

## pTagFP635-clathrin vector

**Cat# FP381**

### Vector description

pTagFP635-clathrin is a mammalian expression vector encoding TagFP635-clathrin fusion protein. The vector can be used for fluorescent labeling of clathrin light chain LCB in living cells.

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

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

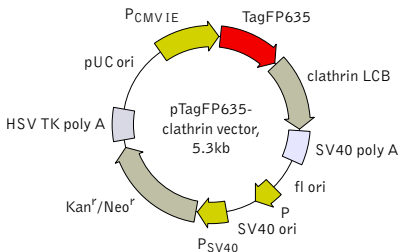
**Note:** The plasmid DNA was isolated from *dam*<sup>+</sup>-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a *dam*<sup>-</sup> host and make fresh DNA.

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

pTagFP635-clathrin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-clathrin 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  
TagFP635  
Start codon (ATG): 613-615  
Last amino acid in TagFP635: 1321-1323  
Clathrin light chain LCB: 1369-2004  
Stop codon: 2002-2004  
SV40 early mRNA polyadenylation signal  
Polyadenylation signals: 2165-2170 2194-2199  
mRNA 3' ends: 2203 2215  
f1 single-strand DNA origin: 2262-2717  
Bacterial promoter for expression of Kan<sup>r</sup> gene  
-35 region: 2779-2784  
-10 region: 2802-2807  
Transcription start point: 2814  
SV40 origin of replication: 3058-3193  
SV40 early promoter  
Enhancer (72-bp tandem repeats): 2891-2962 2963-3034  
21-bp repeats: 3038-3058, 3059-3079 3081-3101  
Early promoter element: 3114-3120  
Major transcription start points: 3110, 3148, 3154 3159  
Kanamycin/neomycin resistance gene  
Neomycin phosphotransferase coding sequences:  
Start codon (ATG): 3242-3244  
Stop codon: 4034-4036  
G->A mutation to remove Pst I site: 3424  
C->A (Arg to Ser) mutation to remove BssH II site: 3770  
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
Polyadenylation signals: 4272-4277 4285-4290  
pUC plasmid replication origin: 4621-5264

## 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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