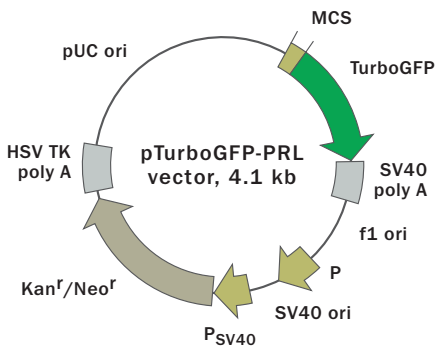


## Promoterless vector pTurboGFP-PRL



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
pTurboGFP-PRL	<b>FP515</b>	20 µg

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

Vector type	promoterless vector
Reporter	TurboGFP
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)

$\xrightarrow{\text{TurboGFP}}$   
 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 . . .  
Eco47III                      XhoI                      PstI\*                      SacII                      SmaI/XmaI

\* — not unique sites.

### Use

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

### Vector description

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

The vector backbone comprises 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) allows proper processing of the 3' end of the reporter mRNA.

SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression 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:** This 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

pTurboGFP-PRL 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.

## Location of features

**MCS:** 12-89

### TurboGFP

Kozak consensus translation initiation site: 90-100

Start codon (ATG): 97-99

Stop codon: 793-795

### SV40 early mRNA polyadenylation signal

Polyadenylation signals: 949-954; 978-983

mRNA 3' ends: 987; 999

**f1 single-strand DNA origin:** 1046-1501

(packages the noncoding strand of TurboGFP)

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

-35 region: 1563-1568

-10 region: 1586-1591

Transcription start point: 1598

**SV40 origin of replication:** 1842-1977

### SV40 early promoter

Enhancer (72-bp tandem repeats): 1675-1746; 1747-1818

21-bp repeats: 1822-1842; 1843-1863; 1865-1885

Early promoter element: 1898-1904

Major transcription start points: 1894; 1932; 1938; 1943

### Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2026-2028

Stop codon: 2818-2820

G->A mutation to remove PstI site: 2208

C->A (Arg to Ser) mutation to remove BssH II site: 2554

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

Polyadenylation signals: 3056-3061; 3069-3074

**pUC plasmid replication origin:** 3405-4048

## 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.