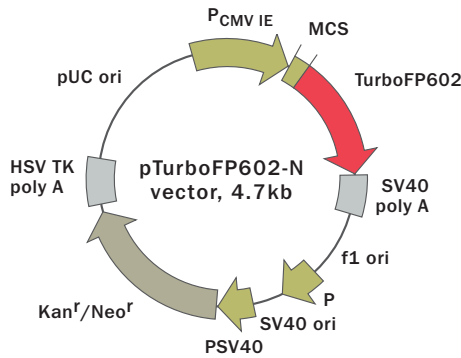


Mammalian expression vector pTurboFP602-N



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

Multiple cloning site (MCS)

<u>NheI</u>	<u>BglII</u>	<u>SacI</u>	<u>HindIII</u>	<u>EcoRI</u>	<u>Sall</u>	<u>KpnI</u>	<u>Apal</u>	<u>BamHI</u>	<u>AgeI</u>	<u>TurboFP602</u>
G. CTA. GCG. CTA. CCG. GAC. TCA. GAT. CTG. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CGG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. G. .										→
<u>AfeI</u>	<u>XhoI</u>			<u>PstI</u>		<u>SacII</u>	<u>SmaI/XmaI</u>			<u>NcoI*</u>

* — not unique sites.

Use

- Generation of fusions to the TurboFP602 N-terminus
- Expression of TurboFP602 or its fusions in mammalian cells

Product	Cat.#	Size
pTurboFP602-N	FP712	20 µg

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

Vector type	mammalian expression vector
Reporter	TurboFP602
Reporter codon usage	mammalian
Promoter for TurboFP602	P _{CMV IE}
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Vector description

pTurboFP602-N is an eukaryotic (mammalian) expression vector encoding true-red fluorescent protein TurboFP602. The vector allows to generate fusions to the TurboFP602 N-terminus and to express TurboFP602 fusions or TurboFP602 alone in eukaryotic (mammalian) cells.

TurboFP602 codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase TurboFP602 translation, Kozak consensus translation initiation site is generated upstream of TurboFP602 sequence (Kozak, 1987). Multiple cloning site (MCS) is located between P_{CMV IE} and TurboFP602 coding sequence.

The vector backbone comprises immediate early promoter of cytomegalovirus (P_{CMV IE}) 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 poly A) 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*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

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 TurboFP602 N-terminus when inserted in the same reading frame as TurboFP602 and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. TurboFP602-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

Notes: 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.

Despite its dimeric structure, TurboFP602 is still suitable for generation of fusions with proteins of interest, however we recommend to use TagRFP or TagFP635 for these purposes.

Expression in mammalian cells

pTurboFP602-N vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985). Unmodified pTurboFP602-N will express TurboFP602, when transfected into eukaryotic (mammalian) cells.

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_{CMV IE}: 1-589

Enhancer region: 59-465;

TATA box: 554-560

Transcription start point: 583

MCS: 592-678

TurboFP602

Kozak consensus translation initiation site: 672-682

Start codon (ATG): 679-681; stop codon: 1384-1386

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1540-1545 & 1569-1574

mRNA 3' ends: 1578 & 1590

f1 single-strand DNA origin: 1637-2092

Bacterial promoter for expression of Kan^r gene

-35 region: 2154-2159;

-10 region: 2177-2182

Transcription start point: 2189

SV40 origin of replication: 2433-2568

SV40 early promoter

Enhancer (72-bp tandem repeats): 2266-2337 & 2338-2409

21-bp repeats: 2413-2433, 2434-2454 & 2456-2476

Early promoter element: 2489-2495

Major transcription start points: 2485, 2523, 2529 & 2534

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2617-2619; Stop codon: 3409-3411

G->A mutation to remove Pst I site: 2799

C->A (Arg to Ser) mutation to remove BssH II site: 3145

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

Polyadenylation signals: 3647-3652 & 3660-3665

pUC plasmid replication origin: 3996-4639

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.