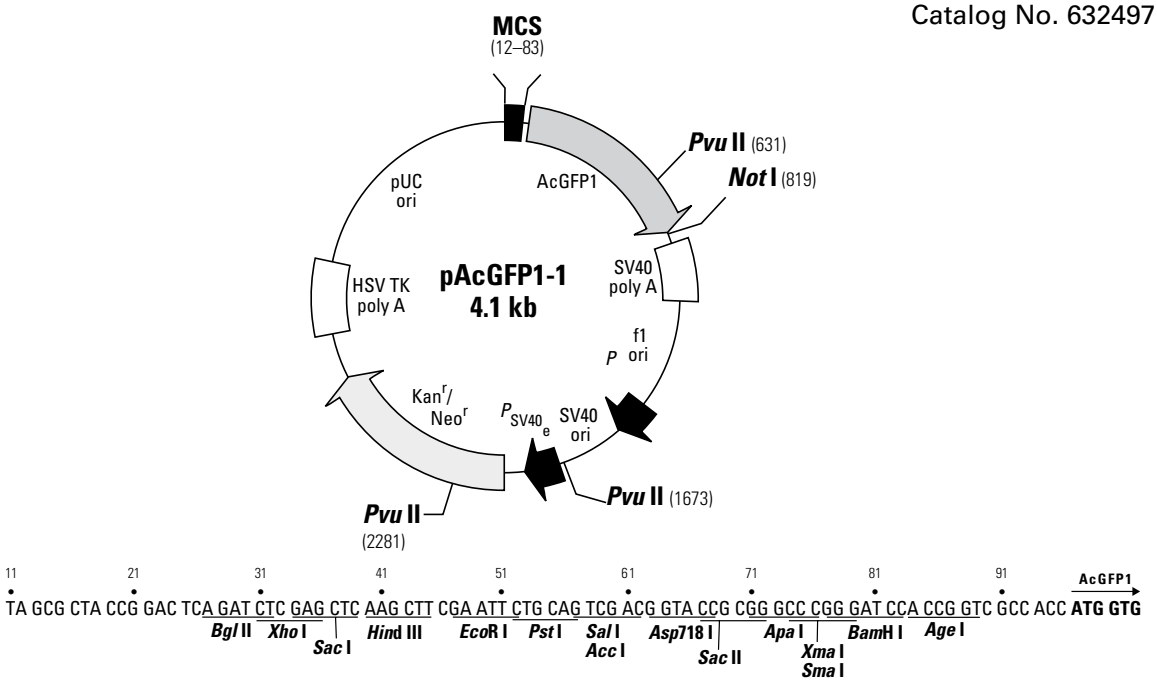
Vector Information

pAcGFP1-1 Vector Information PT3846-5

Catalog No. 632497



Restriction Map and Multiple Cloning Site (MCS) of pAcGFP1-1. All sites shown are unique. 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

pAcGFP1-1 encodes the green fluorescent protein AcGFP1, a derivative of AcGFP from *Aequorea coerulescens*. AcGFP1 has been optimized for brighter fluorescence. (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).

pAcGFP1-1 is a promoterless vector that can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS). Sequences upstream of AcGFP1 have been converted to a Kozak consensus translation initiation site (2) to enhance translation efficiency in eukaryotic cells. SV40 polyadenylation signals downstream of the AcGFP1 gene direct proper processing of the 3' end of the AcGFP1 mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists 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. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Use

AcGFP1 can be used as an *in vivo* reporter of gene expression. Promoters should be cloned into the pAcGFP1-1 MCS upstream from the AcGFP1 coding sequence. Without addition of a functional promoter, this vector will not express AcGFP1. The recombinant AcGFP1 vector can be transfected into mammalian cells using any standard method. If required, stable transfectants can be selected using G418 (3).

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Location of features

- MCS: 12–83
- *Aequorea coerulescens* green fluorescent protein (AcGFP1) gene
  Kozak consensus translation initiation site: 90–100
  Start codon (ATG): 97–99; Stop codon: 814-816
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 969–974 & 998–1003
  mRNA 3' ends: 1007 & 1019
- f1 single-strand DNA origin: 1066–1521
  (Packages noncoding strand of AcGFP1-1.)
- Ampicillin resistance (β-lactamase) promoter
  –35 region: 1583–1588; –10 region: 1606–1611
  Transcription start point: 1618
- SV40 origin of replication: 1862–1997
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 1693–1766 & 1767–1838
  21-bp repeats: 1842–1862, 1863–1883 & 1885–1905
  Early promoter element: 1918–1924
  Major transcription start points: 1914, 1952, 1958 & 1963
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2046–2048; stop codon: 2838–2840
  G→A mutation to remove *Pst* I site: 2228
  C→A (Arg→Ser) mutation to remove *Bss*H II site: 2574
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3076–3081 & 3089–3094
- pUC plasmid replication origin: 3425–4068

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

References

1. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
2. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
3. 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.