shRNA Offers Important Advantages Over siRNA for RNAi Studies: MessageMuter™ shRNAi Production Kit

Introduction

RNA interference (RNAi) is a powerful technique for elucidation of gene function in eukaryotic cells. The RNAi effect can be mediated by transfecting cultured cells with short interfering RNA (siRNA), which is short, double-stranded RNA of 21 to 23 base pairs, or, more recently demonstrated, with short hairpin RNA (shRNA)[1,2,3,4] (Figure 1). Though siRNA and shRNA elicit comparable gene silencing results in RNAi experiments[1,2,5], preparation of shRNA using EPICENTRE's new MessageMuter™ shRNAi Production Kit offers significant advantages compared to either chemical synthesis or *in vitro* transcription of siRNA.

The MessageMuter shRNAi Production Kit produces transfection-ready shRNA in about 4 hours using a simple, 3-step process[4] (see side bar on page 13). Compared to chemical synthesis of siRNA, the kit produces shRNA faster and at a significantly lower cost. Compared to *in vitro* transcription of siRNA, shRNA produced using the MessageMuter Kit is faster and easier to prepare and offers greater flexibility in target sequence selection.

Produce an shRNA from a single oligodeoxynucleotide and a single in vitro transcription reaction

Traditional methods of producing siRNA by *in vitro* transcription requires the user to design and synthesize at least two and, in some procedures, as many as four oligodeoxynucleotides to serve as *in vitro* transcription templates. Once the oligos are annealed, two separate *in vitro* transcription reactions of 2 hours or longer are needed to generate the sense and anti-sense strands of the siRNA.

By comparison, shRNA prepared using the MessageMuter Kit is produced from a single oligodeoxynucleotide. Following a 30-minute "fill-in" reaction which generates the transcription template, i*n vitro* transcription of shRNA is performed using EPICENTRE's new AmpliScribe™ T7-*Flash*™ transcription system. The AmpliScribe T7-*Flash* system enables the user to generate high yields of shRNA in just 30 minutes.

Thus, compared to producing siRNA by *in vitro* transcription, producing shRNA with the MessageMuter kit procedure requires less up-front oligo design and synthesis and uses a shorter, high-yield transcription reaction.

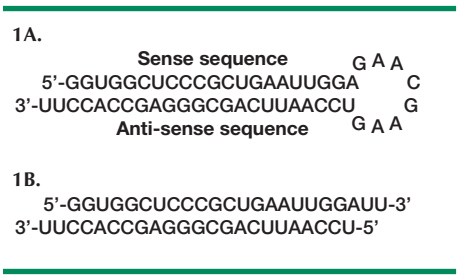

Figure 1. Structural comparison of short hairpin RNA (shRNA) and siRNA directed against the same target sequence of firefly luciferase. **1A.** The shRNA produced using the MessageMuter™ shRNAi Production Kit contains a sequence homologous to the target mRNA (sense sequence), a "loop" region and a sequence complementary to the target sequence (anti-sense sequence). In the example shown, the shRNA targets a 21-base sequence of the firefly luciferase mRNA. **1B.** An siRNA targeting the same 21-base sequence of firefly luciferase.

Advantages of shRNA made with the MessageMuter Kit

- Produced from a single oligodeoxynucleotide
- No need to anneal RNA strands
- Target any sequence 21 – 29 bases long

Producing shRNA does not require annealing of two RNA strands

Preparation of siRNA typically requires overnight annealing of the sense and anti-sense strands of RNA produced by the *in vitro* transcription reactions. The overnight annealing adds a significant amount of time to the production procedure and increases the potential for RNase contamination, which will compromise the yield and integrity of the siRNA.

Once transcribed, shRNA spontaneously forms a hairpin structure in the transcription reaction mix and avoids the need for a long and potentially harmful annealing step. Following clean-up, the shRNA is ready for transfection into cells.

shRNA is readily produced against any target sequence

For efficient *in vitro* transcription initiation, T7 RNA polymerase requires a 'GG' or 'GA' dinucleotide[6] at the start of the transcript. When preparing siRNA by *in vitro* transcription, this limits the selection of mRNA targets to sequences that begin with 'GG' or 'GA'. Alternatively,

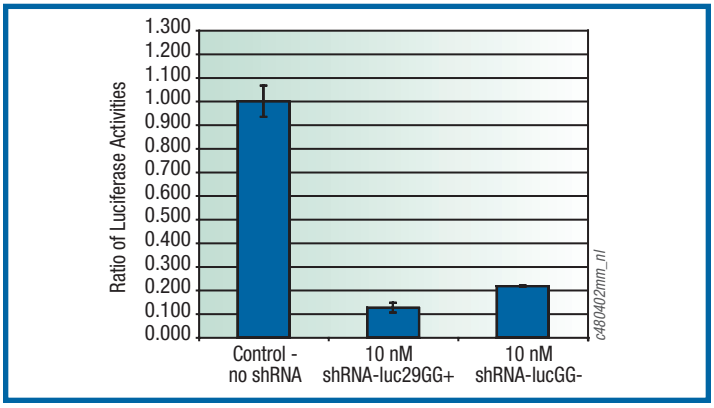

Figure 2. Adding a 5'-GG to the target sequence of a shRNA does not significantly affect effective gene silencing. A 29-base shRNA (shRNA-luc29GG+) was prepared against a 29-base target sequence in firefly luciferase mRNA. The targeted sequence contained a 5'-GG dinucleotide. A 27-base shRNA (shRNA-luc27GG-) was prepared against a 27-base target sequence two bases "upstream" of the shRNA-luc29GG+ target and lacking a 5'-GG or 5'-GA dinucleotide. The dinucleotide 'GG' was added 5' to the target sequence in the user-designed oligo used to produce shRNA-luc27GG-, so this shRNA contains a 'GG' that is not target specific. The shRNA-luc29GG+ and shRNA-luc27GG- were independently co-transfected into HeLa cells in triplicate with firefly and *Renilla* (control) luciferase expression vectors. Results are presented as a ratio of firefly luciferase to *Renilla* luciferase activities. Both shRNA-luc29GG+ and shRNA-luc27GG- reduced firefly luciferase expression by 80% or more.

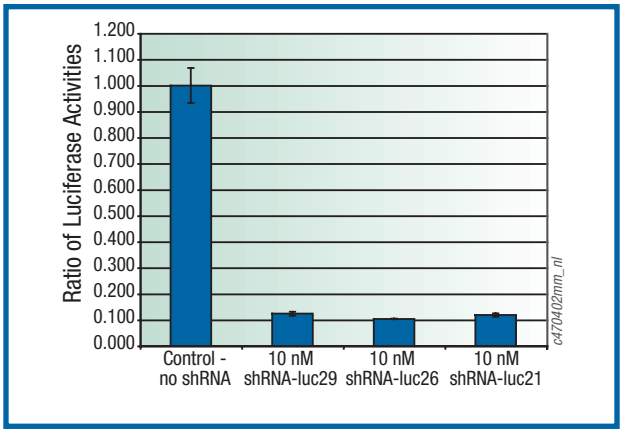

Figure 3. shRNA produced against target sequences ranging from 21 to 29 bases effectively induced silencing of firefly luciferase. The MessageMuter™ Kit was used to produce shRNAs directed against 21-base (shRNA-luc21), 26-base (shRNA-luc26), and 29-base (shRNA-luc29) target sequences in the same region of firefly luciferase mRNA. Transfection of COS-7 cells was done in triplicate with firefly and *Renilla* (control) expression vectors and 10 nM of the respective shRNA-luc. Results are presented as a ratio of firefly luciferase to *Renilla* luciferase activities.

another supplier's siRNA production kit allows the user to synthesize a transcription template that adds a 5′-GG dinucleotide to the target sequence. However, the 5′-GG of the resulting siRNA must be removed prior to cell transfection using a time-consuming and carefully controlled ribonuclease digestion. Thus, *in vitro* transcription of siRNA either limits the user's selection of target mRNA sequences or, if using the other supplier's kit, requires an additional ribonuclease treatment.

In contrast, shRNA can be easily prepared for any target sequence, even those without the 5′-GG or 5′-GA. To prepare MessageMuter shRNA to these target sequences, see the MessageMuter protocol at www.epicentre.com/shRNA.asp. shRNA targeted to these sequences does not require post-transcriptional manipulation.

Additionally, shRNA can be designed against target sequences from 21 to 29 bases long. Targeting larger sequences, in conjunction with cellular processing of the shRNA[1,5] does not alter, and may enhance the overall silencing effect. Using the MessageMuter shRNAi Production Kit, we prepared shRNAs directed against 21-base, 26-base and 29-base target sequences in the same region of firefly luciferase mRNA. These shRNAs demonstrated virtually no difference in their ability to silence expression of firefly luciferase in COS-7 cells (Figure 3). Comparable results were seen in HeLa cells, Normal Rat Kidney (NRK) cells and mouse embryo fibroblast (NIH/3T3) cells (data not shown).

Discussion

EPICENTRE's new MessageMuter™ shRNAi Production Kit produces transfection-ready shRNA, faster and easier than *in vitro* transcription methods for making siRNA and at significantly lower cost than chemical synthesis methods. *In vitro* transcription of shRNA using the MessageMuter Kit enables the user to:

- Eliminate annealing of RNA strands.
- Produce shRNA from a single oligodeoxynucleotide.
- Produce shRNA from a single *in vitro* transcription reaction.
- Target a sequence that does not begin with a 'GG' or 'GA' dinucleotide without the need for a post-transcriptional ribonuclease digestion.
- Target a sequence of 21 to 29 bases in length.

References

1. Paddison, P.J. *e. al.* (2002) *Genes & Development* **16**, 948.
2. Yu, J-Y *et al* (2002) *Proc. Natl. Acad. Sci. USA* **99**(9), 6047.
3. Meis, J.E. (2003) EPICENTRE *Forum* **10**(2), 1.
4. Meis, J.E. (2004) EPICENTRE *Forum* **11**(1), 1.
5. McManus, M.T. *et al.* (2002) *RNA* **8**, 842.
6. Milligan, J.F. *et al.* (1987) *Nuc. Acids Res.* **15**(21), 8783.

Produce transfection-ready shRNA in about 4 hours using the MessageMuter shRNAi Production Kit

The MessageMuter shRNAi Production Kit utilizes a simple and unique 3-step process that yields transfection-ready shRNA in about 4 hours. Each reaction produces enough shRNA for 100's of transfections.

1. Anneal the T7 Promoter Oligo (a short oligodeoxynucleotide containing a phage T7 transcription promoter sequence, provided in the kit) to an oligodeoxynucleotide designed and provided by the user.

2. "Fill-in" the ends of the annealed duplex using the Klenow exo-minus fragment of DNA Polymerase and dNTPs (both provided in the kit) to generate a linear double-stranded DNA template for *in vitro* transcription by T7 RNA Polymerase.

3. Transcribe shRNA from the DNA template in a rapid, high-yield *in vitro* transcription reaction using reagents that are provided in the kit. The transcribed RNA spontaneously forms a hairpin structure (shRNA) in solution. Following clean-up, the shRNA is ready for transfection into cultured cells.

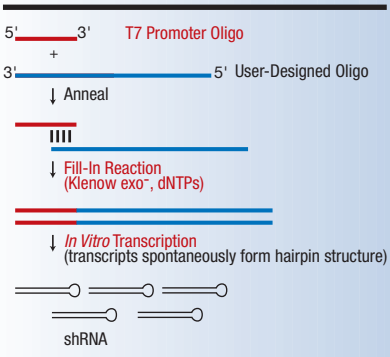

Figure 4. Overview of the method used to produce shRNA using the MessageMuter™ shRNAi Production Kit. All reagents are supplied with the kit except for the user-designed oligo – a 60- to 76-base oligodeoxynucleotide.