P*) upstream of Neo^r expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

pAcGFP1-Mito is designed for fluorescent labeling of mitochondria. The fluorescence from pAcGFP1-Mito expression can be observed within the mitochondrial matrix inside the inner membrane. pAcGFP1-Mito can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (3). pAcGFP1-Mito is not intended as a cloning vector; however, the backbone does contain unique restriction sites upstream and downstream of the AcGFP1-Mito sequence which permit excision of the AcGFP1-Mito sequence.

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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-Mito fusion
  - Start codon: 597–599
  - Mitochondrial targeting sequence: 597–683
  - Start of AcGFP1 coding sequences (ATG): 699–701
  - Insertion of Val at position 2: 702–704
  - Stop codon: 1416–1418
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1571–1576 & 1600–1605; mRNA 3' ends: 1609 &1621
- f1 single-strand DNA origin: 1669–2123 (Packages the noncoding strand of AcGFP1-Mito.)
- Bacterial promoter for expression of Kan^r gene.
  - –35 region: 2185–2190; –10 region: 2208–2213; Transcription start point: 2220
- SV40 origin of replication: 2464–2599
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2297–2368, 2369–2440
  - 21-bp repeats: 2444–2464, 2465–2485 & 2487–2507
  - Early promoter element: 2520–2526
  - Major transcription start points: 2516, 2554, 2560 & 2565
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2548–2550; stop codon: 3440–3442
  - G→A mutation to remove *Pst* I site: 2830
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3176
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3678–3683 & 3691–3696
- pUC plasmid replication origin: 4027–4670

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host such as JM109 or XL1-Blue that contains an F plasmid .
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Rizzuto, R., *et al.* (1995) *Curr. Biol.* **5**:635–642.
2. Rizzuto, R., *et al.* (1989) *J. Biol. Chem.* **246**:10595–10600.
3. 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 sequence.

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