



**Restriction Map of pEYFP-Mito.** All sites shown are unique. 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*<sup>-</sup> host and isolate fresh DNA.

### Description

pEYFP-Mito encodes a fusion of EYFP and the mitochondrial targeting sequence from subunit VIII of human cytochrome c oxidase (1, 2). The EYFP gene (enhanced yellow fluorescent protein) contains four amino acid substitutions previously published as GFP-10C (3), which shift the emission of the chromophore from green to yellow-green. The fluorescence excitation maximum of EYFP is 513 nm, and the emission spectrum has a peak at 527 nm. In addition to the four chromophore mutations, the coding sequence of the EYFP gene contains more than 190 silent base changes corresponding to human codon-usage preferences (4), which increase the translational efficiency of the EYFP transcript.

SV40 polyadenylation signals downstream of the EYFP-Mito fusion direct proper processing of the 3' end of the EYFP-Mito mRNA. The vector backbone also contains an SV40 origin for replication in any mammalian cell line that expresses the SV40 T-antigen. A neomycin resistance cassette (Neo<sup>r</sup>), 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, allow stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*. The pEYFP-Mito backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

**Use**

The pEYFP-Mito Vector is designed to be used for the fluorescent labeling of mitochondria (5). Fluorescence can be observed in living or fixed cells by microscopy, fluorometry, or flow cytometry. pEYFP-Mito can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (6).

**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
- Mitochondrial targeting sequence from subunit VIII of human cytochrome c oxidase  
Start codon (ATG): 597–599  
End of targeting sequence: 683
- Enhanced yellow fluorescent protein (EYFP) gene  
Start codon (ATG): 705–707; stop codon: 1422–1424  
Insertion of Val at position 2: 708–710  
GFP-10C chromophore mutations  
Ser-65 to Gly: 900–902; Val-68 to Leu: 909–911; Ser-72 to Ala: 921–923; Thr-203 to Tyr: 1314–1316  
His-231 to Leu mutation (A→T): 1399
- SV40 early mRNA polyadenylation signal  
Polyadenylation signals: 1578–1583 & 1607–1612; mRNA 3' ends: 1616 & 1628
- f1 single-strand DNA origin: 1675–2130 (packages the noncoding strand of EYFP-Mito)
- Bacterial promoter for expression of Kan<sup>r</sup> gene  
–35 region: 2192–2197; –10 region: 2215–2220  
Transcription start point: 2227
- SV40 origin of replication: 2471–2606
- SV40 early promoter  
Enhancer (72-bp tandem repeats): 2304–2375 & 2376–2447  
21-bp repeats: 2451–2471; 2472–2492 & 2494–2514  
Early promoter element: 2527–2533  
Major transcription start points: 2523, 2561, 2567 & 2572
- Kanamycin/neomycin resistance gene  
Neomycin phosphotransferase coding sequences:  
Start codon (ATG): 2655–2657; stop codon: 3447–3449  
G→A mutation to remove *Pst* I site: 2837  
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3183
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
Polyadenylation signals: 3685–3690 & 3698–3703
- pUC plasmid replication origin: 4034–4677

**Primer Locations**

- EGFP-N Sequencing Primer (#6479-1): 771–750; EGFP-C Sequencing Primer (#6478-1): 1358–1379

**Propagation in *E. coli***

- Suitable host strains: DH5 $\alpha$ , 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  $\mu$ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC; copy number:  $\approx$ 500
- Plasmid incompatibility group: pMB1/ColE1

**References**

1. Rizzuto, R., *et al.* (1995) *Curr. Biol.* **5**:635–642.
2. Rizzuto, R., *et al.* (1989) *J. Biol. Chem.* **264**:10595–10600.
3. Orm $\ddot{o}$ , M., *et al.* (1996) *Science* **273**:1392–1395.
4. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
5. Living Colors™ Subcellular Localization Vectors (October 1998) *Clontechniques XIII*(4):8–9.
6. 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.

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