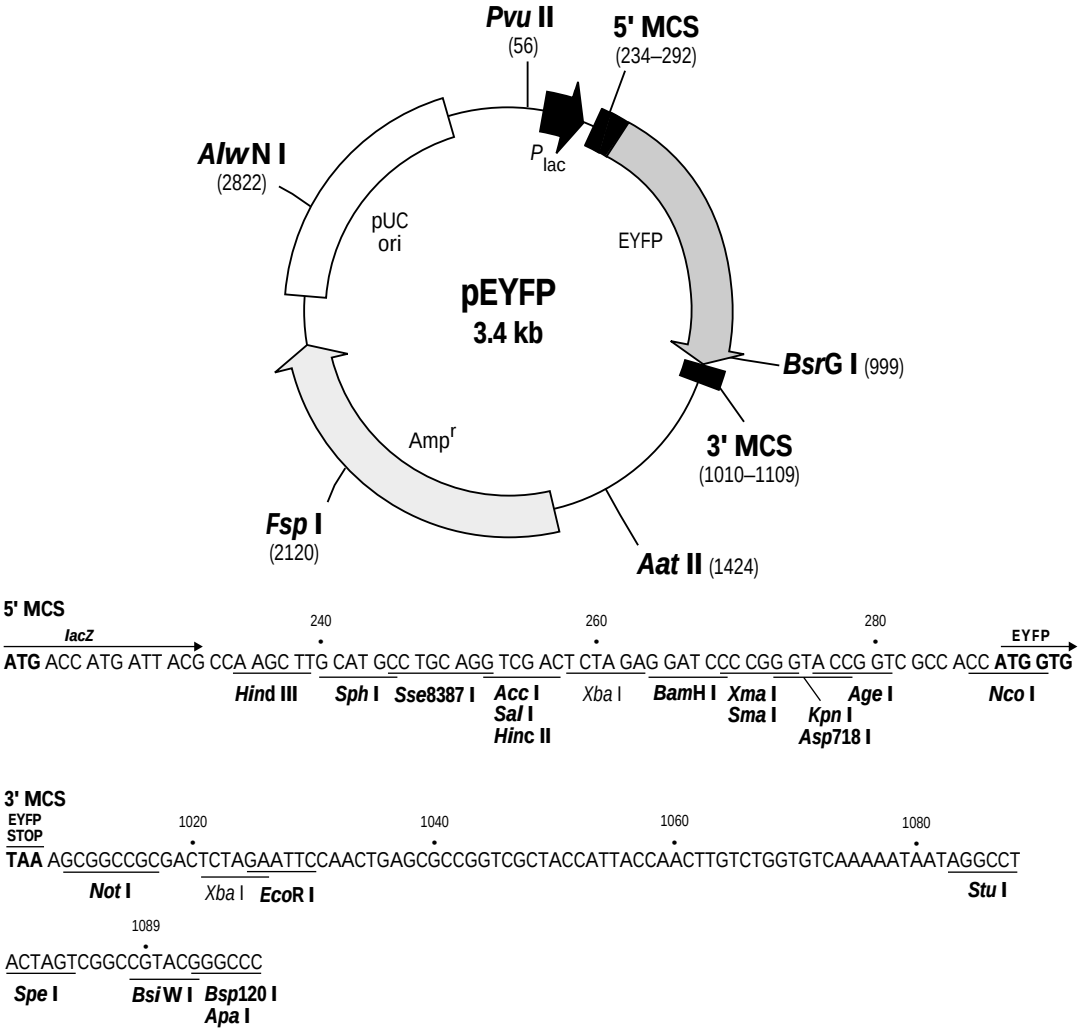

Restriction map and multiple cloning site (MCS) of pEYFP. Unique restriction sites are in bold. The *Xba* I sites in the 5' and 3' MCSs can be used together to excise the EYFP gene.

Description:

pEYFP encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (1): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and Thr-203 to Tyr. 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 fluorescent quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence observed from EYFP is roughly equivalent to that from EGFP.

A mixture of EYFP- and EGFP-expressing cells can be sorted by flow cytometry using a single excitation wavelength (i.e., 488 nm). EYFP emission is detected using a 525-nm dichroic shortpass mirror and a 530/30-nm bandpass filter; EGFP emission is detected using a 510/20-nm bandpass filter.

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.

(PR29943; published 03 October 2002)

The EYFP gene is flanked at the 5' and 3' ends by the two MCSs of the pUC19 derivative pPD16.43 (4). Thus, the EYFP coding sequence can be easily excised from the vector or amplified by PCR. In *E. coli*, EYFP is expressed from the *lac* promoter as a fusion with several additional amino acids, including the first five amino acids of the LacZ protein. Note, however, that if you excise the EYFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., nonfusion) EYFP protein. The pUC19 backbone of EYFP provides a high-copy-number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

Location of features:
- *lac* promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lacZ*–EYFP fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; Stop codon: 1006–1008
- 5' multiple cloning site: 234–281
- Enhanced yellow fluorescent protein (EYFP) gene
  - Kozak consensus translation initiation site: 282–292
  - Start codon (ATG): 289–291; stop codon: 1006–1008
  - Insertion of Val at position 2: 292–294
  - GFP-10C mutations (Ser-65 to Gly: 484–486; Val-68 to Leu: 493–495; Ser-72 to Ala: 505–507; Thr-203 to Tyr: 898–900)
  - His-231 to Leu mutation (A→T): 983
- 3' multiple cloning site: 1010–1109
- Ampicillin resistance gene
  - Promoter: –35 region: 1485–1490; –10 region: 1508–1513
  - Transcription start point: 1520
  - Ribosome binding site: 1543–1547
  - β-lactamase coding sequences
    - Start codon (ATG): 1555–1557; stop codon: 2413–2415
    - β-lactamase signal peptide: 1555–1623
    - β-lactamase mature protein: 1624–2412
- pUC plasmid replication origin: 2563–3206

Primer location:
- EGFP-N Sequencing Primer (#6479-1): 355–334
- EGFP-C Sequencing Primer (#6478-1): 942–963

Propagation in *E. coli*:
- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *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. Fire, A., *et al.* (1990) *Gene* **93**:189–198.

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.