pEGFP-C1 Vector Information

Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C1. All restriction sites shown are unique. The Xba I and Bcl I sites (*) are methylated in the DNA provided by BD Biosciences 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.

Description

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 virus 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.

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**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’ gene**  
  –35 region: 2164–2169; –10 region: 2187–2192  
  Transcription start point: 2199
- **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
- **Kanamycin/neomycin resistance gene**  
  Neomycin phosphotransferase coding sequences:  
  Start codon (ATG): 2627–2629; stop codon: 3419–3421  
  G→A mutation to remove *Pst I* site: 2809  
  C→A (Arg to Ser) mutation to remove *Bss H II* site: 3155
- **Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal**  
  Polyadenylation signals: 3657–3662 & 3670–3675
- **pUC plasmid replication origin**: 4006–4649

**Primer Locations**
- **EGFP-N Sequencing Primer (#6479-1)**: 679–658
- **EGFP-C Sequencing Primer (#6478-1)**: 1266–1287

**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 JM109 or XL1-Blue.
- **Selectable marker**: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- ***E. coli* replication origin**: pUC  
  - Copy number: ≈500  
  - Plasmid incompatibility group: pMB1/ColE1

**References**
**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 BD Biosciences Clontech. This vector has not been completely sequenced.

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