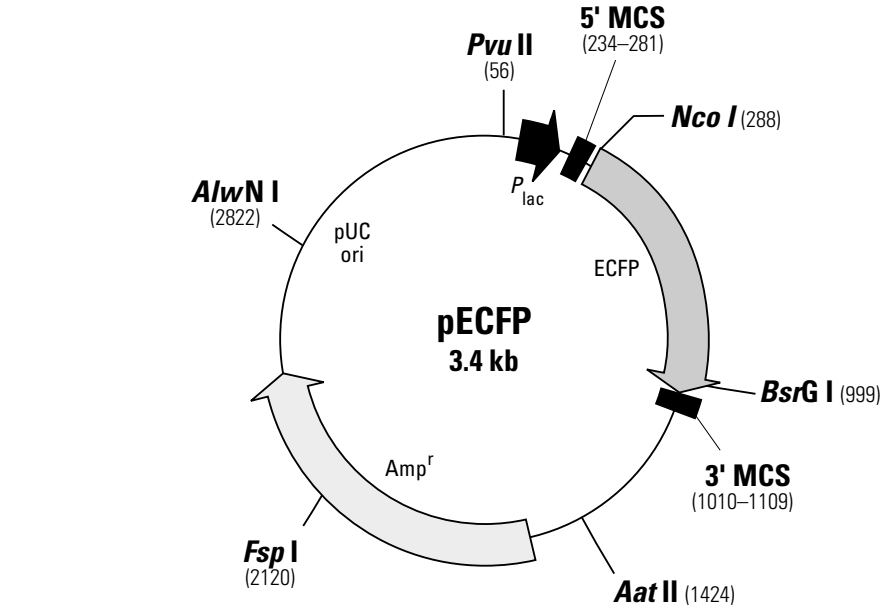

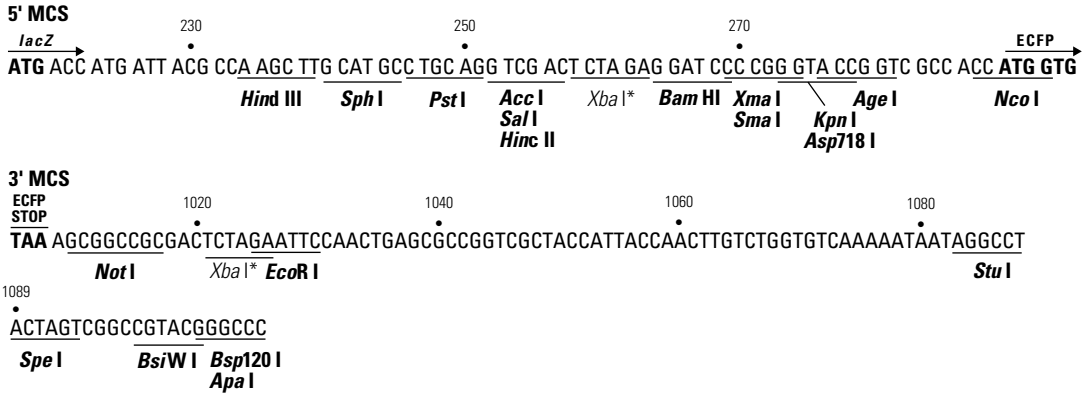

Restriction map and multiple cloning site (MCS) of pECFP. Unique restriction sites are in bold. The *Xba* I sites in the 5' and 3' MCSs can be used to excise the ECFP gene.

Description:

pECFP encodes an enhanced cyan fluorescent variant of the *Aequorea victoria* green fluorescent protein gene (GFP). The ECFP gene contains six amino acid substitutions. The Tyr-66 to Trp substitution gives ECFP fluorescence excitation (major peak at 433 nm and a minor peak at 453 nm) and emission (major peak at 475 nm and a minor peak at 501 nm) similar to other cyan emission variants (1–3). The other five substitutions (Phe-64 to Leu; Ser-65 to Thr; Asn-146 to Ile; Met-153 to Thr; and Val-163 to Ala) enhance the brightness and solubility of the protein, primarily due to improved protein folding properties and efficiency of chromophore formation (2, 4, 5).

In addition to the chromophore mutations, ECFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (6). Furthermore, upstream sequences flanking ECFP have been converted to a Kozak consensus translation initiation site (7). These changes increase the translational efficiency of the ECFP mRNA and consequently the expression of ECFP in mammalian and plant cells.

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The ECFP gene is flanked at the 5' and 3' ends by the two MCSs of the pUC19 derivative pPD16.43 (8). Thus, the ECFP coding sequence can be easily excised from the vector or amplified by PCR. In *E. coli*, ECFP 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 ECFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., nonfusion) ECFP protein. The pUC19 backbone of ECFP provides a high-copy-number origin of replication and an ampicillin resistance gene for propagation and selection, respectively 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*–ECFP 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 cyan fluorescent protein (ECFP) 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
  - ECFP mutations (Phe-64 to Leu, Ser-65 to Thr, and Tyr-66 to Trp): 481–489; Asn-146 to Ile: 727–729; Met-153 to Thr: 748–750; Val-163 to Ala: 778–780.
  - 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 Locations:

- 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

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8. 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.

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