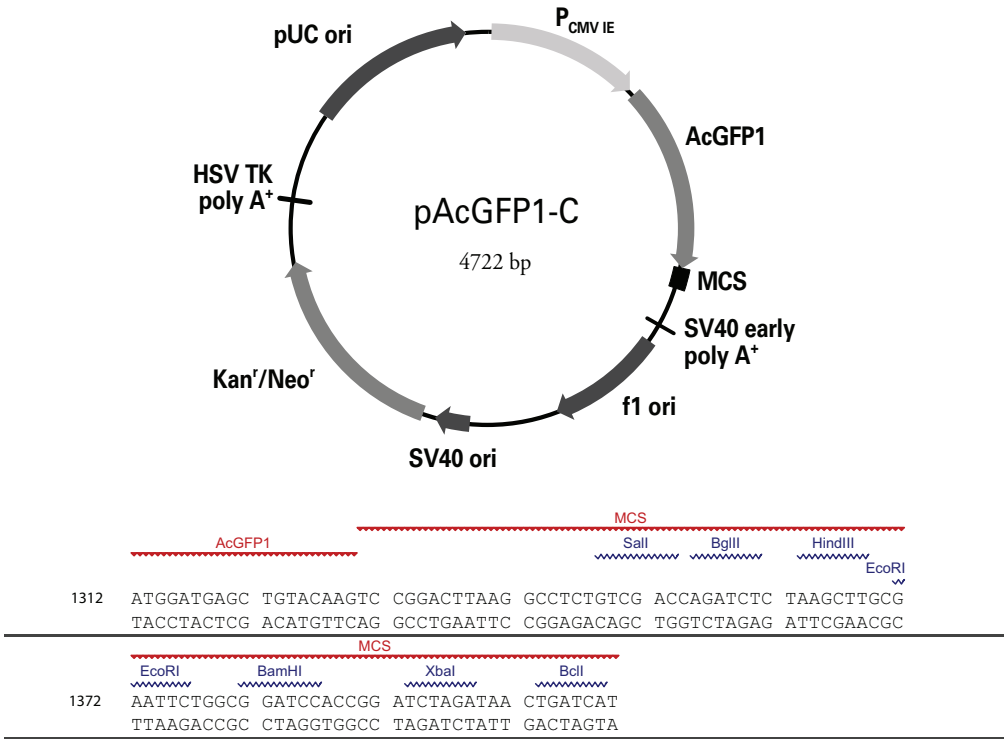
Vector Information

pAcGFP1-C Vector Information PT3933-5

Sold as part of Catalog No. 630458



Map of pAcGFP1-C Vector. All restriction sites shown are unique.

Description

pAcGFP1-C Vector is a mammalian expression vector that encodes a green fluorescent protein (GFP) from *Aequorea coerulescens.* The 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. Sequences flanking AcGFP1 have been converted to a Kozak consensus translation initiation site (1) to further increase translation efficiency in eukaryotic cells. A gene of interest can be added in-frame downstream of the AcGFP1 coding sequence and expressed as a fusion protein to the C-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 SV40 T-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 expresses kanamycin resistance in *E. coli.* pAcGFP-C Vector also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

Provided with the Matchmaker™ Chemiluminescent CoIP Vector Set (Cat. No. 630458), the pAcGFP1-C Vector allows the generation of a N-terminal AcGFP1-bait fusion. Any bait sequence in our current Matchmaker Yeast Two Hybrid System 3 pGBKT7 bait vector can be PCR-amplified using the AcGFP BD FWD/REV universal In-Fusion primer set, and directionally and efficiently cloned into the SalI/HindIII sites of the pAcGFP1-C using our In-Fusion Dry-Down PCR Cloning Kit (Cat. No. 639602). The recombinant AcGFP1 vector can be transfected into mammalian cells using any standard transfection method. The resulting expressed AcGFP1-bait fusion protein will automatically be in-frame and can be used to monitor transfection efficiency as well as AcGFP1-bait fusion expression and localization *in vivo* since the AcGFP1-bait fusion retains the fluorescent properties of the native AcGFP1 protein.

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Location of features

- P CMV ie
  Start: 1 End: 589
- AcGFP1
  Start: 613 End: 1329
- SV40 early poly A
  Start: 1541 End: 1591
- f1 ori
  Start: 1638 End: 2093
- SV40 ori
  Start: 2434 End: 2569
- Kan R/Neo R
  Start: 2618 End: 3410
- Kanamycin/neomycin resistance gene:
  Neomycin phosphotransferase coding sequences:
  start codon (ATG): 2629–2631
  stop codon: 3421–3423
  G to A mutation to remove Pst I site: 2811
  C to A mutation to remove BssH II site: 3157
- HSV TK poly A
  Start: 3648 End: 3666
- pUC ori
  Start: 3997 End: 4640

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 JM101 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
- Plasmid incompatibility group: pMB1/ColE1

References

1. Kozak, M. (1987) Nucleic Acids Res. **15**(20):8125–8148.
2. Gorman, C. (1985) In *DNA cloning: A practical approach, Vol. II.* Ed. D.M. Glover. (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. This vector has not been completely sequenced.

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CMV Sequence

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