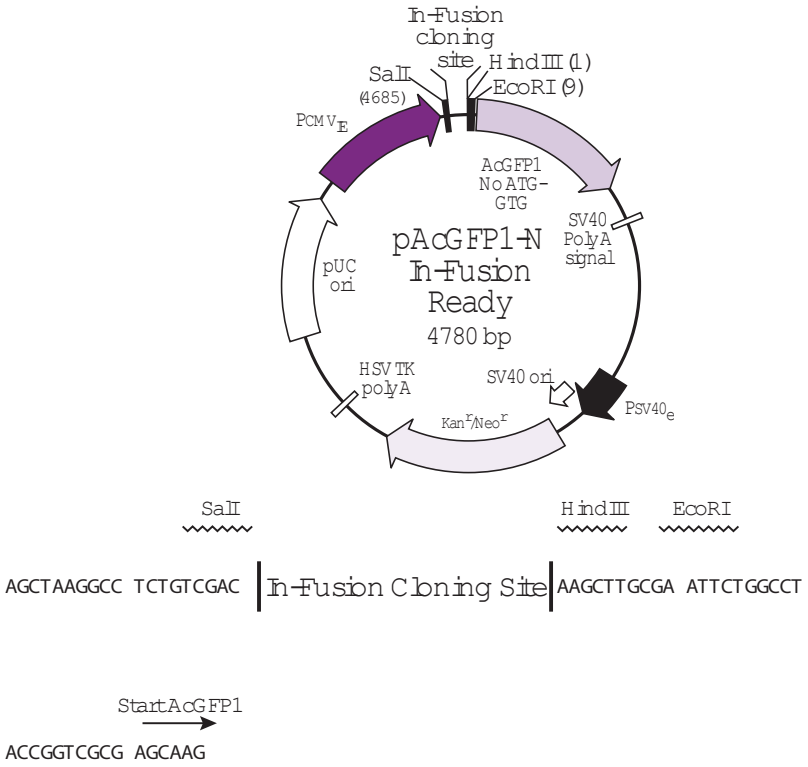
Vector Information

pAcGFP1-N In-Fusion® Ready Vector Information PT3862-5

Catalog No. 632501



Restriction Map and Multiple Cloning Site (MCS) of pAcGFP1-N In-Fusion Ready (Linear) Vector. All sites shown are unique.

Description

pAcGFP1-N In-Fusion Ready vector is a *linearized* mammalian expression vector that encodes a Green Fluorescent Protein (GFP) from *Aequorea coerulescens*. This fluorescent protein coding sequence in this construct has been human codon-optimized for efficient expression and enhanced brightness. AcGFP1 protein has an excitation maximum at 475 nm and an emission maximum at 505 nm. The linearized vector allows direct cloning of PCR products without any need for restriction digestion when using Clontech's In-Fusion HD Cloning Plus (638909). This is accomplished by the use of a specific 15 nucleotide long sequence within the sense and antisense primers that overlap with the cut ends created by initial digestion of the vector with *Sal* I and *Hin*d III.

The primers that will be used to amplify In-Fusion Ready PCR products require the following 15 nucleotides on their 5′ends:

Sense primer: 5′ AAGGCCTCTGTCGAC followed by sequence of amplification target 3′

Antisense primer: 5′ AGAATTCGCAAGCTT followed by sequence of amplification target 3′

If the sequence of the gene of interest is added in-frame immediately after the 15 nucleotides mentioned above, this sequence will automatically be in-frame with the AcGFP1 sequence upstream and therefore be expressed as a fusion protein to the N-terminus of AcGFP1.

SV40 polyadenylation signals downstream of the AcGFP1 gene direct proper processing of the 3′ end of the mRNA transcript. In addition, the vector also contains a SV40 origin of replication in mammalian cells expressing the SV40T-antigen. A neomycin resistance cassette (Neo^r), containing an SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows selection of stable transformants in eukaryotic cells using G418. A bacterial promoter upstream of the gene allows the plasmid to express kanamycin resistance in *E. coli*. The pAcGFP1-N In-Fusion Ready Vector also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(071113)

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Use

Fusions to the N-terminus of AcGFP1 retain the fluorescent properties of the native protein and allow monitoring of fusion protein localization *in vivo.* The PCR-amplified gene of interest is directly cloned into pAcGFP1-N In-Fusion Ready vector so that it is in-frame with the AcGFP1 coding sequence with no intervening in-frame stop codons. This can be accomplished by using the suggested primers. The inserted gene must include the initiating ATG codon. The recombinant AcGFP1 vector can be transfected into mammalian cells using any standard transfection method. Stable transformants can be selected using G418 (2).

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 4078–4666
  Enhancer region:4136–4542; TATA box: 4631–4637
  Transcription start point: 4660
  CØG mutation to remove *Sac* I site: 4646
- *Aequorea coerulescens* Green Fluorescent Protein (AcGFP1): 30–740 (No ATG–GTG)
  First codon: 30–32
  Stop codon: 741–743
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 896–901 & 925–930; mRNA 3′ ends: 934 & 946
- SV40 origin of replication: 1789–1924
- SV40 early promoter: 1622–1832
- Kanamycin/neomycin resistance gene:
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 1973–1975
  Stop codon: 2765–2767
  GA mutation to remove *Pst* I site: 2155
  CØA (Arg to Ser) mutation to remove *Bss*HII site: 2501
- pUC plasmid replication origin: 3352–3995

Propagation in *E. coli*

- Suitable host strains: Stellar™ Competent Cells.
- 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.

This document has been reviewed and approved by the Clontech Quality Assurance Department.