

## pTagRFP-laminB1 vector

**Cat# FP370**

### Vector description

pTagRFP-laminB1 is a mammalian expression vector encoding TagRFP-laminB1 fusion protein. The vector can be used for fluorescent labeling of lamin B1 in living cells.

TagRFP codon usage is optimized for high expression in mammalian cells, i.e. humanized [Haas *et al.*, 1996]. Human lamin B1 is fused to the TagRFP C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TagRFP-laminB1 coding sequence [Kozak, 1987].

pTagRFP-laminB1 can be used as a source of TagRFP-laminB1 hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

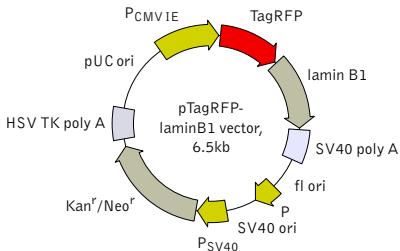
**Note:** The plasmid DNA was isolated from *dam*<sup>+</sup>-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a *dam*<sup>-</sup> host and make fresh DNA.

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-laminB1 can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the TagRFP-laminB1 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

TagRFP

Start codon (ATG): 613-615

Last amino acid in TagRFP: 1321-1323

Lamin B1: 1354-3114

Stop codon: 3112-3114

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 3275-3280 3304-3309

mRNA 3' ends: 3313 3325

f1 single-strand DNA origin: 3372-3827

Bacterial promoter for expression of Kan<sup>r</sup> gene

-35 region: 3889-3894

-10 region: 3912-3917

Transcription start point: 3924

SV40 origin of replication: 4168-4303

SV40 early promoter

Enhancer (72-bp tandem repeats): 4001-4072 4073-4144

21-bp repeats: 4148-4168, 4169-4189 4191-4211

Early promoter element: 4224-4230

Major transcription start points: 4220, 4258, 4264 4269

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 4352-4354

Stop codon: 5144-5146

G->A mutation to remove Pst I site: 4534

C->A (Arg to Ser) mutation to remove BssH II site: 4880

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

Polyadenylation signals: 5382-5387 5395-5400

pUC plasmid replication origin: 5731-6374

## 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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