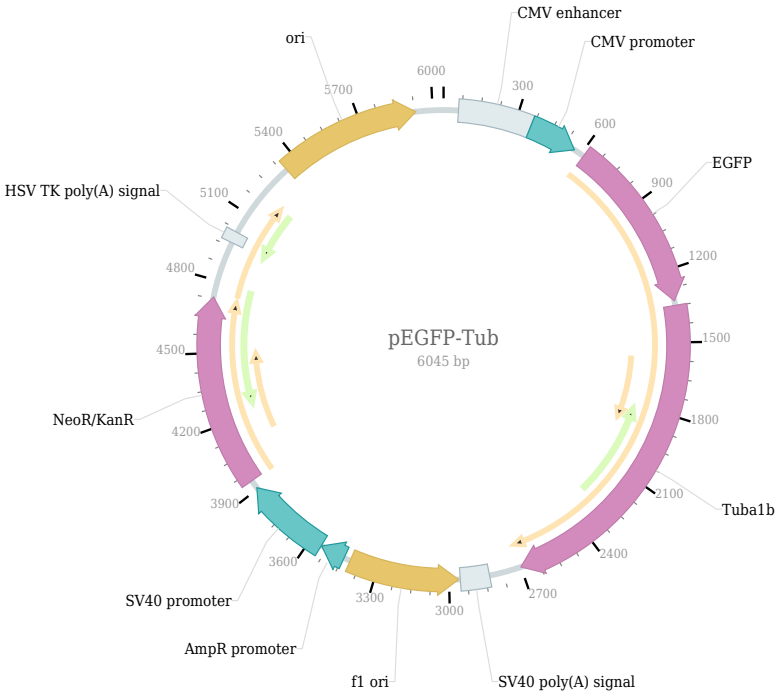
pEGFP-Tub

Vector ID	V000717
Antibiotic Resistance	Kanamycin
Length	6045 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	EGFP
Comment	pEGFP-Tub encodes a fusion protein consisting of the red-shifted, human codon-optimized variant of green fluorescent protein (EGFP; 1–3) and the gene encoding human α-tubulin (4, 5). EGFP, a derivative of the GFPmut1 variant (6), has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) This variant contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr, as well as more than 190 silent base changes that correspond to human codon-usage preferences (7). SV40 polyadenylation signals downstream of the EGFP-Tub fusion direct proper processing of the 3' end of the mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neor), 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 pEGFP-Tub backbone also provides a pUC origin of replication for propagation in E. coli and an f1 origin for single-stranded DNA production.The pEGFP-Tub Vector is designed for expressing the EGFP-tubulin fusion protein in mammalian cells. The protein incorporates directly into microtubules and thereby allows the observation of microtubules in living or fixed cells by fluorescence microscopy (5, 8). pEGFP-Tub can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (9).

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 ℃.