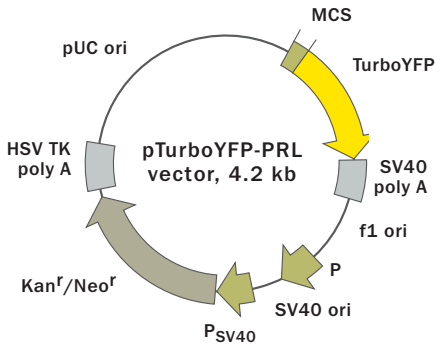


Promoterless vector pTurboYFP-PRL



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

Product	Cat.#	Size
pTurboYFP-PRL	FP615	20 µg

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

Vector type	promoterless vector
Reporter	TurboYFP
Reporter codon usage	mammalian
Promoter for TurboGFP	NO
Host cells	mammalian, bacterial
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. . . . TurboYFP
 Afel XhoI PstI SacII* SmaI/XmaI

* — not unique sites.

Use

- Monitoring the activity of promoter or promoter/enhancer combination cloned into vector MCS

Vector description

pTurboYFP-PRL is a promoterless expression vector encoding yellow fluorescent protein TurboYFP, which can be used as *in vivo* reporter of gene expression. TurboYFP codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). To increase TurboRFP mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TurboRFP 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 TurboYFP.

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 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

pTurboYFP-PRL 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.

Location of features

MCS: 12-89

TurboYFP

Kozak consensus translation initiation site: 90-100

Start codon (ATG): 97-99

Stop codon: 826-828

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 982-987 & 1011-1016

mRNA 3' ends: 1020 & 1032

f1 single-strand DNA origin: 1079-1534

Bacterial promoter for expression of Kan^r gene

-35 region: 1596-1601

-10 region: 1619-1624

Transcription start point: 1631

SV40 origin of replication: 1875-2010

SV40 early promoter

Enhancer (72-bp tandem repeats): 1708-1779 & 1780-1851

21-bp repeats: 1855-1875, 1876-1896 & 1898-1918

Early promoter element: 1931-1937

Major transcription start points: 1927, 1965, 1971 & 1976

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2059-2061

Stop codon: 2851-2853

G->A mutation to remove Pst I site: 2241

C->A (Arg to Ser) mutation to remove BssH II site: 2587

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

Polyadenylation signals: 3089-3094 & 3102-3107

pUC plasmid replication origin: 3438-4081

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.

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To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.