pTagFP635-tubulin vector

Cat# FP391

Vector description

pTagFP635-tubulin is a mammalian expression vector encoding TagFP635-tubulin fusion protein. The vector can be used for fluorescent labeling of α-tubulin in living cells.

TagFP635 codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas *et al.*, 1996]. Human α-tubulin is fused to the TagFP635 C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagFP635-tubulin coding sequence [Kozak, 1987].

pTagFP635-tubulin can be used as a source of TagFP635-tubulin hybrid sequence. The vector backbone contains unique restriction sites that permit it excision and further insertion into expression vector of choice.

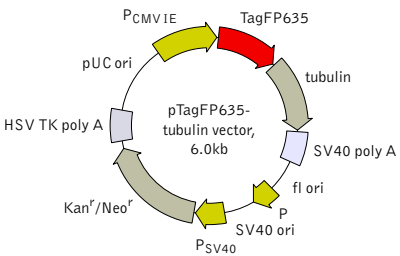
Note: The plasmid DNA was isolated from dam^+-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

The vector backbone also 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.

Vector map

For vector sequence, please visit our Web site at
http://www.evrogen.com/support/vector-info.shtml



Expression in mammalian cells

pTagFP635-tubulin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-tubulin fusion in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman, 1985].

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
TagFP635
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615
Stop codon: 2695-2697
Last amino acid in TagFP635: 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
Bacterial 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

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. High efficiency gene transfer into mammalian cells. In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.). 1985; 143-90.

Haas J, Park EC, Seed B. Codon usage limitation in the expression of HIV-1 envelope glycoprotein. Curr Biol. 1996; 6 (3):315-24. / pmid: 8805248

Kozak M. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res. 1987; 15 (20):8125-48. / pmid: 3313277