pTagFP635-zyxin vector

Cat# FP389

Vector description

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

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

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

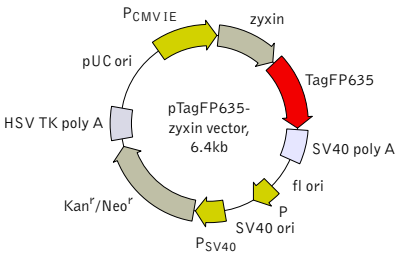
Note: The plasmid DNA was isolated from dam^+-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

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-zyxin can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-zyxin 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
Zyxin: 636-2348
TagFP635: 2370-3083
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 3236-3241 3265-3270
mRNA 3' ends: 3274 3286
f1 single-strand DNA origin: 3333-3788
Bacterial promoter for expression of Kan^r gene
-35 region: 3850-3855
-10 region: 3873-3878
Transcription start point: 3885
SV40 origin of replication: 4129-4264
SV40 early promoter
Enhancer (72-bp tandem repeats): 3962-4033 4034-4105
21-bp repeats: 4109-4129, 4130-4150 4152-4172
Early promoter element: 4185-4191
Major transcription start points: 4181, 4219, 4225 4230
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 4313-4315
Stop codon: 5105-5107
G->A mutation to remove Pst I site: 4495
C->A (Arg to Ser) mutation to remove BssH II site: 4841
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 5343-5348 5356-5361
pUC plasmid replication origin: 5692-6335

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