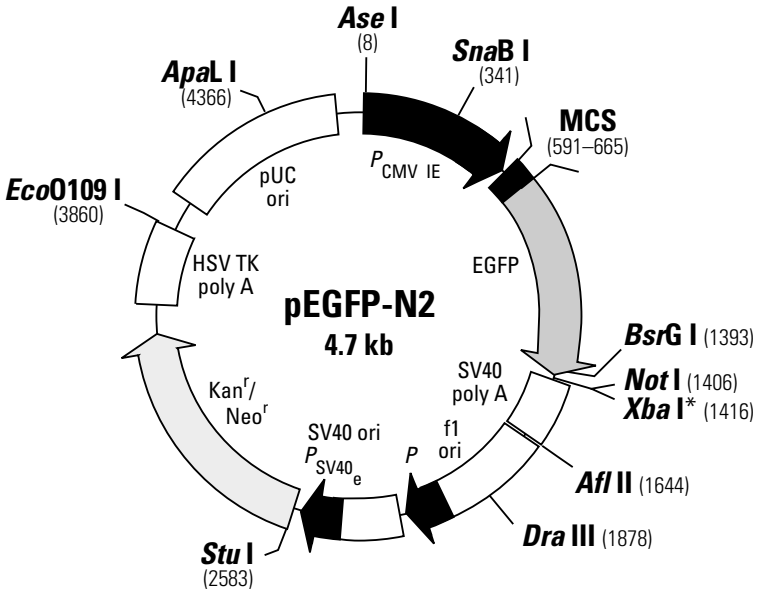
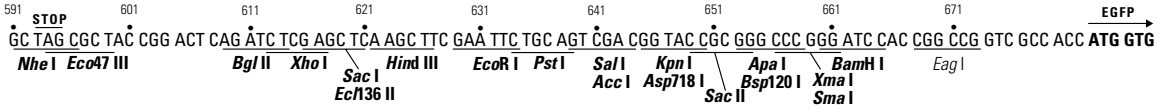
Vector Information

pEGFP-N2 Vector Information PT3053-5

GenBank Accession #: U57608 Catalog #6081-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N2 Unique restriction sites are in bold. The *Not* I site follows the EGFP stop codon. The *Nhe* I site cannot be used for fusions since it contains an in-frame stop codon. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences Clontech. 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-N2 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-N2 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-N2 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N 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 (Neor), 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-N2 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR29968; published 03 October 2002)

Use:
Fusions to the N 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-N2 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. 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-N2 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
- MCS: 591–665
- Enhanced green fluorescent protein gene
  Kozak consensus translation initiation site: 676–686
  Start codon (ATG): 683–685; Stop codon: 1400–1402
  Insertion of Val at position 2: 686–688
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 875–880
  His-231 to Leu mutation (A→T): 1377
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1556–1561 & 1585–1590; mRNA 3' ends: 1594 & 1606
- f1 single-strand DNA origin: 1653–2108 (Packages the noncoding strand of EGFP)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2170–2175; –10 region: 2193–2198
  Transcription start point: 2205
- SV40 origin of replication: 2449–2584
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2282–2353 & 2354–2425
  21-bp repeats: 2429–2449, 2450–2470 & 2472–2492
  Early promoter element: 2505–2511
  Major transcription start points: 2501, 2539, 2545 & 2550
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2633–2635; stop codon: 3425–3427
  G→A mutation to remove *Pst* I site: 2815
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3161
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3663–3668 & 3676–3681
- pUC plasmid replication origin: 4012–4655

Primer Locations:
- EGFP-N Sequencing Primer (#6479-1): 749–728
- EGFP-C Sequencing Primer (#6478-1): 1336–1357

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:
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* **173**:33–38.
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. Glover, D. M. (IRL Press, Oxford, UK) 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.