pTagFP635-EB3 vector

Cat# FP385

Vector description

pTagFP635-EB3 is a mammalian expression vector encoding TagFP635-EB3 fusion protein. The vector can be used for fluorescent labeling of EB3 protein in living cells.

TagFP635 codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas *et al.*, 1996). Human microtubule-associated EB3 protein is fused to the TagFP635 N-terminus.

pTagFP635-EB3 can be used as a source of TagFP635-EB3 hybrid sequence. The vector backbone contains unique restriction sites that permit it excision and further insertion into expression vector of choice.

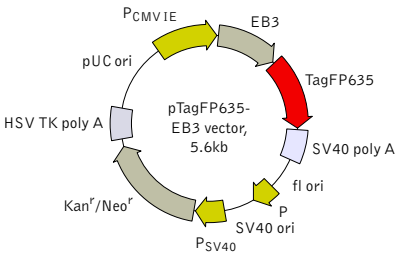
Note: The plasmid DNA was isolated from dam^+-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

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-EB3 can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-EB3 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
EB3: 652-1494
TagFP635: 1516-2229
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2382-2387 2411-2416
mRNA 3' ends: 2420 2432
f1 single-strand DNA origin: 2479-2934
Bacterial promoter for expression of Kan^r gene
-35 region: 2996-3001
-10 region: 3019-3024
Transcription start point: 3031
SV40 origin of replication: 3275-3410
SV40 early promoter
Enhancer (72-bp tandem repeats): 3108-3179 3180-3251
21-bp repeats: 3255-3275, 3276-3296 3298-3318
Early promoter element: 3331-3337
Major transcription start points: 3327, 3365, 3371 3376
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3459-3461
Stop codon: 4251-4253
G->A mutation to remove Pst I site: 3641
C->A (Arg to Ser) mutation to remove BssH II site: 3987
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4489-4494 4502-4507
pUC plasmid replication origin: 4838-5481

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