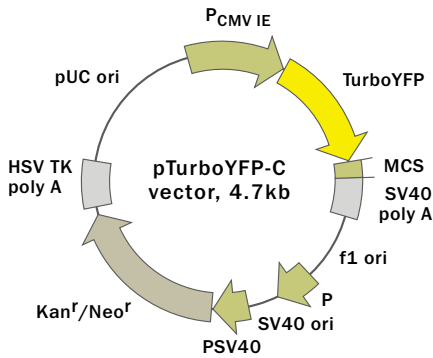


Mammalian expression vector pTurboYFP-C



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

Multiple cloning site (MCS)

TurboYFP → GGT. CTC. AGA. TCT. CGA. GCT. CAA. GCT. TCG. AAT. TCT. GCA. GTC. GAC. GGT. ACC. GCG. GGC. CCG. GGA. TCC. ACC. GGA. TCT. AGA. TAA. CTG. ATC. A

BglII
SacI
EcoRI
Sall
KpnI
ApaI
BamHI
STOPs

XhoI
HindIII
PstI
SacII*
SmaI/XmaI
XbaI#
BclI#

* - not unique sites. # - sites are blocked by methylation.

Use

- Generation of fusions to the TurboYFP C-terminus
- Expression of TurboYFP or its fusions in mammalian cells

Vector description

pTurboYFP-C is an eukaryotic (mammalian) expression vector encoding true yellow fluorescent protein, TurboYFP. The vector allows to generate fusions to the TurboYFP C-terminus and to express TurboYFP fusions or TurboYFP alone in mammalian cells.

TurboYFP codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase TurboYFP translation, Kozak consensus translation initiation site is generated upstream of the TurboYFP sequence (Kozak, 1987). Multiple cloning site (MCS) is located between TurboYFP 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 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 TurboYFP C-terminus when inserted in the same reading frame as TurboYFP and no intervening stop codons are present. TurboYFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

Notes: 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.

Despite its dimeric structure, TurboYFP is still suitable for generation of fusions with proteins of interest, however we recommend that you use TagYFP for these purposes.

Product	Cat.#	Size
pTurboYFP-C	FP611	20 µg
Please contact your local distributor for exact prices and delivery information.		
Vector type	mammalian expression vector	
Reporter	TurboYFP	
Reporter codon usage	mammalian	
Promoter for TagYFP	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)	
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori	

Expression in mammalian cells

The vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985). Unmodified vector will express TurboYFP, 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

TurboYFP

Kozak consensus translation initiation site: 606 - 616

Start codon (ATG): 613 - 615

Stop codon: 1420 - 1422

Last amino acid in TurboYFP: 1339 - 1341

MCS: 1342 - 1419

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1562 - 1567; 1591 - 1596

mRNA 3' ends: 1600; 1612

f1 single-strand DNA origin: 1659 - 2114

Bacterial promoter for expression of Kan^r gene

-35 region: 2176 - 2181; -10 region: 2199 - 2204

Transcription start point: 2211

SV40 origin of replication: 2455 - 2590

SV40 early promoter

Enhancer (72-bp tandem repeats): 2288 - 2359; 2360 - 2431

21-bp repeats: 2435 - 2455; 2456 - 2476; 2478 - 2498

Early promoter element: 2511 - 2517

Major transcription start points: 2507; 2545; 2551; 2556

Kanamycin/neomycin resistance gene

Start codon (ATG): 2639 - 2641

Stop codon: 3431 - 3433

G->A mutation to remove Pst I site: 2821

C->A (Arg to Ser) mutation to remove BssH II site: 3167

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

Polyadenylation signals: 3669 - 3674; 3682 - 3687

pUC plasmid replication origin: 4018 - 4661

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:

Evrogen Fluorescent Protein Products (the 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).

CMV promoter: 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.