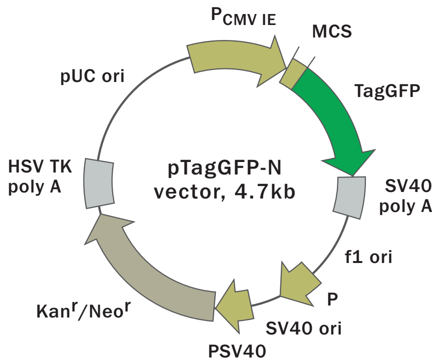
Mammalian expression vector pTagGFP-N



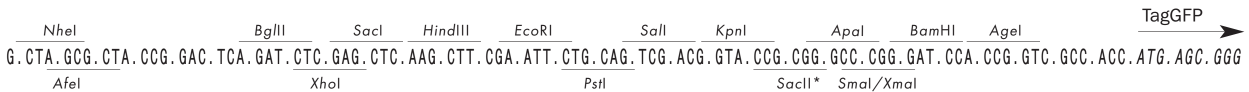
For vector sequence, please visit our Web site at
www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pTagGFP-N	**FP122**	20 µg

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

Vector type	mammalian expression vector
Reporter	TagGFP
Reporter codon usage	mammalian
Promoter for TagGFP	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)



* — not unique sites.

Use

- Generation of fusions to the TagGFP N-terminus
- Expression of TagGFP or its fusions in mammalian cells

Vector description

pTagGFP-N is an eukaryotic (mammalian) expression vector encoding green fluorescent protein TagGFP. The vector is designed to generate TagGFP-tagged fusions or to express TagGFP in mammalian cells.

TagGFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase TagGFP translation, Kozak consensus translation initiation site is generated upstream of TagGFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between $P_{CMV\ IE}$ and TagGFP 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 polyA) 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 the TagGFP N-terminus when inserted in the same reading frame as TagGFP and no intervening stop codons are present. The inserted sequence should contain an initiating ATG codon.

TagGFP fusions retain fluorescent properties of the native protein allowing fusion protein 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

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

Unmodified pTagGFP-N will express TagGFP, 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: 592-678
TagGFP
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681; stop codon: 1393-1395
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1549-1554 & 1578-1583
mRNA 3' ends: 1587 & 1599
f1 single-strand DNA origin: 1646-2101
Bacterial promoter for expression of Kan^r gene
-35 region: 2163-2168;
-10 region: 2186-2191
Transcription start point: 2198
SV40 origin of replication: 2442-2577
SV40 early promoter
Enhancer (72-bp tandem repeats): 2275-2346 & 2347-2418
21-bp repeats: 2422-2442, 2443-2463 & 2465-2485
Early promoter element: 2498-2504
Major transcription start points: 2494, 2532, 2538 & 2543
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2626-2628; stop codon: 3418-3420
G->A mutation to remove Pst I site: 2808
C->A (Arg to Ser) mutation to remove BssH II site: 3154
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3656-3661 & 3669-3674
pUC plasmid replication origin: 4005-4648

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.

Notice to Purchaser:
TagGFP-related products: These products are intended for research use only and covered by Evrogen Patents and/or Patent applications pending. By use of these products, you accept the terms and conditions of the applicable Limited Use Label License (enclosed).
The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MATERIAL SAFETY DATA SHEET INFORMATION
To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.