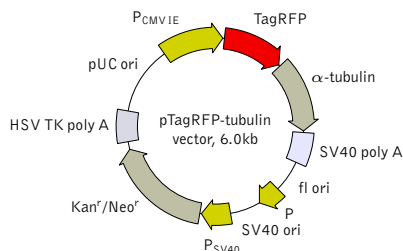


pTagRFP-tubulin vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.



For vector sequence, please visit our Web site at <http://www.evrogen.com/products/vectors.shtml>

Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 TagRFP
 Kozak consensus translation initiation site: 606-616
 Start codon (ATG): 613-615; Stop codon: 2695-2697
 Last amino acid in TagRFP: 1321-1323
 Tubulin: 1342-2697
 SV40 early mRNA polyadenylation signal
 Polyadenylation signals: 2858-2863 & 2887-2892
 mRNA 3' ends: 2896 & 2908
 f1 single-strand DNA origin: 2955-3410
 Eukaryotic promoter for expression of Kan^r gene
 -35 region: 3472-3477; -10 region: 3495-3500
 Transcription start point: 3507
 SV40 origin of replication: 3751-3886
 SV40 early promoter
 Enhancer (72-bp tandem repeats): 3584-3655 & 3656-3727
 21-bp repeats: 3731-3751, 3752-3772 & 3774-3794
 Early promoter element: 3807-3813
 Major transcription start points: 3803, 3841, 3847 & 3852
 Kanamycin/neomycin resistance gene
 Neomycin phosphotransferase coding sequences:
 Start codon (ATG): 3935-3937; Stop codon: 4727-4729
 G->A mutation to remove Pst I site: 4117
 C->A (Arg to Ser) mutation to remove BssH II site: 4463
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 4965-4970 & 4978-4983
 pUC plasmid replication origin: 5314-5957

Product	Cat.#	Size
pTagRFP-tubulin vector	FP145	20 μg
Vector type	mammalian expression vector	
Reporter	TagRFP	
Reporter codon usage	mammalian	
Promoter for TagRFP	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	red (orange) fluorescent labeling of α-tubulin filaments	

Vector description

pTagRFP-tubulin is a mammalian expression vector encoding TagRFP-tubulin fusion protein. The vector can be used for fluorescent labeling of α-tubulin in living cells.

TagRFP codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Human α-tubulin is fused to the TagRFP C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the TagRFP-tubulin coding sequence [Kozak 1987].

pTagRFP-tubulin vector can be used as a source of TagRFP-tubulin hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

Note: The plasmid DNA was isolated from dam⁺-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam⁻ host and make fresh DNA.

The vector backbone contains immediate early promoter of cytomegalovirus (P_{CMV IE}) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli*, and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA.

SV40 early promoter (P_{SV40}) provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Expression in mammalian cells

pTagRFP-tubulin vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagRFP-tubulin fusion in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman 1985].

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

References

- Gorman, C. (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143-190.
- Haas, J. et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248
- Kozak, M. (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." *Nucleic Acids Res*, 15 (20): 8125-8148 / pmid: 3313277

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