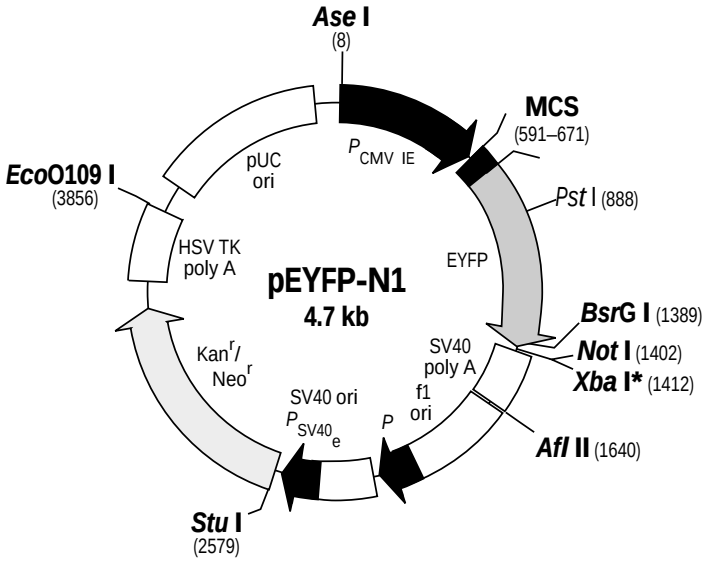

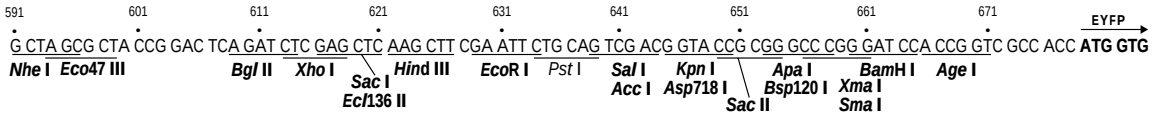

Restriction Map and Multiple Cloning Site (MCS) of pEYFP-N1. Unique restriction sites are in bold. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest this vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEYFP-N1 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 $cm^{-1}M^{-1}$ 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.

The MCS in pEYFP-N1 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the EYFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EYFP if they are in the same reading frame as EYFP and there are no intervening stop codons. The inserted gene should include an initiating ATG codon. EYFP with N-terminal fusion moieties retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin of replication and a neomycin resistance (Neo^r) gene for selection (using G418) in mammalian cells. A bacterial promoter upstream of this cassette (*P*) 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). pEYFP-N1 can also be used simply to express EYFP in a cell line of interest (e.g., as a transfection marker). EGFP, EYFP, and EBFP variants can be used independently or in combination for flow cytometry analysis.

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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
- MCS: 591–671
- Enhanced yellow fluorescent protein (EYFP) gene
  Kozak consensus translation initiation site: 672–682
  Start codon (ATG): 679–681; stop codon: 1396–1398
  Insertion of Val at position 2: 682–684
  GFP-10C mutations (Ser-65 to Gly: 874–876; Val-68 to Leu: 883–885; Ser-72 to Ala: 895–897; Thr-203 to Tyr: 1288–1290)
  His-231 to Leu mutation (A→T): 1373
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1552–1557 & 1581–1586
  mRNA 3' ends: 1590 & 1602
- f1 single-strand DNA origin: 1649–2104
  (Packages the noncoding strand of EYFP.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2166–2171; –10 region: 2189–2194
  Transcription start point: 2201
- SV40 origin of replication: 2445–2580
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2278–2349 & 2350–2421
  21-bp repeats: 2425–2445, 2446–2466, & 2468–2488
  Early promoter element: 2501–2507
  Major transcription start points: 2497, 2535, 2541 & 2546
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2629–2631; stop codon: 3421–3423
  G→A mutation to remove *Pst* I site: 2811
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3157
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3659–3664 & 3672–3677
- pUC plasmid replication origin: 4008–4651

Primer Locations:
- EGFP-N Sequencing Primer (#6479-1): 745–724
- EGFP-C Sequencing Primer (#6478-1): 1332–1353

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 JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μg/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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