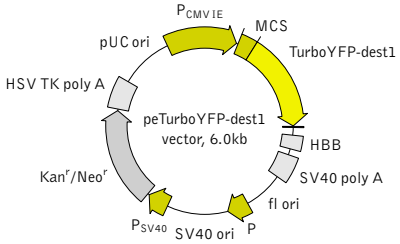
peTurboYFP-dest1 vector

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.

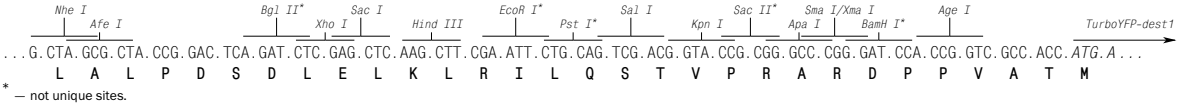


For vector sequence, please visit our Web site at http://www.evrogen.com/products/vectors.shtml

Product	Cat.#	Size
peTurboYFP-dest1 vector	**FP617**	20 μg

Vector type	mammalian expression vector
Reporter	TurboYFP
Reporter codon usage	mammalian
Promoter for TurboYFP	$P_{CMV\ IE}$
Host cells	mammalian
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	TurboYFP expression in mammalian cells; generation of fusions to the TurboYFP-dest1 N-terminus

Multiple cloning site (MCS)



* — not unique sites.

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
MCS: 591-671
TurboYFP-dest1
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681
Last amino acid in TurboYFP: 1405-1407
Amino acid residues of mouse ornithine decarboxylase (MODC) PEST sequence: 1429-1548
Stop codon: 1549-1551
Fragment of human beta globin (HBB) gene
Last 35 bp of HBB exon 2 : 1560-1594
HBB intron 2: 1595-2445
First 233 bp of HBB exon 3: 2446-2678
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2820-2825 & 2849-2854
mRNA 3' ends: 2858 & 2870
f1 single-strand DNA origin: 2917-3372
Bacterial promoter for expression of Kan^r gene
-35 region: 3434-3439; -10 region: 3457-3462
Transcription start point: 3469
SV40 origin of replication: 3713-3848
SV40 early promoter
Enhancer (72-bp tandem repeats): 3546-3617 & 3618-3689
21-bp repeats: 3693-3713, 3714-3734 & 3736-3756
Early promoter element: 3769-3775
Major transcription start points: 3765, 3803, 3809 & 3814
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3897-3899; Stop codon: 4689-4691
G->A mutation to remove Pst I site: 4079
C->A (Arg to Ser) mutation to remove BssH II site: 4425
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4927-4932 & 4940-4945
pUC plasmid replication origin: 5276-5919

References

Gorman, C. (1985). In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143–190.

Haas, J. et al. (1996). Curr Biol, 6 (3): 315–324 / pmid: 8805248

Kozak, M. (1987). Nucleic Acids Res, 15 (20): 8125–8148 / pmid: 3313277

Li, X. et al. (1998). J Biol Chem, 273 (52): 34970–34975 / pmid: 9857028

Vector description

peTurboYFP-dest1 is a mammalian expression vector encoding destabilized variant of the yellow fluorescent protein TurboYFP. To generate TurboYFP-dest1 variant, residues 422-461 of mouse ornithine decarboxylase (MODC) were fused to the TurboYFP C-terminus. This MODC region contains a PEST amino acid sequence that targets the protein for degradation and provides for rapid protein turnover [Li et al. 1998]. TurboYFP-dest1 retains fluorescent properties of the native protein and has a half-life of approximately 1-1.5 hours, as measured by fluorescence intensity of cells treated with the protein synthesis inhibitor, cycloheximide.

peTurboYFP-dest1 carries synthetic version of the TurboYFP-dest1 gene which 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 TurboYFP-dest1 coding sequence [Kozak 1987]. Fragments of exons 2 and 3 and intron 2 of human beta globin gene are added in the 3' UTR of TurboYFP-dest1 coding sequence in order to increase the protein expression level.

peTurboYFP-dest1 vector can be used to express TurboYFP-dest1 in eukaryotic (mammalian) cells. For example it can be used as a positive control with a peTurboYFP-PRL-dest1 promoterless vector (Cat.# FP617). The vector can be also used to generate destabilized TurboYFP-tagged fusion proteins. Multiple cloning site (MCS) is located upstream of TurboYFP-dest1 coding sequence.

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 TurboYFP-dest1-tagged fusions

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 TurboYFP-dest1 N-terminus when inserted in the same reading frame as TurboYFP and no in-frame stop codons are present. TurboYFP-dest1-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified vector will express TurboYFP-dest1 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

peTurboYFP-dest1 vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of TurboYFP-dest1 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.