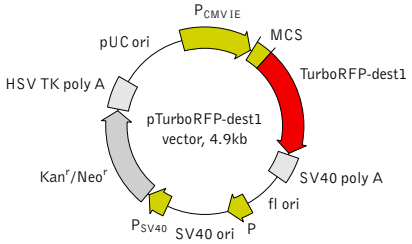
pTurboRFP-dest1 vector

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
pTurboRFP-dest1 vector	**FP239**	20 μg

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

Multiple cloning site (MCS)

Afe I | Xho I | Hind III | Pst I* | Kpn I | Apa I | BamH I | TurboRFP-dest1

. . . 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 . . .

Nhe I | Bgl II* | Sac I | EcoR I | Sal I | Sac II | Sma I/Xma I | Age I

* — 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
TurboRFP
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681
Last amino acid in TurboRFP: 1405-1407
Stop codon: 1519-1521
MODC PEST sequence: 1399-1518
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1676-1681 & 1705-1710
mRNA 3' ends: 1714 & 1726
f1 single-strand DNA origin: 1773-2228
Eukaryotic promoter for expression of Kan^r gene
-35 region: 2290-2295; -10 region: 2313-2318
Transcription start point: 2325
SV40 origin of replication: 2569-2704
SV40 early promoter
Enhancer (72-bp tandem repeats): 2402-2473 & 2474-2545
21-bp repeats: 2549-2569, 2570-2590 & 2592-2612
Early promoter element: 2625-2631
Major transcription start points: 2621, 2659, 2665 & 2670
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2753-2755; Stop codon: 3545-3547
G->A mutation to remove Pst I site: 2935
C->A (Arg to Ser) mutation to remove BssH II site: 3281
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3783-3788 & 3796-3801
pUC plasmid replication origin: 4132-4775

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

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

Vector description

pTurboRFP-dest1 is a mammalian expression vector encoding destabilized red (orange) fluorescent protein TurboRFP. To generate TurboRFP-dest1 variant, residues 422-461 of mouse ornithine decarboxylase (MODC) were fused to the TurboRFP 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]. TurboRFP-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.

pTurboRFP-dest1 carries synthetic version of the TurboRFP-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 TurboRFP-dest1 coding sequence [Kozak 1987].

pTurboRFP-dest1 vector can be used to express TurboRFP-dest1 in eukaryotic (mammalian) cells. For example it can be used as a positive control with a pTurboRFP-PRL-dest1 promoterless vector (Cat.# FP238). The vector can be also used to generate destabilized TurboRFP-tagged fusion proteins. Multiple cloning site (MCS) is located upstream of TurboRFP-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 TurboRFP-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 TurboRFP-dest1 N-terminus when inserted in the same reading frame as TurboRFP and no in-frame stop codons are present. TurboRFP-dest1-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified pTurboRFP-dest1 vector will express TurboRFP-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

pTurboRFP-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.