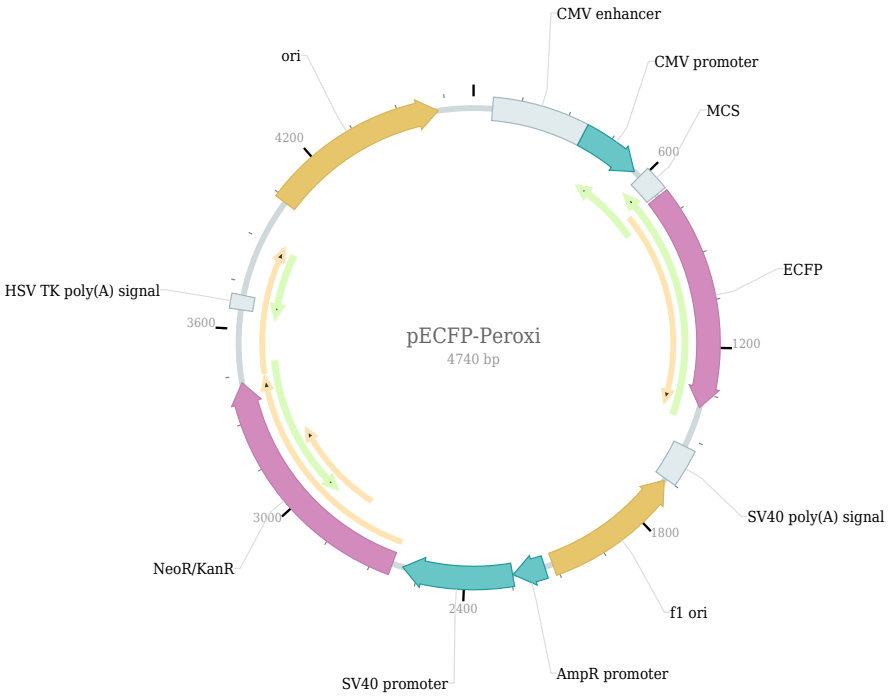
pECFP-Peroxi

Vector ID	V001407
Antibiotic Resistance	Kanamycin
Length	4740 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	ECFP
Comment	pECFP-Peroxi encodes a fusion of the enhanced cyan fluorescent variant of the Aequorea victoria green fluorescent protein gene (GFP) and the peroximal targeting signal 1 (PTS1). The PTS1 sequence is fused to the 3' end of ECFP and encodes the tripeptide SKL, which targets the fusion protein to the matrix of peroxisomes (1–4). 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 (5, 6). The other five substitutions enhance the brightness and solubility of the protein, primarily due to improved protein folding properties and efficiency of chromophore formation (5, 7, 8).In addition to the chromophore mutations, ECFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (9, 10). Furthermore, upstream sequences flanking ECFP have been converted to a Kozak consensus translation initiation site (11). These changes increase the translational efficiency of the ECFP-PTS1 mRNA and consequently the expression of ECFP-PTS1 in mammalian and plant cells.The expression of ECFP-PTS1 is driven by the immediate early promoter of CMV (PCMV IE). pECFPPeroxi contains an MCS at the 5' end of the ECFP-PTS1 sequence. Genes of interest cloned into the MCS will be expressed as fusions to the N-terminus of ECFP-PTS1 if they are in the same reading frame as ECFP-PTS1 and there are no intervening stop codons.The vector contains an SV40 origin of replication and a neomycin resistance (Neor) 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.Expression of ECFP-PTS1 can be used simply for the labeling of peroxisomes. The MCS at the 5' end of ECFP-PTS1 may be used to fuse genes of interest to ECFP-PTS1. The inserted gene should include the initiation ATG codon and be in frame with ECFP-PTS1. The recombinant ECFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (12). ECFP can be used for double-or triple-labeling experiments together with EYFP and DsRed using standard fluorescence microscopy and the appropriate filter sets.

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 ℃.