

## pTagFP635-H2B vector

**Cat# FP386**

### Vector description

pTagFP635-H2B is a mammalian expression vector encoding TagFP635-H2B fusion protein. The vector can be used for fluorescent labeling of histone H2B in living cells.

TagFP635 codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas *et al.*, 1996). Human histone H2B is fused to the TagFP635 N-terminus.

pTagFP635-H2B can be used as a source of TagFP635-H2B hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into an expression vector of choice.

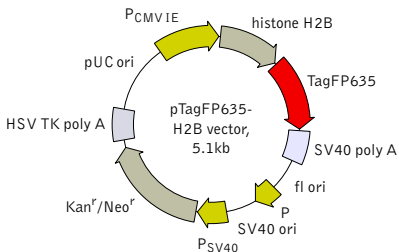
**Note:** The plasmid DNA was isolated from  $dam^+$ -methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a  $dam^-$  host and make fresh DNA.

The vector backbone also contains an 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-H2B can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-H2B 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  
Histone H2B: 657-1034  
TagFP635: 1053-1766  
SV40 early mRNA polyadenylation signal  
Polyadenylation signals: 1919-1924 1948-1953  
mRNA 3' ends: 1957 1969  
f1 single-strand DNA origin: 2016-2471  
Bacterial promoter for expression of Kan<sup>r</sup> gene  
-35 region: 2533-2538  
-10 region: 2556-2561  
Transcription start point: 2568  
SV40 origin of replication: 2812-2947  
SV40 early promoter  
Enhancer (72-bp tandem repeats): 2645-2716 2717-2788  
21-bp repeats: 2792-2812, 2813-2833 2835-2855  
Early promoter element: 2868-2874  
Major transcription start points: 2864, 2902, 2908 2913  
Kanamycin/neomycin resistance gene  
Neomycin phosphotransferase coding sequences:  
Start codon (ATG): 2996-2998  
Stop codon: 3788-3790  
G->A mutation to remove Pst I site: 3178  
C->A (Arg to Ser) mutation to remove BssH II site: 3524  
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
Polyadenylation signals: 4026-4031 4039-4044  
pUC plasmid replication origin: 4375-5018

## 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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