



Restriction map and multiple cloning site (MCS) of pECFP-C1. Unique restriction sites are in bold. The *Xba I* and *Bcl I* sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pECFP-C1 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.

The MCS in pECFP-C1 is between the ECFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of ECFP if they are in the same reading frame as ECFP and there are no intervening in-frame stop codons. ECFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (Neo^r) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of Neo^r 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.

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The recombinant ECFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (8). pECFP-C1 can also be used simply to express ECFP 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
- Enhanced cyan fluorescent protein gene
Kozak consensus translation initiation site: 606–616
Start codon (ATG): 613–615; stop codon: 1408–1410
Insertion of Val at position 2: 616–618
ECFP mutations (Phe-64 to Leu; Ser-65 to Thr; and Tyr-66 to Trp): 805–813; Asn-146 to Ile: 1051-1053;
Met-153 to Thr: 1072–1074; Val-163 to Ala: 1102-1104
His-231 to Leu mutation (A→T): 1307
Last amino acid in ECFP coding region: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of ECFP.)
- Bacterial promoter for expression of Kan^r gene.
–35 region: 2164–2169; –10 region: 2187–2192
Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
21-bp repeats: 2423–2443, 2444–2464 & 2466–2486
Early promoter element: 2499–2505
Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2627–2629; stop codon: 3419–3421
G→A mutation to remove *Pst* I site: 2809
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

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:

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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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