



GenScript Corporation

PO Box 855, Scotch Plains
NJ 07076-0855

Tel: 732-357-3839
Fax: 732-210-0262
E-mail: order@genscript.com

Product Information for siRLuc Constructs

A. Generate large amount of DNA:

The vector DNA is delivered as lyophilized form (5 µg). Before use, add 20 µl water to dissolve it. You can use it directly if you only need limited DNA. For large amount of DNA, please dilute the plasmid 100 fold, and transform competent DH5α or TOP10 cells to make a Maxprep using Qiagen Maxprep kit. siRLuc constructs are all Amp resistant.

B. The insert information for siRLuc:

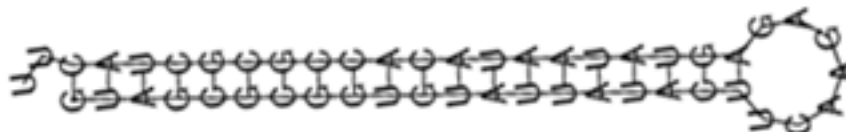
SiRLuc is a siRNA construct designed to knock down Renilla luciferase. The insert sequence is the following:

BamH I		HindIII
GGATCCCGTAGCGGGTGTATTATAC	TTCAAGAGAGTATAATACACCGCGCTACTTTTTTCCAAAAGCTT	
	Sense	Loop antisense Termination

The target sequence (GTAGCGCGGTGTATTATAC) is from reference Nature Biotechnology, 19:497. The target sequence matches pRL-TK, and is designed to silence Renilla luciferase expressed by a co-transfected pRL-TK vector. Here is the alignment:

```
target:      1 gtagcgcggtgtattatac 19
              |||||
pRL-TK:    1241 gtagcgcggtgtattatac 1259
```

Predicted secondary structure of the hairpin structure:



C. Vector information:

The siRLuc insert has been cloned into pRNA series vector using BamH I and Hind III sites and have been sequencing verified. Please see attached map for reference.

D. Transfecting mammalian cells:

A variety of protocols such as lipofection and electroporation have been used successfully to transfect vector-based siRNA constructs into mammalian cells. The transfection procedures are identical to the way you have used for DNA plasmid transfection. The choice of transfection procedures will depend on the mammalian cell line used. For general cell line, we recommend to use Lipofectamine-2000 from Invitrogen. The information and protocol can be found using this link:

<http://www.lifetech.com/content.cfm?pageid=93>.

Here is a brief protocol for siRLuc construct transfection.

1. To use siRLuc construct, pGL-3 control vector (Promega, Cat. #E1741) and pRL-TK vector (Promega, Cat. #E2241) need to be purchased from Promega.
2. To observe the silence effect of siRLuc, three sets of transfections are needed: a. pGL-3 control and pRL-TK vector alone; b. pGL-3 control and pRL-TK plus siRLuc; c. pGL-3 control and pRL-TK plus an empty pRNA vector.
3. For cell transfection, 12-well plates can be used. For 293SFM cell from Invitrogen, 20,000 cell can be seeded the day before transfection.
4. The amount of siRLuc plasmid used for transfection should be 10-30 fold higher than pGL-3 control plasmid. For 293SFM, 0.16 μ g of pGL-3 control and 0.16 μ g of pRL-TK vector were used, 1.6 μ g of siRLuc construct or empty vector are used for each well.
5. The plasmid can be transfected into mammalian cells using Lipofectamine-2000 following the protocol.
6. The Firefly and Renilla luciferase activities can be measured using Dual Luciferase assay kit from Promega (Cat. #E1910) after 24 hrs of transfection.
7. The activities of Renilla luciferase need to be normalized using Firefly luciferase activity.
8. Typical inhibition of Renilla luciferase by siRLuc construct is about 80%.

E. References:

1. Elbashir SM et al, (2001) Nature, 411, 494-498.
2. Sui G et al, (2002) PNAS, 99, 5515-5520.
3. Miyagishi et al, (2002) Nature Biotechnology, 19, 497-500.