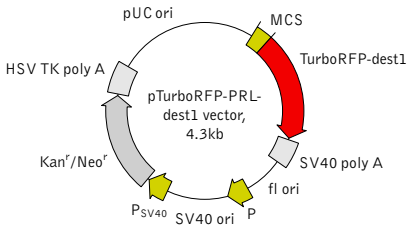
pTurboRFP-PRL-dest1 vector

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-PRL-dest1 vector	**FP238**	20 μg

Vector type	promoterless expression vector
Reporter	TurboRFP
Reporter codon usage	mammalian
Promoter for TurboRFP	NO
Host cells	mammalian, prokaryotic
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori
Use	Monitoring of activity of different promoters and promoter/enhancer combinations

Multiple cloning site (MCS)

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

...A.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*...

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

* — not unique sites.

Location of features

MCS: 12-89
TurboRFP-dest1
Kozak consensus translation initiation site: 90-100
Start codon (ATG): 97-99
Last amino acid in TurboRFP: 799-801
Stop codon: 937-939
MODC PEST sequence: 817-939
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1094-1099 & 1123-1128
mRNA 3' ends: 1132 & 1144
f1 single-strand DNA origin: 1191-1646
Eukaryotic promoter for expression of Kan^r gene
-35 region: 1708-1713; -10 region: 1731-1736
Transcription start point: 1743
SV40 origin of replication: 1987-2122
SV40 early promoter
Enhancer (72-bp tandem repeats): 1820-1891 & 1892-1963
21-bp repeats: 1967-1987, 1988-2008 & 2010-2030
Early promoter element: 2043-2049
Major transcription start points: 2039, 2077, 2083 & 2088
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2171-2173; Stop codon: 2963-2965
G->A mutation to remove Pst I site: 2353
C->A (Arg to Ser) mutation to remove BssH II site: 2699
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3201-3206 & 3214-3219
pUC plasmid replication origin: 3550-4193

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

Vector description

pTurboRFP-PRL-dest1 is a promoterless vector encoding destabilized variant of the red (orange) fluorescent protein, TurboRFP, which can be used as *in vivo* reporter of promoter activity. 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. Rapid TurboRFP-dest1 turnover allows accurate analysis of changes in gene regulation.

TurboRFP-dest1 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].

Multiple cloning site (MCS) is located upstream of the Kozak consensus translation initiation site and can be used to clone a promoter or a promoter/enchancer combination of interest. Without the addition of a functional promoter, this vector will not express TurboRFP-dest1.

The vector backbone contains 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.

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

The vector will express TurboRFP-dest1 under the control of functional promoter cloned into the vector's MCS. pTurboRFP-PRL-dest1 vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 [Gorman 1985].

Note: pTurboRFP-dest1 vector (Cat.# FP239) expressing TurboRFP-dest1 under the control of CMV promoter can be used as a positive control to pTurboRFP-PRL-dest1 vector.

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.