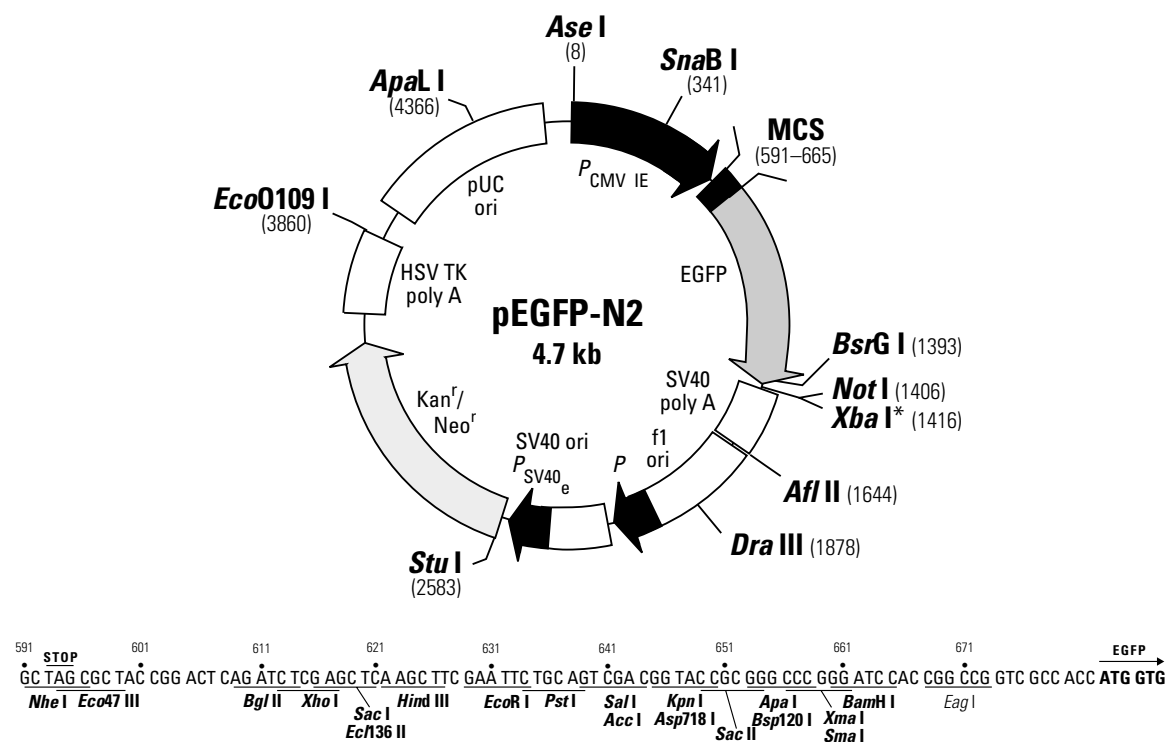




Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N2 Unique restriction sites are in bold. The *Not* I site follows the EGFP stop codon. The *Nhe* I site cannot be used for fusions since it contains an in-frame stop codon. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences 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 (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-N2 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

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: DH5 α , 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 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: $\approx$ 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.

Notice to Purchaser

Use of BD Biosciences Clontech's Living Colors™ products containing DNA sequences coding for mutant *Aequorea victoria* green fluorescent protein (GFP) variants or proteins thereof requires a license from Amersham Biosciences under U.S. Patent Nos. 5,625,048; 5,777,079; 6,054,321 and other pending U.S. and foreign patent applications. In addition, certain BD Biosciences Clontech products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

Not-For-Profit research institutes or entities are granted an automatic license with the purchase of this product for use in non-commercial internal research purposes, the terms of which are disclosed in detail in the license that accompanies the shipment of this product. Such license specifically excludes the right to sell or otherwise transfer this product or its components to third parties.

For-Profit research institutes or entities must obtain a license from Amersham Biosciences. E-mail: gfp@amershambiosciences.com

Please contact BD Biosciences Clontech directly for any other assistance, including purchasing and technical support. All companies and institutions purchasing Living Colors™ products will be included in a quarterly report to Aurora Biosciences, as required by the BD Biosciences Clontech/Aurora Biosciences license agreement.

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. BD Biosciences Clontech products may not be resold, modified for resale, or used to manufacture commercial products without written approval of BD Biosciences Clontech.

© 2002, Becton, Dickinson and Company