pGFP-B-RS Vector – Application Guide

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Package Contents and Storage Conditions
1 vial of pGFP-B-RS Blasticidin vector, (5ug, dried)
1 copy of Application Guide

Product is shipped at room temperature, but should be stored at – 20ºC.

Product Description
The pGFP-B-RS vector has a blasticidin resistance gene replaced with puromycin that is available in OriGene's standard cataloged vectors. Following the protocol provided, the shRNA insert from any OriGene's standard vectors can be easily transferred to this vector to establish stable clones in single or dual knockdown experiments. The pGFP-B-RS vector contains the kanamycin resistance gene for the selection of *E. coli* transformants and for plasmid DNA propagation in *E. coli*. The vector can be used to clone shRNA cassettes and also serves as a negative control for transient transfection experiments.

Production and Quality Assurance
The circular plasmid DNA has been purified from an *E. coli* host strain using a commercial plasmid purification kit. The DNA is suitable for transformation into *E. coli*, transfection into mammalian cells, and other molecular manipulations. It has been tested to be free of nuclease activity. The EcoR I and Hind III digested vector has been tested to successfully re-ligate to pre-cut shRNA cassettes and produce sufficient colonies when transformed into *E. coli* competent cells. The self-ligation background (vector re-ligating to itself without an insert) is less than 5% of transformants.

Methods
Maintenance of pGFP-B-RS vector:
When transforming the DNA, a recA- and endA- E. *coli* host strain should be used to avoid recombination and endonuclease activities. Transformants should be selected on LB kanamycin-agar plates as described in the manufacturer's protocol. Single colonies can be cultured for larger scale plasmid vector purification. For downstream applications, DNA must be clean and in some cases free of endotoxin. (Several commercial purification kits are available which provide transfection-grade purities and achievable endotoxin levels.) A portion of the bacterial culture should be brought to 15% v/v glycerol and stored at –80ºC for long-term maintenance. Re-suspended DNA should be stored at –20ºC.

Preparation of restriction enzyme digested vector from uncut pGFP-B-RS:
1. Digest 1 ug DNA with EcoR I+ Hind III using conditions recommended by the restriction enzyme provider.
2. Add 10% volume of 1M Tris. HCl, pH 8.3 to the digestion tube.
3. Add 5 units of Calf intestine alkaline phosphatase (CIP).
4. Incubate at 37 ºC for 30 min, or as recommended by the manufacturer.
5. Perform agarose gel electrophoresis to separate the insert and vector fragments.
6. Use a gel purification kit to purify the vector fragment.
7. Estimate the recovered DNA quantity by spectrophotometric analysis or by running an agarose gel and by comparing to a reference DNA such as DNA Quanti-Ladder (cat #QLD200).

Shuttling shRNA cassette from entry vector to pGFP-B-RS vector

1. Digest the shRNA insert from entry vector:

Item	Vol/Sample
ddH2O	7ul
NEBuffer II	1ul
EcoR1 (HF)	0.5ul
Hind III	0.5ul
Entry vector with shRNA	1ul

Incubate at 37°C for 1 hr. Incubate at 65°C for an additional 20 minutes. Once digested, proceed with this mixture to do a ligation using components below.

2. Set up a ligation reaction:

Item	Vol/Sample
ddH2O	6.5ul
T4 DNA ligation Buffer	1ul
T4 DNA ligase	0.5ul
pGFP-B-RS (pre-cut 20ng/ul)	1.0ul
Digest from step 1	1.0ul

Incubate the ligation reaction at room temperature for 1 hour.

5. Transform the ligation reaction into high-efficiency, competent *E. coli* cells (≥ 1×108 CFU/µg DNA) following the appropriate transformation protocol. Plate the transformants on LB-agar plates with 25ug/ml kanamycin.
6. Pick at least four colonies for subsequent DNA purification and screening. Amplify and purify the selected clone(s) by growing overnight in liquid LB-Kan media, then isolating the DNA using standard plasmid purification procedures.
7. Confirm the insert by restriction digestion and/or shRNA cassette sequencing.

Features for pGFP-B-RS vector:

Start	End	Description
1	6	EcoR I
75	331	U6 promoter
335	340	BamH I
379	385	Hind III
386	391	Sal I
413	604	SV40 promoter
674	1072	Blasticidin resistance gene
1251	1744	3' LTR
2101	2720	pBR322 ORI
2779	3365	Poly A signal
3648	4380	tGFP
4269	4894	CMV Promoter
5007	5897	Kanamycin resistance
6033	6503	5' LTR