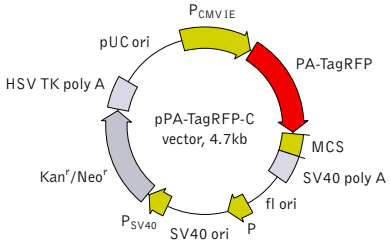
pPA-TagRFP-C vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.

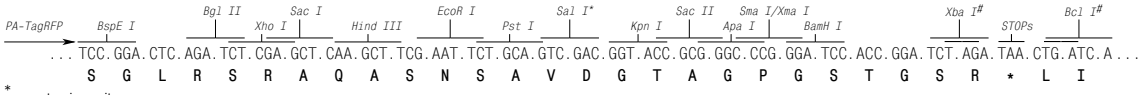


For vector sequence, please visit our Web site at http://www.evrogen.com/products/vectors.shtml

Product	Cat.#	Size
pPA-TagRFP-C vector	**FP811**	20 μg

Vector type	mammalian expression vector
Reporter	PA-TagRFP
Reporter codon usage	mammalian
Promoter for PA-TagRFP	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	PA-TagRFP expression in mammalian cells; generation of fusions to the PA-TagRFP C-terminus

Multiple cloning site (MCS)



* — not unique sites.
\# — sites are blocked by *dam* methylation. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
Kozak consensus translation initiation site: 600-610
PA-TagRFP
Start codon (ATG): 607-609; Stop codon: 1402-1404
Last amino acid in PA-TagRFP: 1309-1311
MCS: 1324-1401
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1544-1549 & 1573-1578
mRNA 3' ends: 1582 & 1594
f1 single-strand DNA origin: 1641-2096
Bacterial promoter for expression of Kan^r gene
-35 region: 2158-2163; -10 region: 2181-2186
Transcription start point: 2193
SV40 origin of replication: 2437-2572
SV40 early promoter
Enhancer (72-bp tandem repeats): 2270-2341 & 2342-2413
21-bp repeats: 2417-2437, 2438-2458 & 2460-2480
Early promoter element: 2493-2499
Major transcription start points: 2489, 2527, 2533 & 2538
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2621-2623; Stop codon: 3413-3415
G->A mutation to remove Pst I site: 2803
C->A (Arg to Ser) mutation to remove BssH II site: 3149
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3651-3656 & 3664-3669
pUC plasmid replication origin: 4000-4643

References

Gorman, C. (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II.* Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143–190.

Haas, J. et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." Curr Biol, 6 (3): 315–324 / pmid: 8805248

Kozak, M. (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." Nucleic Acids Res, 15 (20): 8125–8148 / pmid: 3313277

Subach, FV et al. (2010) "Bright monomeric photoactivatable red fluorescent protein for two-color super-resolution sptPALM of live cells." J Am Chem Soc, 132 (18): 6481–91 / pmid: 20394363

Vector description

pPA-TagRFP-C is a mammalian expression vector encoding photoactivatable red fluorescent protein PA-TagRFP. The vector allows generation of fusions to the PA-TagRFP C-terminus and expression of PA-TagRFP fusions or PA-TagRFP alone in eukaryotic (mammalian) cells.

Note: The pPA-TagRFP-C vector encodes the PA-TagRFP protein in which the amino acid sequence at C-terminus is modified compared to the originally reported sequence [Subach et al. 2010]. These modifications do not influence the fluorescent properties of the PA-TagRFP, but improves its performance in fusions.

PA-TagRFP codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the PA-TagRFP coding sequence [Kozak 1987]. Multiple cloning site (MCS) is located between PA-TagRFP coding sequence and SV40 polyadenylation signal (SV40 polyA).

The vector backbone contains 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 (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.

Generation of PA-TagRFP fusion proteins

A localization signal or a gene of interest can be cloned into MCS of the vector. It will be expressed as a fusion to the PA-TagRFP C-terminus when inserted in the same reading frame as PA-TagRFP and no in-frame stop codons are present. PA-TagRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express PA-TagRFP when transfected into eukaryotic (mammalian) cells.

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.

Expression in mammalian cells

pPA-TagRFP-C vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of PA-TagRFP or its fusions in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman 1985].

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.