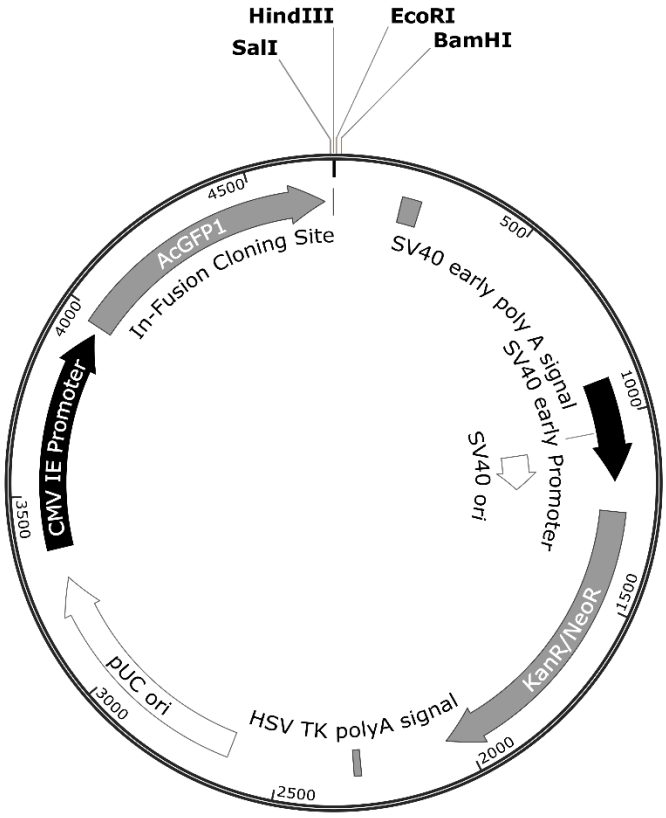
pAcGFP1-C In-Fusion® Ready Vector

Catalog No.
632500



pAcGFP1-C In-Fusion Ready Vector
4707 bp

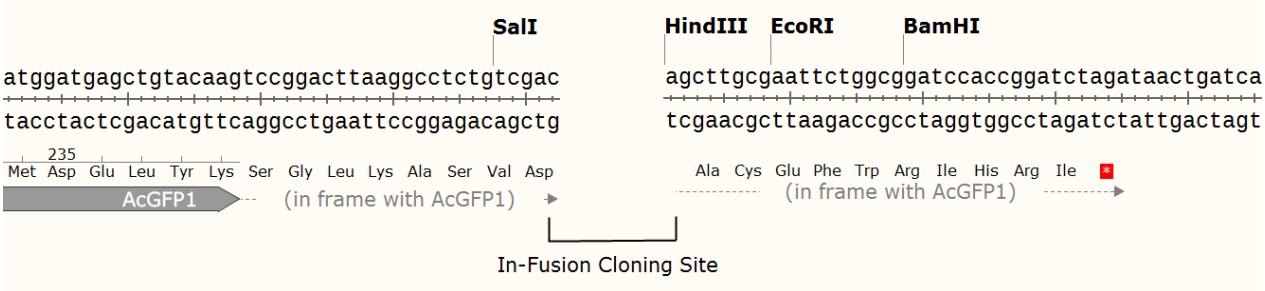


Figure 1. pAcGFP1-C In-Fusion Ready Vector map and In-Fusion cloning site. This vector is provided pre-linearized. Both the SalI and HindIII sites are incomplete. The last and first nucleotides for SalI and HindIII, respectively, are lost in vector linearization, and therefore not included in the vector backbone. These nucleotides must be introduced by the PCR primers designed for the amplification of the gene of interest. (See primer details on the following page.)

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(072816)

Vector Map

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Description

This linearized vector allows direct cloning of PCR products without any need for restriction digest when used in conjunction with In-Fusion HD Cloning Plus. This is accomplished by the use of specific 15-nucleotide sequences within the sense and antisense amplification primers. These sequences overlap with the cut vector ends created by initial digestion with SalI and HindIII. The primers used to amplify In-Fusion Ready PCR products require the following 15 nucleotides on their 5' ends:

Sense primer: 5' - AAGGCCTCTGTCGAC - target sequence - 3'
Antisense primer: 5' - AGAATTCGCAAGCTT - target sequence - 3'

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

Location of Features: pAcGFP1-C In-Fusion Ready Vector

- Human cytomegalovirus (CMV) immediate early promoter: 3360–3948
  - Enhancer region: 3418–3824; TATA box: 3913–3919
  - Transcription start point: 3942
  - CØG mutation to remove Sac I site: 3928
- *Aequorea coerulescens* green fluorescent protein (AcGFP1) gene
  - Kozak consensus translation initiation site: 3965–3975
  - Start codon (ATG): 3972–3974; last codon: 4686–4688
  - Stop codons after In-Fusion Cloning site: 32–34; 36–38; 40–42
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 178–183 & 207–212; mRNA 3' ends: 216 & 212
- SV40 origin of replication: 1071–1206
- SV40 early promoter: 904–1172
- Kan^R/Neo^R (kanamycin/neomycin resistance gene):
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 1255–1257; stop codon: 2047-2049
  - GØA mutation to remove Pst I site: 1437
  - CØA Arg to Ser mutation to remove BssH II site: 1783
- ori (high-copy-number ColE1/pMB1/pBR322/pUC origin of replication): 2634–3277

NOTES:

The GenBank file provided for this vector does not show the overhangs of the cloning site. Complementary bases fill in the gaps of the sequence file, though they are not present in the actual linearized vector.

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.

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