

pTagFP635-vinculin vector

Cat# FP388

Vector description

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

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

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

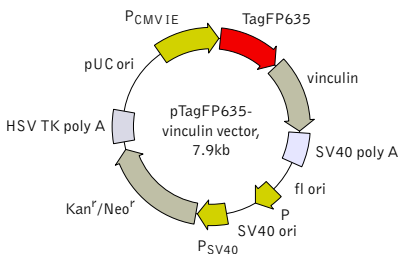
Note: The plasmid DNA was isolated from *dam*⁺-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

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

pTagFP635-vinculin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-vinculin 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
Vinculin: 1393-4593
Stop codon: 4591-4593
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 4746-4751 4775-4780
mRNA 3' ends: 4784 4796
f1 single-strand DNA origin: 4843-5298
Bacterial promoter for expression of Kan^r gene
-35 region: 5360-5365
-10 region: 5383-5388
Transcription start point: 5395
SV40 origin of replication: 5639-5774
SV40 early promoter
Enhancer (72-bp tandem repeats): 5472-5543 5544-5615
21-bp repeats: 5619-5639, 5640-5660 5662-5682
Early promoter element: 5695-5701
Major transcription start points: 5691, 5729, 5735 5740
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 5823-5825
Stop codon: 6615-6617
G->A mutation to remove Pst I site: 6005
C->A (Arg to Ser) mutation to remove BssH II site: 6351
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 6853-6858 6866-6871
pUC plasmid replication origin: 7202-7845

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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