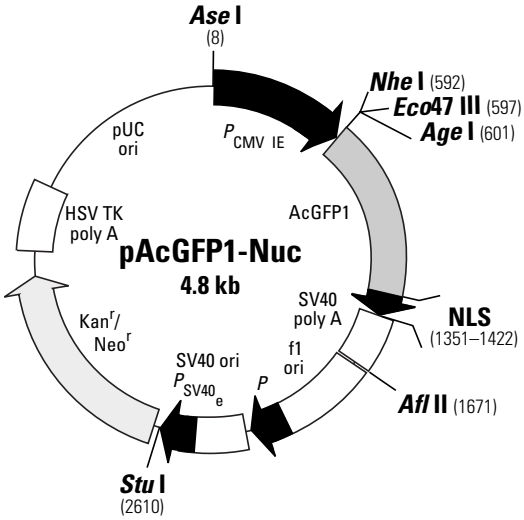
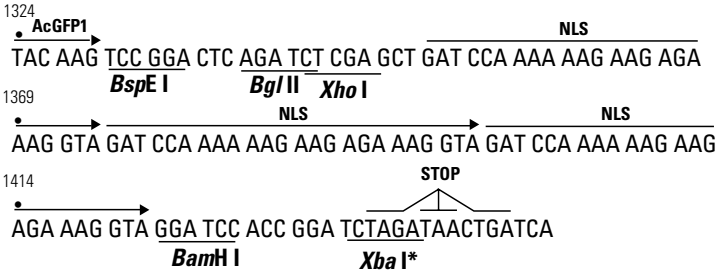
Vector Information

pAcGFP1-Nuc Vector Information PT3729-5

Cat. No. 632431





Map and Nuclear Localization Signal (NLS) of pAcGFP1-Nuc. NLS = three tandem repeats of the nuclear localization signal from simian virus large T-antigen. All 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-Nuc encodes a green fluorescent protein (GFP) from *Aequorea coerulescens* (Excitation maximum = 475 nm; emission maximum = 505 nm) with three copies of the nuclear localization signal (NLS) of the simian virus 40 large T-antigen fused to its C-terminus (1, 2). The reiteration of the NLS sequence significantly increases the efficiency of translocation of AcGFP1 into the nucleus of mammalian cells (3).

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. A bacterial promoter (*P*) upstream of Neo^r 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. The pAcGFP1-Nuc vector can be transfected into mammalian cells using any standard transfection method. If desired, stable transfectants can be selected using G418 (4).

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

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Use

pAcGFP1-Nuc can be used for the localized expression of AcGFP1 in the nucleus of mammalian cells. It allows the visualization of the nucleus in living and fixed cells using fluorescence microscopy. pAcGFP1-Nuc is not meant to be used as a cloning vector; however, unique restriction sites at the 5' end of AcGFP1, between AcGFP1 and the three copies of the NLS, and at the 3' end of the fusion protein, allow excision or insertion of DNA.

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
- *Aequorea coerulescens* Green fluorescent protein (AcGFP1) gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; stop codon: 1441–1443
  Insertion of Val at position 2: 616–618
  Last amino acid: 1327–1329
- Tandem repeat of the nuclear localization signal (NLS): 1351–1422
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1583–1588 & 1612–1617; mRNA 3' ends: 1621 & 1633
- f1 single-strand DNA origin: 1680–2135 (Packages the noncoding strand of pAcGFP1-Nuc.)
- Bacterial promoter for expression of Kan^r gene.
  –35 region: 2197–2202; –10 region: 2220–2225
  Transcription start point: 2232
- SV40 origin of replication: 2476–2611
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2309–2380 & 2381–2452
  21-bp repeats: 2456–2476, 2477–2497 & 2499–2519
  Early promoter element: 2532–2538
  Major transcription start points: 2528, 2566, 2572 & 2577
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2660–2662; stop codon: 3452–3454
  G→A mutation to remove *Pst* I site: 2842
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3188
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3690–3695 & 3703–3708
- pUC plasmid replication origin: 4039–4682

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. Kalderon, D., *et al.* (1984) *Cell* **39**:499–509.
2. Lanford, R. E., *et al.* (1986) *Cell* **46**: 575–582.
3. Fischer-Fantuzzi, L. & Vesco, C. (1988) *Mol. Cell. Biol.* **8**:5495–5503.
4. 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 sequenced.