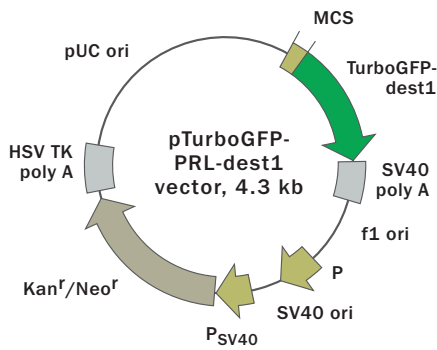


Promoterless vector pTurboGFP-PRL-dest1



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pTurboGFP-PRL-dest1	FP518	20 µg

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

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

Multiple cloning site (MCS)

$\xrightarrow{\text{TurboGFP}}$
 ACTA. 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. GAG. AGC

 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 of interest cloned into vector MCS. Rapid turnover of TurboGFP-dest1 allows exact measuring of changes in gene expression

Vector description

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

To generate destabilized variant of TurboGFP, residues 422-461 of mouse ornithine decarboxylase (MODC) were fused to the TurboGFP 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 mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TurboGFP-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/enhancer combination of interest. Without the addition of a functional promoter, this vector will not express TurboGFP-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_{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 can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985).

Note: pTurboGFP-dest1 vector (Cat.# FP519) expressing TurboGFP-dest1 under the control of CMV promoter can be used as a positive control to pTurboGFP-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

TurboGFP-dest1

Kozak consensus translation initiation site: 90-100

Start codon (ATG): 97-99

Last amino acid in TurboGFP: 790-792

Stop codon: 928-930

MODC PEST sequence: 808-930

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1085-1090; 1114-1119

mRNA 3' ends: 1123; 1135

f1 single-strand DNA origin: 1182-1637

(packages the noncoding strand of TurboGFP-dest1)

Bacterial promoter for expression of Kan^r gene

-35 region: 1699-1704; -10 region: 1722-1727

Transcription start point: 1734

SV40 origin of replication: 1978-2113

SV40 early promoter

Enhancer (72-bp tandem repeats): 1811-1882; 1883-1954

21-bp repeats: 1958-1978; 1979-1999; 2001-2021

Early promoter element: 2034-2040

Major transcription start points: 2030; 2068; 2074; 2079

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences

Start codon (ATG): 2162-2164

Stop codon: 2954-2956

G->A mutation to remove PstI site: 2344

C->A (Arg to Ser) mutation to remove BssHII site: 2690

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

Polyadenylation signals: 3192-3197; 3205-3210

pUC plasmid replication origin: 3541-4184

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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