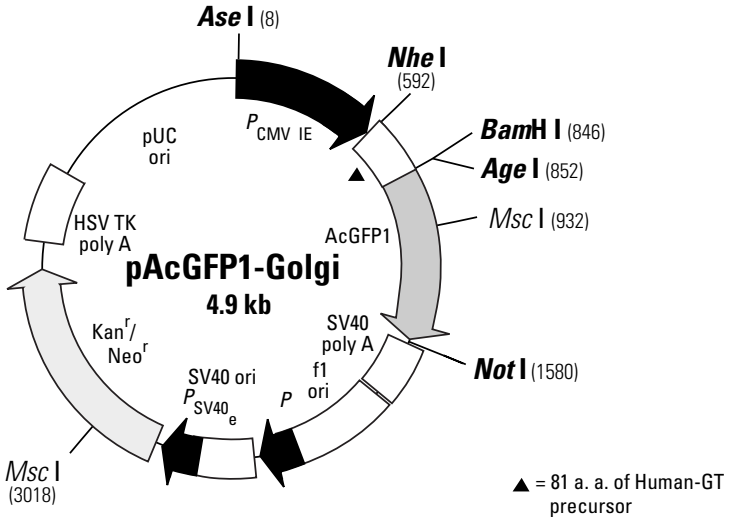
Vector Information

pAcGFP1-Golgi Vector Information PT3776-5

Cat. No. 632464



Restriction map of pAcGFP1-Golgi Vector. Restriction sites in bold are unique.

Description

pAcGFP1-Golgi Vector encodes a fusion protein consisting of the N-terminal 81 amino acids of the human beta 1,4-galactosyltransferase (GT; 1) directly upstream of the green fluorescent protein (GFP) from *Aequorea coerulescens* (AcGFP1). The human beta 1,4-galactosyltransferase sequence contains a membrane-anchoring signal peptide that targets the protein to the transmedial region of the Golgi aparatus (2–4). AcGFP1 (excitation maximum = 475 nm; emission maximum = 505 nm) contains silent mutations that create an open reading frame comprised almost entirely of optimized human codons. These changes increase the translational efficiency of the AcGFP1 mRNA and, consequently, the expression of AcGFP1 in mammalian and plant cells.

SV40 polyadenylation signals downstream of the protein coding region direct proper processing of the 3' end of the mRNA. The vector contains an SV40 origin for replication and a neomycin resistance (Neo^r) gene for selection (using G418) in eukaryotic cells (5). A bacterial promoter (*P*) upstream of Neo^r expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

pAcGFP1-Golgi Vector is designed for fluorescent labeling of the Golgi apparatus. pAcGFP1-Golgi can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (5). pAcGFP1-Golgi is not intended as a cloning vector; however, the backbone does contain unique restriction sites upstream and downstream of the AcGFP1-Golgi sequence which permit its excision.

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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
- Human beta 1,4-GT-AcGFP1 fusion
  Start codon: 597–599
  N-terminal 81 a. a. of human beta 1,4-GT: 597–842
  Start of AcGFP1 coding sequences (ATG): 858–1574
  Insertion of Val at position 2: 861–863
  Stop codon: 1575–1577
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1730–1735 & 1759–1764; mRNA 3' ends: 1768 &1780
- f1 single-strand DNA origin: 1827–2282 (packages the noncoding strand of AcGFP1-Golgi)
- Bacterial promoter for expression of Kan^r gene.
  –35 region: 2344–2349; –10 region: 2367–2372; Transcription start point: 2379
- SV40 origin of replication: 2623–2758
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2456–2527, 2528–2599
  21-bp repeats: 2603–2623, 2624–2644 & 2646–2666
  Early promoter element: 2679–2685
  Major transcription start points: 2675, 2713, 2719 & 2724
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2807–2809; stop codon: 3599–3601
  G→A mutation to remove *Pst* I site: 2989
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3335
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3837–3842 & 3850–3855
- pUC plasmid replication origin: 4186–4829

Propagation in *E. coli*

- Suitable host strains: Fusion-Blue™ competent cells (Cat. No. 636700), DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host such as JM109 or XL1-Blue that contains an F plasmid.
- 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. Watzele, G. & Berger, E. G. (1990) *Nucleic Acids. Res.* **18**:7174.
2. Llopis, J., *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**:6803–6808.
3. Yamaguchi, N. & Fukuda, M. N. (1995) *J. Biol. Chem.* **270**:12170–12176.
4. Gleeson, P. A., *et al.* (1994) *Glycoconjugate J.* **11:**381–394.
5. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (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 Laboratories, Inc. This vector has not been completely sequenced.