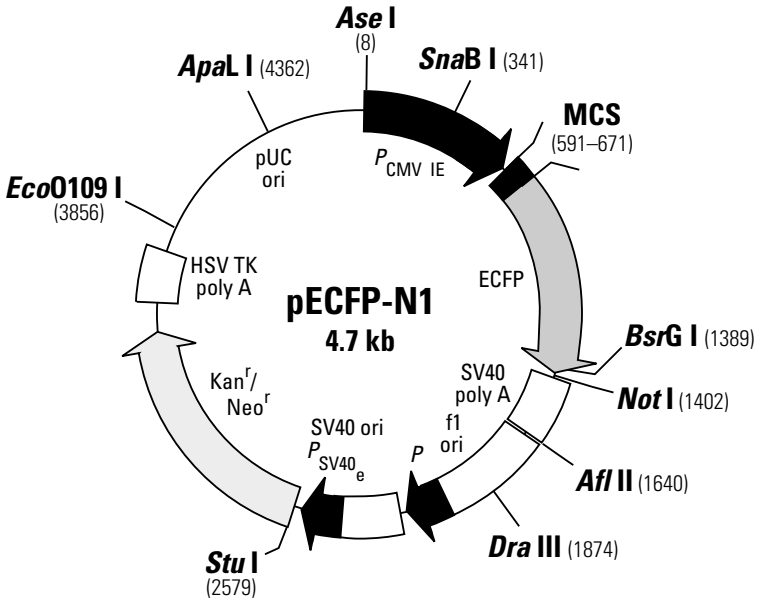

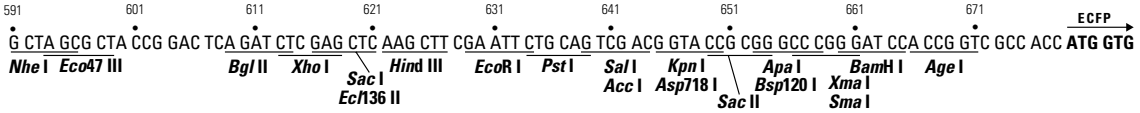

Restriction Map and Multiple Cloning Site (MCS) of pECFP-N1. All restriction sites shown are unique.

Description

pECFP-N1 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, 2). 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 (1, 3, 4). In addition to the chromophore mutations, ECFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (5, 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.

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

Use

The recombinant ECFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (8). pECFP-N1 can also be used simply to express ECFP in a cell line of interest (e.g., as a transfection marker). ECFP can be used for double-labeling experiments together with EYFP using standard fluorescence microscopy and the appropriate filter sets.

(PR29991; published 04 October 2002)

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 cyan fluorescent protein (ECFP) 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
  - ECFP mutations:
    - Phe-64 to Leu, Ser-65 to Thr, and Tyr-66 to Trp: 871–879
    - Asn-146 to Ile: 1117–1119
    - Met-153 to Thr: 1138–1140
    - Val-163 to Ala: 1168–1170
  - 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 ECFP.)
- 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/Col E1

References

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8. 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.