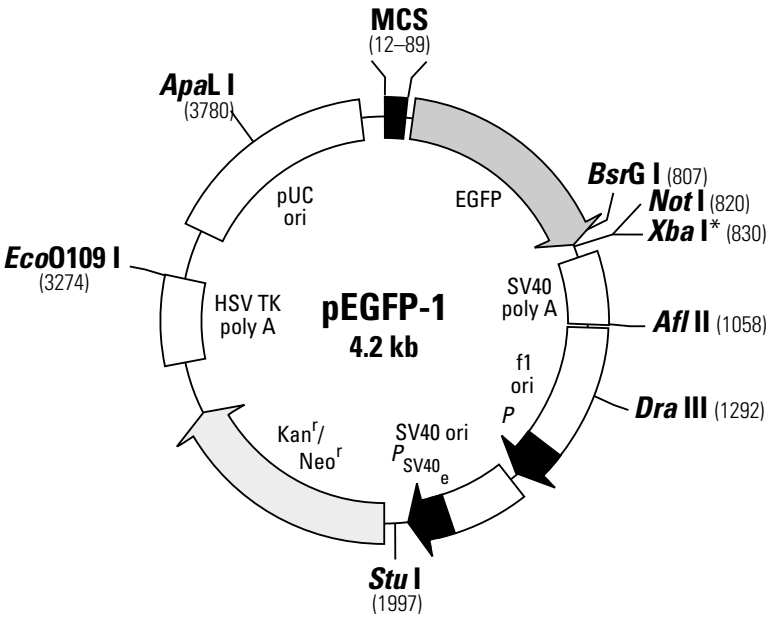
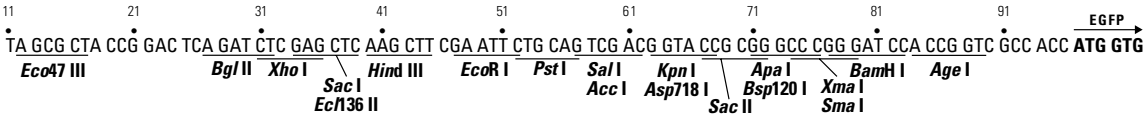
Vector Information

pEGFP-1 Vector Information PT3026-5

GenBank Accession #: U55761 Catalog #6086-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-1. All restriction sites shown are unique. The *Not* I site follows the EGFP stop codon. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pEGFP-1 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-1 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. pEGFP-1 is a promoterless EGFP vector which can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the MCS located upstream of the EGFP coding sequence. 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) allows stably transfected eukaryotic cells to be selected using G418. The Neo^r cassette consists 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. A bacterial promoter upstream of this cassette confers kanamycin resistance in *E. coli*. The pEGFP-1 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

EGFP can be used as an *in vivo* reporter of gene expression (2). Promoters should be cloned into the pEGFP-1 MCS upstream from the EGFP coding sequences. Without the addition of a functional promoter, this vector will not express EGFP. 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).

Location of features

- MCS: 12–89
- Enhanced green fluorescent protein (EGFP) gene
  Kozak consensus translation initiation site: 90–100
  Start codon (ATG): 97–99; Stop codon: 814–816
  Insertion of Val at position 2: 100–102
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 289–294
  His-231 to Leu mutation (A→T): 791
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 970–975 & 999–1004
  mRNA 3' ends: 1008 & 1020
- f1 single-strand DNA origin: 1067–1522
  (Packages noncoding strand of EGFP.)
- Ampicillin resistance (β-lactamase) promoter
  –35 region: 1584–1589; –10 region: 1607–1612
  Transcription start point: 1619
- SV40 origin of replication: 1863–1998
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 1694–1767 & 1768–1839
  21-bp repeats: 1843–1863, 1864–1884 & 1886–1906
  Early promoter element: 1919–1925
  Major transcription start points: 1915, 1953, 1959 & 1964
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2047–2049; stop codon: 2839–2841
  G→A mutation to remove *Pst* I site: 2229
  C→A (Arg→Ser) mutation to remove *Bss*H II site: 2575
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3077–3082 & 3090–3095
- pUC plasmid replication origin: 3426–4069

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 163–142
- EGFP-C Sequencing Primer (#6478-1): 750–771

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 XL-1 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

1. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
2. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
4. Cormack, B., *et al.* (1996) *Gene* (in press).
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
7. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.), 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 BD Biosciences Clontech. This vector has not been completely sequenced.

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