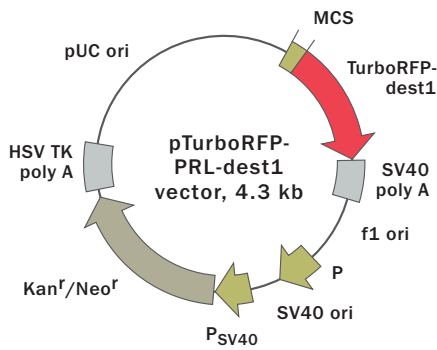


## Promoterless vector pTurboRFP-PRL-dest1



For vector sequence, please visit our Web site at [www.evrogen.com/support/vector-info.shtml](http://www.evrogen.com/support/vector-info.shtml)

Product	Cat.#	Size
pTurboRFP-PRL-dest1	<b>FP238</b>	20 µg

Please contact your local distributor for exact prices and delivery information.

Vector type	promoterless vector
Reporter	destabilized TurboRFP (TurboRFP-dest1)
Reporter codon usage	mammalian
Promoter for TurboRFP-dest1	NO
Host cells	mammalian, prokaryotic
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

### Multiple cloning site (MCS)

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. . . . TurboRFP-dest1

BglII\*
SacI
HindIII
EcoRI
Sall
KpnI
ApaI
BamHI
AgeI

AfeI
XhoI
PstI\*
SacII
SmaI/XmaI

\* — not unique sites.

### Use

- Monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS. Rapid turnover of TurboRFP-dest1 allows exact measuring of changes in gene expression

### Vector description

pTurboRFP-PRL-dest1 is a promoterless vector encoding destabilized red (orange) fluorescent protein TurboRFP-dest1, which can be used as *in vivo* reporter of promoter activity. TurboRFP-dest1 codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996).

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 results in rapid protein turnover (Li *et al.*, 1998).

To increase TurboRFP-dest1 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 upstream of the Kozak consensus translation initiation site and can be used to clone a promoter or a promoter/enhancer 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 polyA) direct proper processing of the 3'-end of 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.

**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

pTurboRFP-PRL-dest1 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.

## Location of features

**MCS:** 12-89

### TurboRFP-dest1

Kozak consensus translation initiation site: 90-100

Start codon (ATG): 97-99

Last amino acid in TurboGFP: 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

### Bacterial promoter for expression of Kan<sup>r</sup> 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 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.

Li X. *et al.* (1998) *J. Biol. Chem.* 273:34970-34975.

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