p2FP-RNAi vector

Cat. #FP981

Vector description

p2FP-RNAi vector is a mammalian expression vector designed for RNA interference studies.

The vector encodes two fluorescent proteins: TurboGFP and JRed-neomycin phosphotransferase II protein fusion (JRed-Neo^r). Both proteins are under the control of immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$): the first regulates TurboGFP expression, while the second provides JRed-Neo^r expression.

Codon usages of both TurboGFP and JRed proteins are humanized, i.e. optimized for high expression in mammalian cells.

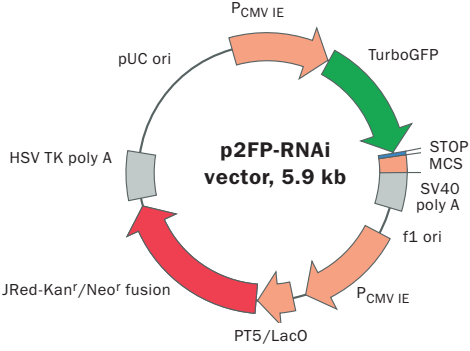
p2FP-RNAi vector backbone also contains pUC origin of replication for propagation in *E. coli*, and f1 origin for single-stranded DNA production. Bacterial T5 promoter allows the expression of kanamycin resistance gene (fusion with JRed) in *E. coli*. To increase TurboGFP mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TurboGFP coding sequence.

Multiple cloning site (MCS) is located between the TurboGFP stop codon and polyadenylation signals allowing to clone a DNA fragment of interest into the TurboGFP 3' untranslated region (3'UTR).

Vector map

For vector sequence, please visit our Web site at www.evrogen.com/p2FP.shtml.

Note: The plasmid DNA was isolated from dam+-methylated *E. coli*. Therefore some restriction sites are bloked 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.



Multiple cloning site (MCS)

STOP#	Bgl II	Sac I	Sal I	Sma I
AA.TGA.TCC.CGG.AAG.ATC.TCG.AGC.TCA.AGC.TTC.GTC.GAC.GGT.ACC.GCC.CGG.GA				
		Xho I*	Hind III	Kpn I

STOP# — in-frame STOP codon of TurboGFP; * — not unique site.

Location of features:

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465; TATA box: 554-560;
Transcription start point: 583
TurboGFP
Kozak consensus translation initiation site: 606-616
Start codon (ATG): 613-615; Stop codon: 1309-1311
MCS: 1312-1360
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1505-1510 & 1534-1539
mRNA 3' ends: 1543 & 1555
f1 single-strand DNA origin: 1602-2057
(Packages the noncoding strand of TurboGFP.)
$P_{CMV\ IE}$: 2231-2812
Enhancer region: 2282-2688; TATA box: 2777-2783;
Transcription start point: 2806
T5 promoter/lac operator element: 2894-2974
T5 transcription start: 2948
JRed-Neo^r
Start codon (ATG): 3002-3004; JRed: 3038-3760
Kanamycin/neomycin resistance gene: 3785-4576; Stop codon: 4574-4576
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4812-4817 & 4825-4830
pUC plasmid replication origin: 5161-5804

Reporters

TurboGFP is an improved variant of the green fluorescent protein from copepoda *Pontellina plumata*. TurboGFP exhibits bright green fluorescence and maturates noticeably faster than EGFP in eukaryotic cells.

JRed is a red fluorescent protein obtained by mutagenesis of Anthomedusae jellyfish chromoprotein. To generate JRed-Neo^r fusion, JRed protein coding sequence was operatively linked to the 5'-end of the Neo^r gene. JRed-Neo^r expression results in red cell fluorescence as well as neomycin resistance allowing selection of stably transfected eukaryotic cells using G418.

Vector use

Schematic representation of the procedure for siRNA testing using p2FP-RNAi vector is shown in **Fig. 1**. A DNA fragment of interest should be cloned into MCS of the vector. When transfected into eukaryotic cells in the absence of a functional siRNA, the vector will express both JRed-Neo^r and TurboGFP proteins. In this case the brightness of green fluorescence significantly prevails.

In the presence of siRNA directed against the cloned DNA fragment, TurboGFP expression and fluorescence are knocked down. JRed expression remains unchanged or (in some experimental systems) even increases due to translational competition.

Thus, p2FP-RNAi vector makes it possible to trace RNA interference in the transfected cells by turning off/on green fluorescence against a background of red fluorescence.

Short description of selected applications

p2FP-RNAi vector can be used in different RNAi-related applications, for example, to test the ability of synthetic siRNA oligonucleotides to knock down the expression of a gene of your interest. The whole gene of interest or its part should be cloned into MCS of the vector. Then the vector must be delivered into the cells together with tested siRNAs. Increasing red/green fluorescence intensity ratio comparing to control experiment indicates successful performance of siRNAs.

p2FP-RNAi vector can be also used as an internal control in the gene silencing experiments. The whole gene of interest or its part containing the siRNA recognition site should be cloned into MCS of the vector. The cells transfected with the resulted construct will fluoresce in green and red channels due to TurboGFP and JRed-Neor expression. Since the cloned fragment is located in the 3' UTR, it is not translated and does not affect functions of neither TurboGFP nor other genes in a cell.

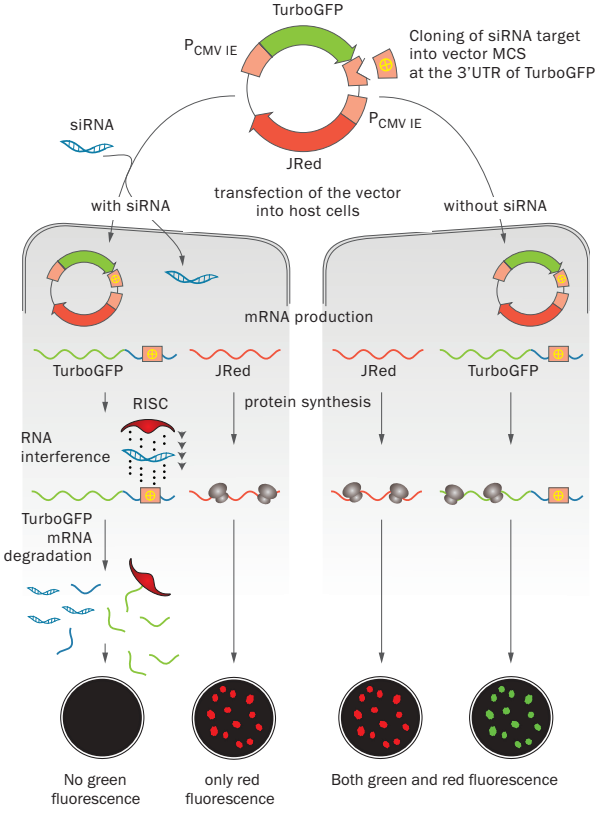


Fig. 1. Schematic representation of the p2FP-RBNAi vector use.

If functional siRNAs are delivered together with p2FP-RNAi vector or already existed in the cells, TurboGFP expression is knocking down and the green/red fluorescence ratio in the cell population switches to red. Hence, decrease of green fluorescence indicates that expression of investigated gene is also knocked down.

Expression in mammalian cells

p2FP-RNAi vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418.

Note: Due to lower phosphotransferase activity of the JRed-Neo^r fusion protein in comparison with neomycin phosphotransferase II alone, working concentration of the neomycin for cultivation of mammalian cells comprising p2FP-RNAi vector must be 2-4 fold lower than standard one for cells with neomycin resistance.

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.

Model experiment

p2FP-RNAi vector was used to test functionality of synthetic siRNA directed against TurboGFP sequence (Fig. 2). Phoenix Eco cells were transfected with the p2FP-RNAi vector mixed with dsRNA directed against TurboGFP sequence. Transfection was performed using Lipofectamine™ 2000 reagent (Invitrogen) with the following protocol:

1. Phoenix Eco cells were plated on 35 mm Petri dishes ($5x10^5$ cells per dish) the day before transfection in growth medium (DMEM) containing serum and antibiotics.

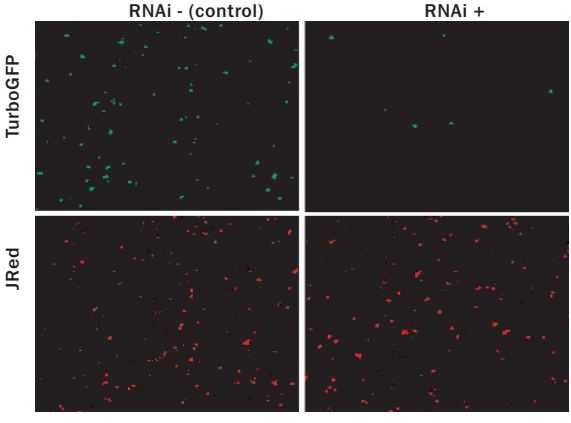


Fig. 2. Fluorescence of mammalian cells transfected with p2FP-RNAi vector with or without siRNA directed against TurboGFP (second day of cell growth). In the presence of siRNA, TurboGFP fluorescence are suppressed almost completely while JRed fluorescence is slightly increased due to the translational competition.

2. Two hours before transfection the growth medium was replaced with 2 ml of DMEM without serum and antibiotics.

3. The following reagents were mixed in four separate tubes with 50 µl of DMEM without serum and antibiotics (volumes per one 35 mm Petri dish):

a. LipofectamineTM 2000 reagent (3 µl)

b. p2FP-RNAi vector (1.5 µg)

c. LipofectamineTM 2000 reagent (4 µl)

d. Synthetic dsRNA (400 pM)

4. After 5 min of incubation, the contents of tubes a and b were mixed together, the same was done with tubes c and d.

5. After 20 min of incubation, the contents of both resulting tubes was added to cells.

6. After 4 hr, the growth medium was replaced with 2 ml of DMEM with serum (10%) serum and antibiotics.

Notice to Purchaser:

TagGFP-related products: These products are intended for research use only and covered by Evrogen Patents and/or Patent applications pending. By use of these products, you accept the terms and conditions of the applicable Limited Use Label License (enclosed).

The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MATERIAL SAFETY DATA SHEET INFORMATION: 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.