

pTagRFP-Cx26 vector

Cat# FP362

Vector description

pTagRFP-Cx26 is a mammalian expression vector encoding TagRFP-Cx26 fusion protein. The vector can be used for fluorescent labeling of connexin 26 in living cells.

TagRFP codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas et al., 1996). Rat connexin 26 is fused to the TagRFP N-terminus.

pTagRFP-Cx26 can be used as a source of TagRFP-Cx26 hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

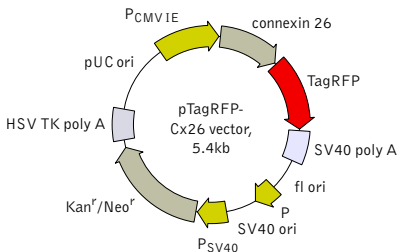
Note: The plasmid DNA was isolated from dam^+ -methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam^- host and make fresh DNA.

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

pTagRFP-Cx26 can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagRFP-Cx26 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 26: 683-1360
TagRFP: 1382-2095
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2248-2253 2277-2282
mRNA 3' ends: 2286 2298
f1 single-strand DNA origin: 2345-2800
Bacterial promoter for expression of Kan^r gene
-35 region: 2862-2867
-10 region: 2885-2890
Transcription start point: 2897
SV40 origin of replication: 3141-3276
SV40 early promoter
Enhancer (72-bp tandem repeats): 2974-3045 3046-3117
21-bp repeats: 3121-3141, 3142-3162 3164-3184
Early promoter element: 3197-3203
Major transcription start points: 3193, 3231, 3237 3242
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3325-3327
Stop codon: 4117-4119
G->A mutation to remove Pst I site: 3507
C->A (Arg to Ser) mutation to remove BssH II site: 3853
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4355-4360 4368-4373
pUC plasmid replication origin: 4704-5347

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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