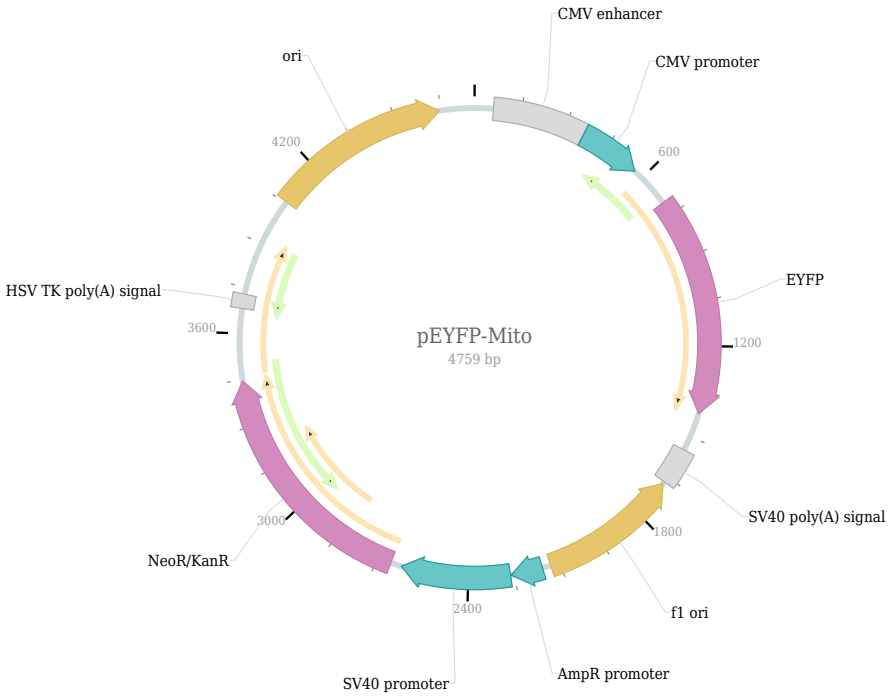
pEYFP-Mito

Vector ID	V001112
Antibiotic Resistance	Kanamycin
Length	4759 bp
Vector Type	Fluorescent Protein Reporter Vectors

Product Information

Replication Origin	ori
Selection Marker	Neomycin
Promoter	CMV
Cloning Method	Enzyme digestion and ligation
Fusion Tag	EYFP
Growth Strain(s)	stbl3
Growth Temperature	37℃
Comment	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 r), 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.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).

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 μl for 5 μg plasmid, and 100 μl for 100 μg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.