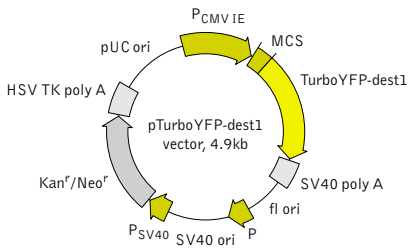


## pTurboYFP-dest1 vector

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/support/vector-info.shtml>

### Multiple cloning site (MCS)

... G. CTA. 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. A . . .

*Afe I* *Xho I* *Hind III* *Pst I*\* *Kpn I* *Apa I* *BamH I* *TurboYFP-dest1*  
*Nhe I* *Bgl II*\* *Sac I* *EcoR I* *Sal I* *Sac II*\* *Sma I/Xma I* *Age I*

\* — not unique sites.

### Location of features

PCMV IE: 1-589  
 Enhancer region: 59-465  
 TATA box: 554-560  
 Transcription start point: 583  
 MCS: 591-671  
 TurboYFP  
 Kozak consensus translation initiation site: 672-682  
 Start codon (ATG): 679-681  
 Last amino acid in TurboYFP: 1405-1407  
 Stop codon: 1549-1551  
 MODC PEST sequence: 1426-1548  
 SV40 early mRNA polyadenylation signal  
 Polyadenylation signals: 1706-1711 & 1735-1740  
 mRNA 3' ends: 1744 & 1756  
 f1 single-strand DNA origin: 1803-2258  
 Eukaryotic promoter for expression of Kan<sup>r</sup> gene  
 -35 region: 2320-2325; -10 region: 2343-2348  
 Transcription start point: 2355  
 SV40 origin of replication: 2599-2734  
 SV40 early promoter  
 Enhancer (72-bp tandem repeats): 2432-2503 & 2504-2575  
 21-bp repeats: 2579-2599, 2600-2620 & 2622-2642  
 Early promoter element: 2655-2661  
 Major transcription start points: 2651, 2689, 2695 & 2700  
 Kanamycin/neomycin resistance gene  
 Neomycin phosphotransferase coding sequences:  
 Start codon (ATG): 2783-2785; Stop codon: 3575-3577  
 G->A mutation to remove Pst I site: 2965  
 C->A (Arg to Ser) mutation to remove BssH II site: 3311  
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal  
 Polyadenylation signals: 3813-3818 & 3826-3831  
 pUC plasmid replication origin: 4162-4805

### References

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

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

Li et al. (1998) "Generation of destabilized green fluorescent protein as a transcription reporter." *J Biol Chem*, 273 (52): 34970-34975 / pmid: 9857028

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Product	Cat.#	Size
pTurboYFP-dest1 vector	FP619	20 µg
The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.		
Vector type	mammalian expression vector	
Reporter	TurboYFP	
Reporter codon usage	mammalian	
Promoter for TurboYFP	P <sub>CMV IE</sub>	
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	

### Vector description

pTurboYFP-dest1 is a mammalian expression vector encoding destabilized 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.

pTurboYFP-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 TurboYFP-dest1 coding sequence [Kozak 1987].

pTurboYFP-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 pTurboYFP-PRL-dest1 promoterless vector (Cat.# FP618). 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<sub>CMV IE</sub>) 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<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in *E. coli*. Kan<sup>r</sup>/Neo<sup>r</sup> 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 should 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 pTurboYFP-dest1 vector will express TurboYFP-dest1 when transfected into eukaryotic (mammalian) cells.

**Note:** The plasmid DNA was isolated from dam<sup>+</sup>-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<sup>-</sup> host and make fresh DNA.

### Expression in mammalian cells

pTurboYFP-dest1 vector can be transfected into mammalian cells by any known transfection method. 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.