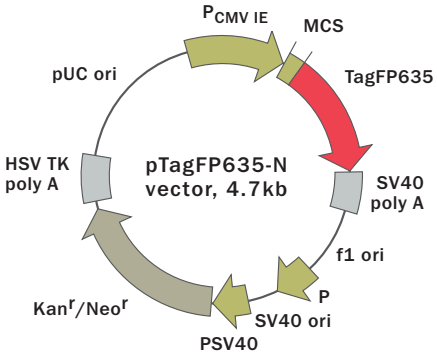
# Mammalian expression vector pTagFP635-N




For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

| Product | Cat.# | Size |
|---|---|---|
| pTagFP635-N | **FP162** | 20 µg |

Please contact your local distributor for exact prices and delivery information.

| | |
|---|---|
| Vector type | mammalian expression vector |
| Reporter | TagFP635 |
| Reporter codon usage | mammalian |
| Promoter for TagFP635 | P $_{CMV\ IE}$ |
| Host cells | mammalian |
| Selection | prokaryotic — kanamycin<br>eukaryotic — neomycin (G418) |
| Replication | prokaryotic — pUC ori<br>eukaryotic — SV40 ori |

### Multiple cloning site (MCS)

NheI / AflI — BglII — SacI / XhoI — HindIII — EcoRI — SalI / PstI — KpnI / SacII — ApaI / SmaI/XmaI — BamHI — AgeI — TagFP635 / NcoI*

GCTA.GCG.CTA.CCG.GAC.TCA.GAT.CTC.GAG.CTC.AAG.CTT.CGA.ATT.CTG.CAG.TCG.ACG.GTA.CCG.CGG.GCC.CGG.GAT.CCA.CCG.GTC.GCC.ACC.*ATG.G*...

* — not unique sites.

## Use

**- Generation of fusions to the TagFP635 N-terminus**

**- Expression of TagFP635 or its fusions in mammalian cells**

## Vector description

pTagFP635-N is an eukaryotic (mammalian) expression vector encoding monomeric far-red fluorescent protein TagFP635. The vector allows generation of fusions to the TagFP635 N-terminus and expression of TagFP635 fusions or TagFP635 alone in mammalian cells.

TagFP635 codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase TagFP635 translation, Kozak consensus translation initiation site is generated upstream of TagFP635 sequence (Kozak, 1987). Multiple cloning site (MCS) is located between $P_{CMV\ IE}$ and TagFP635 coding sequence.

The vector backbone comprises 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 provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan$^r$/Neo$^r$ gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

### Generation of fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to TagFP635 N-terminus when inserted in the same reading frame as TagFP635 and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. TagFP635-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

**Note:** The plasmid DNA was isolated from dam$^+$-methylated *E.coli*. Therefore some restriction sites are bloked 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.

## Expression in mammalian cells

pTagFP635-N can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985).

Unmodified pTagFP635-N will express TagFP635, when transfected into eukaryotic (mammalian) cells.

## 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.

## Location of features

**$P_{CMV\ IE}$:** 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
**MCS:** 591-671
**TagFP635**
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681
Stop codon: 1390-1392
**SV40 early mRNA polyadenylation signal**
Polyadenylation signals: 1546-1551 & 1575-1580
mRNA 3' ends: 1584 & 1596
**f1 single-strand DNA origin:** 1643-2098
**Bacterial promoter for expression of $Kan^r$ gene**
-35 region: 2160-2165; -10 region: 2183-2188
Transcription start point: 2195
**SV40 origin of replication:** 2439-2574
**SV40 early promoter**
Enhancer (72-bp tandem repeats): 2272-2343 & 2344-2415
21-bp repeats: 2419-2439, 2440-2460 & 2462-2482
Early promoter element: 2495-2501
Major transcription start points: 2491, 2529, 2535 & 2540
**Kanamycin/neomycin resistance gene**
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2623-2625
Stop codon: 3415-3417
G->A mutation to remove Pst I site: 2805
C->A (Arg to Ser) mutation to remove BssH II site: 3151
**Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal**
Polyadenylation signals: 3653-3658 & 3666-3671
**pUC plasmid replication origin:** 4002-4645

## References


Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

Haas J. *et al*. (1996) Curr. Biol. 6: 315-324.

Kozak M. (1987) Nucleic Acids Res. 15:8125-8148.