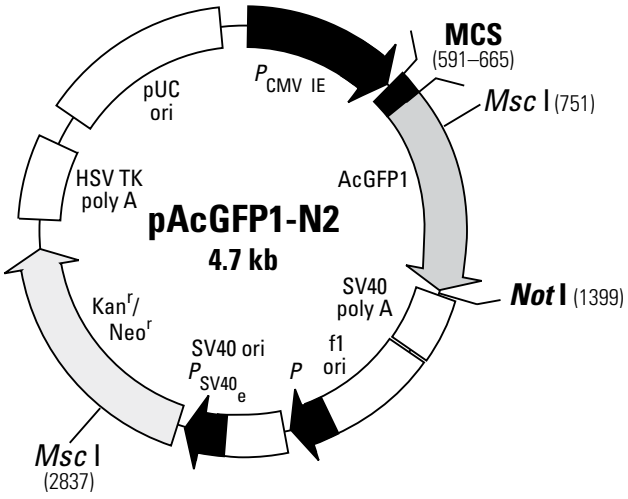
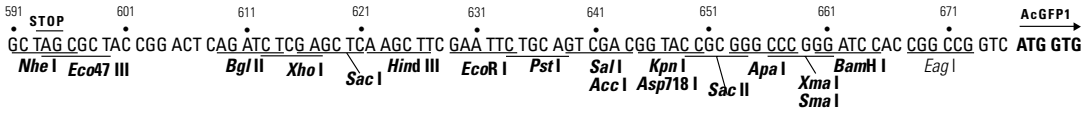
Vector Information

pAcGFP1-N2 Vector Information PT3833-5

Cat. Nos. 632483
& 632485





Restriction Map and Multiple Cloning Site (MCS) of pAcGFP1-N2. Unique restriction sites are in bold. The *Not* I site follows the AcGFP1 stop codon. The *Nhe* I site cannot be used for fusions because it contains an in-frame stop codon. NOTE: The *Xba* I and *Bcl* I sites are methylated in the DNA provided by Clontech Laboratories, Inc. If you wish to digest the vector with these enzymes, you will need to transform the vector into a dam– host and make fresh DNA.

Description:

pAcGFP1-N2 encodes a Green Fluorescent Protein (GFP) from *Aequorea coerulescens*. (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 (1). The MCS in pAcGFP1-N2 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the AcGFP1 coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of AcGFP1 if they are in the same reading frame as AcGFP1 and there are no intervening stop codons. SV40 polyadenylation signals downstream of the AcGFP1 gene direct proper processing of the 3' end of the AcGFP1 mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo^r)—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 the gene expresses kanamycin resistance in *E. coli*. The pAcGFP1-N2 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use:

Fusions to the N-terminus of AcGFP1 retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo* . The target gene should be cloned into pAcGFP1-N2 such that it is in frame with the AcGFP1 coding sequences and contains no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant AcGFP1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (2). pAcGFP1-N2 can also be used simply to express AcGFP1 in a cell line of interest (e.g., as a transfection marker).

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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
- MCS: 591–665
- *Aequorea coerulescens* green fluorescent protein gene
  Start codon (ATG): 677–679; Stop codon: 1394–1396
  Insertion of Val at position 2: 680–682
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1549–1554 & 1578–1583; mRNA 3' ends: 1587 & 1599
- f1 single-strand DNA origin: 1646–2101 (Packages the noncoding strand of AcGFP1)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2163–2168; –10 region: 2186–2191
  Transcription start point: 2198
- SV40 origin of replication: 2442–2577
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2275–2346 & 2347–2418
  21-bp repeats: 2422–2442, 2443–2463 & 2465–2485
  Early promoter element: 2498–2504
  Major transcription start points: 2494, 2532, 2538 & 2543
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2626–2628; stop codon: 3418–3420
  G→A mutation to remove *Pst* I site: 2808
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3154
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3656–3661 & 3669–3674
- pUC plasmid replication origin: 4005–4648

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 JM109 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. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
2. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) 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.