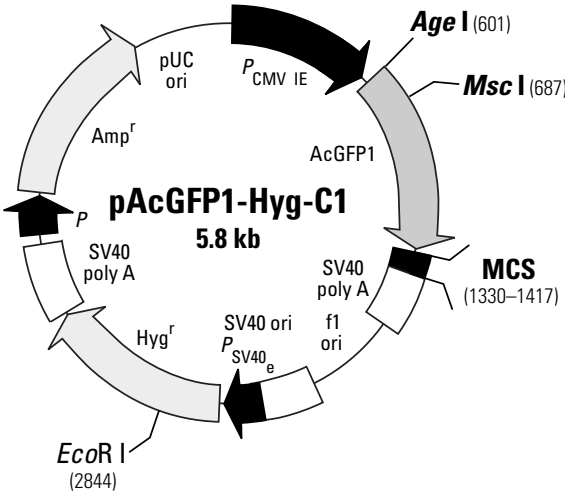
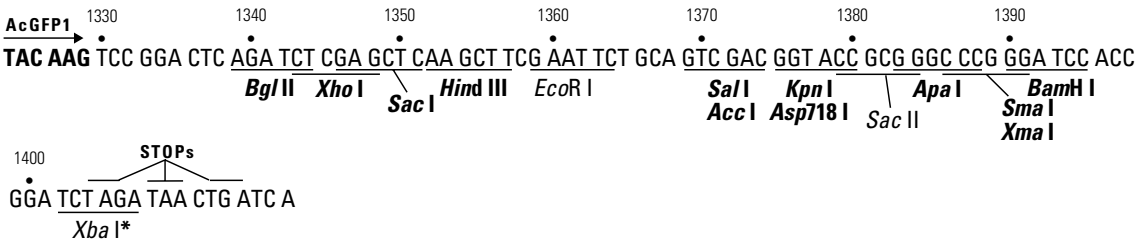
Vector Information

pAcGFP1-Hyg-C1 Vector Information PT3844-5

Catalog No. 632492





Restriction Map and Multiple Cloning Site (MCS) of pAcGFP1-Hyg-C1. Restriction sites shown in bold are unique. The *Xba* I site (*) is methylated in the DNA provided by Clontech Laboratories, Inc.. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and isolate fresh DNA.

Description

pAcGFP1-Hyg-C1 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-Hyg-C1 is located downstream of the AcGFP1 coding region, allowing the construction of a C-terminal fusion protein with AcGFP1 when genes are cloned 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 hygromycin resistance cassette (Hygʳ), consisting of the SV40 early promoter, the hygromycin resistance gene, and SV40 polyadenylation signals, allows stably transfected eukaryotic cells to be selected using hygromycin. A bacterial promoter upstream of the ampicillin gene expresses ampicillin resistance in *E. coli*. The pAcGFP1-Hyg-C1 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 C 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-Hyg-C1 so that it is in frame with the AcGFP1 coding sequences, with no intervening in-frame stop codons. The recombinant AcGFP1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using hygromycin. pAcGFP1-Hyg-C1 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
- *Aequorea coerulescens* green fluorescent protein (AcGFP1) gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615
  Insertion of Val at position 2: 616–618
  Last amino acid of AcGFP1: 1327–1329
  Stop codons: 1404–1406, 1408–1410 & 1412–1414
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of AcGFP.)
- SV40 origin of replication: 2443–2578
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
  21-bp repeats: 2423–2443, 2444–2464, & 2466–2486
  Early promoter element: 2499–2505
  Major transcription start points: 2495, 2533, 2539 & 2544
- Hygromycin resistance gene:
  Start codon (ATG): 2600–2602; stop codon: 3623–3625
- SV40 early mRNA polyadenylation signal: 3772–3777 & 3801–3806; mRNA 3' ends: 3810 & 3822
- Bacterial promoter for expression of Amp^r gene:
  –35 region: 3972–3977; –10 region: 3995–4000
- Ampicillin resistance gene:
  Start codon (ATG): 4042–4044; stop codon: 4900–4902
- pUC plasmid replication origin: 5065–5708

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Reference

1. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.

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.