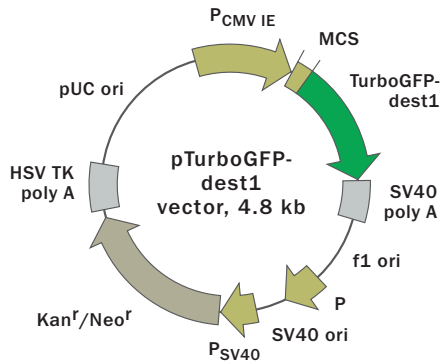


# Mammalian expression vector pTurboGFP-dest1



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

## Multiple cloning site (MCS)

$\xrightarrow{\text{NheI}}$  GCTA . GCG . CTA . CCG . GAC . TCA . GAT .  $\xrightarrow{\text{BglII}^*}$  CTC . GAG . CTC . AAG . CTT . CGA . ATT .  $\xrightarrow{\text{SmaI}}$  CTG . CAG . TCG . ACG . GTA .  $\xrightarrow{\text{HindIII}}$  CCG . CGG . GCC . CGG . GAT . CCA . CCG . GTC . GCC . ACC . ATG . GAG .  $\xrightarrow{\text{EcoRI}}$  AGC  $\xrightarrow{\text{Sall}}$   $\xrightarrow{\text{KpnI}}$   $\xrightarrow{\text{ApaI}^*}$   $\xrightarrow{\text{BamHI}}$   $\xrightarrow{\text{AgeI}}$   $\xrightarrow{\text{TurboGFP-dest1}}$   
 $\xrightarrow{\text{AfeI}}$   $\xrightarrow{\text{XhoI}^*}$   $\xrightarrow{\text{PstI}^*}$   $\xrightarrow{\text{SacI}}$   $\xrightarrow{\text{SmaI/XmaI}}$

\* — not unique sites.

## Use

- Generation of fusions to the TurboGFP-dest1 N-terminus
- Expression of TurboGFP-dest1 or its fusions in mammalian cells
- Positive control for the pTurboGFP-PRL-dest1 vector (Cat.# FP518)

Product	Cat.#	Size
pTurboGFP-dest1	FP519	20 µg

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

Vector type	mammalian expression vector
Reporter	destabilized TurboGFP (TurboGFP-dest1)
Reporter codon usage	mammalian
Promoter for TurboGFP	P <sub>CMV IE</sub>
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

## Vector description

pTurboGFP-dest1 vector is an eukaryotic (mammalian) expression vector encoding destabilized green fluorescent protein TurboGFP-dest1. To generate TurboGFP-dest1 variant, residues 422-461 of mouse ornithine decarboxylase (MODC) were fused to the TurboGFP C-terminus. This MODC region targets the protein for degradation and provides for rapid protein turnover (Li *et al.*, 1998).

TurboGFP-dest1 codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). To increase TurboGFP-dest1 mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TurboGFP-dest1 coding sequence (Kozak, 1987).

The vector can be used to express TurboGFP-dest1 in mammalian cells. For example it can be used as a positive control with a pTurboGFP-PRL-dest1 promoterless vector (Cat.# FP518). The vector can be also used to generate destabilized TurboGFP-tagged fusion proteins. Multiple cloning site (MCS) is located upstream of TurboGFP-dest1 coding sequence.

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) for protein expression, 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 provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*.

## Generation of fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to the TurboGFP-dest1 N-terminus when inserted in the same reading frame as TurboGFP-dest1 and no in-frame stop codons are present. TurboGFP-dest1-tagged fusions retain fluorescent properties of the TurboGFP allowing fusion localization *in vivo*. Furthermore we expect that these fusions should be destabilized. Unmodified pTurboGFP-dest1 vector will express TurboGFP-dest1, when transfected into eukaryotic (mammalian) cells.

**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

The vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of TurboGFP-dest1 or its fusions in many cell types. 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

**P<sub>CMV IE</sub>:** 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

**MCS:** 591-671

**TurboGFP-dest1**

Kozak consensus translation initiation site: 672-682

Start codon (ATG): 679-681

Last amino acid in TurboGFP: 1372-1374

Stop codon: 1510-1512

MODC PEST sequence: 1390-1512

**SV40 early mRNA polyadenylation signal**

Polyadenylation signals: 1667-1672; 1696-1701

mRNA 3' ends: 1705; 1717

**f1 single-strand DNA origin:** 1764-2219

(packages the noncoding strand of TurboGFP-dest1)

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

-35 region: 2281-2286; -10 region: 2304-2309

Transcription start point: 2316

**SV40 origin of replication:** 2560-2695

**SV40 early promoter (P<sub>SV40e</sub>)**

Enhancer (72-bp tandem repeats): 2393-2464; 2465-2536

21-bp repeats: 2540-2560; 2561-2581; 2583-2603

Early promoter element: 2616-2622

Major transcription start points: 2612; 2650; 2656; 2661

**Kanamycin/neomycin resistance gene**

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2744-2746; Stop codon: 3536-3538

G->A mutation to remove PstI site: 2926

C->A (Arg to Ser) mutation to remove BssHII site: 3272

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

Polyadenylation signals: 3774-3779; 3787-3792

**pUC plasmid replication origin:** 4123-4766

## 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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