



Restriction Map and Multiple Cloning Site (MCS) of pEYFP-1. Unique restriction sites are in bold. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest this vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEYFP-1 encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains four amino acid substitutions previously published as GFP-10C (1). The fluorescence excitation maximum of EYFP is 513 nm, and the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513 nm, the E_m of EYFP is 36,500 $\text{cm}^{-1}\text{M}^{-1}$ and the fluorescence quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence level observed from EYFP is roughly equivalent to that from EGFP.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

pEYFP-1 is a promoterless EYFP vector which can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the MCS located upstream of the EYFP coding sequence. Without the insertion of a functional promoter in this site, the vector will not express EYFP.

The vector contains an SV40 origin of replication and a neomycin resistance (*Neo*^r) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of *Neo*^r expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

The recombinant EYFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4). EGFP, EYFP, and EBFP variants can be used independently or in combination with each other for flow cytometry analysis.



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Location of features:

- MCS: 12–89
- Enhanced yellow fluorescent protein (EYFP) 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
 - GFP-10C mutations (Ser-65 to Gly: 292–294; Val-68 to Leu: 301–303; Ser-72 to Ala: 313–315; Thr-203 to Tyr: 706–708)
 - 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 EYFP.)
- Bacterial promoter for expression of Kan^r gene
 - 35 region: 1584–1589; –10 region: 1607–1612
 - Transcription start point: 1621
- SV40 origin of replication: 1863–1998
- SV40 early promoter
 - Enhancer (72-bp tandem repeats): 1696–1767 & 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*:

- Recommended host strain: DH5 α , 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 μ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Ormö, M. *et al.* (1996) *Science* **273**:1392–1395.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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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