Vector Info

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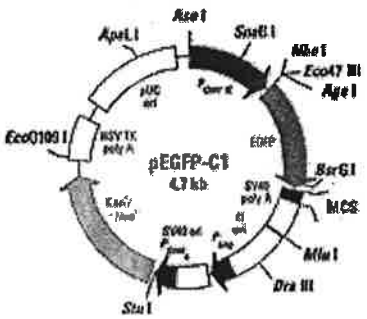
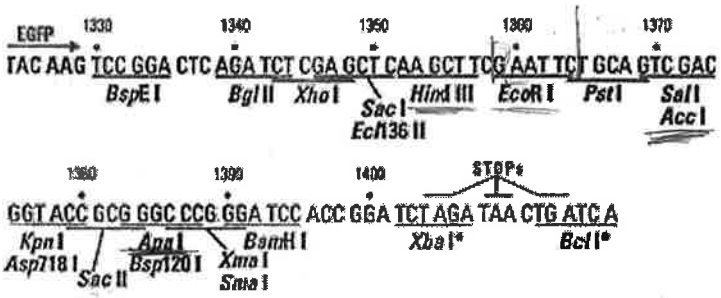
pEGFP-C1





Restriction Map and Multiple Cloning Site of pEGFP-C1. (Unique restriction sites are in bold.) The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by CLONTECH. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*- host and make fresh DNA.

Note: The vector sequence file has been compiled from information in the sequence database, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.

Description

TOP

pEGFP-C1 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-C1 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-C1 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40

T-antigen. A neomycin resistance cassette (neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-C1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Vector	Size	Cat. #	GenBank Accession #
pEGFP-C1	20 µg	6084-1	U55763

Use

Fusions to the C-terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-C1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-C1 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

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
- Enhanced green fluorescent protein gene
  - Kozak consensus translation initiation site: 606–616
  - Start codon (ATG): 613–615; Stop codon: 1408–1410
  - Insertion of Val at position 2: 616–618
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
  - His-231 to Leu mutation (A–>T): 1307
  - Last amino acid in wild-type GFP: 1327–1329
- 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 EGFP.)
- Bacterial promoter for expression of Kan^r gene
  - –35 region: 2164–2169; –10 region: 2187–2192
  - Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter