

## pTagRFP-profilin vector

**Cat# FP371**

### Vector description

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

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

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

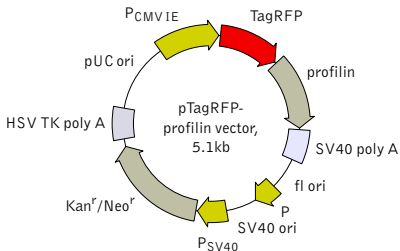
**Note:** The plasmid DNA was isolated from  $dam^+$ -methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a  $dam^-$  host and make fresh DNA.

The vector backbone also contains immediate early promoter of cytomegalovirus ( $P_{CMVIE}$ ) 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

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

## Location of features

PCMV IE: 1-589  
Enhancer region: 59-465  
TATA box: 554-560  
Transcription start point: 583  
Kozak consensus translation initiation site: 606-616  
TagRFP  
Start codon (ATG): 613-615  
Last amino acid in TagRFP: 1321-1323  
Profilin: 1354-1776  
Stop codon: 1774-1776  
SV40 early mRNA polyadenylation signal  
Polyadenylation signals: 1937-1942 1966-1971  
mRNA 3' ends: 1975 1987  
f1 single-strand DNA origin: 2034-2489  
Bacterial promoter for expression of Kan<sup>r</sup> gene  
-35 region: 2551-2556  
-10 region: 2574-2579  
Transcription start point: 2586  
SV40 origin of replication: 2830-2965  
SV40 early promoter  
Enhancer (72-bp tandem repeats): 2663-2734 2735-2806  
21-bp repeats: 2810-2830, 2831-2851 2853-2873  
Early promoter element: 2886-2892  
Major transcription start points: 2882, 2920, 2926 2931  
Kanamycin/neomycin resistance gene  
Neomycin phosphotransferase coding sequences:  
Start codon (ATG): 3014-3016  
Stop codon: 3806-3808  
G->A mutation to remove Pst I site: 3196  
C->A (Arg to Ser) mutation to remove BssH II site: 3542  
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
Polyadenylation signals: 4044-4049 4057-4062  
pUC plasmid replication origin: 4393-5036

## 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

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