

pTagFP635-Cx43 vector

Cat# FP384

Vector description

pTagFP635-Cx43 is a mammalian expression vector encoding TagFP635-Cx43 fusion protein. The vector can be used for fluorescent labeling of connexin 43 in living cells.

TagFP635 codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas et al., 1996). Rat connexin 43 is fused to the TagFP635 N-terminus.

pTagFP635-Cx43 can be used as a source of TagFP635-Cx43 hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

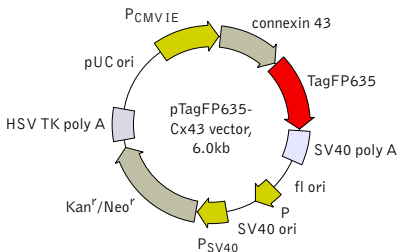
Note: The plasmid DNA was isolated from dam^+ -methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

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-Cx43 can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagFP635-Cx43 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
Connexin 43: 824-1969
TagFP635: 1991-2704
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2857-2862 2886-2891
mRNA 3' ends: 2895 2907
f1 single-strand DNA origin: 2954-3409
Bacterial promoter for expression of Kan^r gene
-35 region: 3471-3476
-10 region: 3494-3499
Transcription start point: 3506
SV40 origin of replication: 3750-3885
SV40 early promoter
Enhancer (72-bp tandem repeats): 3583-3654 3655-3726
21-bp repeats: 3730-3750, 3751-3771 3773-3793
Early promoter element: 3806-3812
Major transcription start points: 3802, 3840, 3846 3851
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3934-3936
Stop codon: 4726-4728
G->A mutation to remove Pst I site: 4116
C->A (Arg to Ser) mutation to remove BssH II site: 4462
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4964-4969 4977-4982
pUC plasmid replication origin: 5313-5956

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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