

High Throughput Screening For RNAi Using the MessageMuter™ shRNAi Production Kit

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 double-stranded, short interfering RNA (siRNA) or, as recently demonstrated, with short hairpin RNA (shRNA).^{1,2,3,4,5} Figure 1 compares the structures of an shRNA and an siRNA directed against firefly luciferase. Figure 2 shows the silencing effect of an shRNA directed against firefly luciferase and transfected into a variety of mammalian cells.³

Though siRNA and shRNA elicit comparable results in RNAi experiments, preparation of shRNA using EPICENTRE's MessageMuter™ shRNAi Production Kit (see box) has significant advantages over both *in vitro* transcription or chemical synthesis of siRNA.⁵ In addition, before investing time and expense in an

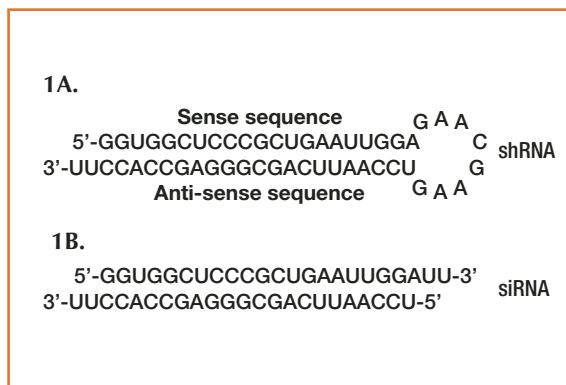


Figure 1. Structural comparison of short hairpin RNA (shRNA) and short interfering (siRNA) directed against the same target sequence of firefly luciferase. **1A.** The shRNA produced using the MessageMuter™ shRNAi Production Kit contains a sequence homologous to the target mRNA (sense sequence), a “loop” 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.

in vivo RNAi expression strategy, the MessageMuter Kit provides an ideal method to rapidly and easily prepare and screen a variety of specific RNA sequences for effective gene silencing. For high throughput RNAi studies, the MessageMuter Kit offers:

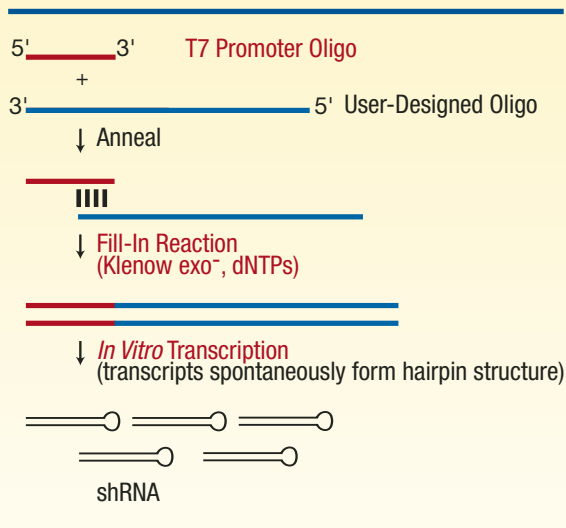
• **shRNA production in about 4 hours**

The MessageMuter Kit produces shRNA in about 4 hours with minimal hands-on time (see box). The siRNA produced by other *in vitro* methods can take up to 24 hours.

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*⁻ 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.



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.

• **No long, inefficient annealing step**

Once transcribed, shRNA, produced using the MessageMuter process, spontaneously forms the functional hairpin structure (Figure 1) in the transcription reaction mix. In contrast, individual siRNA transcripts prepared by *in vitro* transcription require overnight annealing of the sense and anti-sense RNA strands, which is time-consuming and increases the potential for RNase degradation.

• **shRNA production from a single oligodeoxynucleotide and a single *in vitro* transcription reaction.**

shRNA prepared using the MessageMuter Kit is produced from a single user-supplied oligodeoxynucleotide and a single *in vitro* transcription reaction (see box). Other siRNA construction methods require as many as four user-supplied oligos and two *in vitro* transcription reactions.

• **High throughput RNAi screening at significantly lower cost than chemical synthesis of siRNA.**

Each MessageMuter reaction produces sufficient shRNA for 100's of transfections. Compared to chemical synthesis of double-stranded siRNA, each shRNA is produced at a fraction of the cost.

References

1. Paddison, P.J. et 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. Meis, J.E. (2004) *EPICENTRE Forum* **11**(2), 12.

www.epicentre.com/messagemuter.asp

MessageMuter™ shRNAi Production Kit
MM031110 10 Reactions

Each kit provides reagents to prepare 10 different shRNA in sufficient quantity for 100's of transfections. User provides one oligodeoxynucleotide.

See also:

**Product Data Sheet
on Page 14**

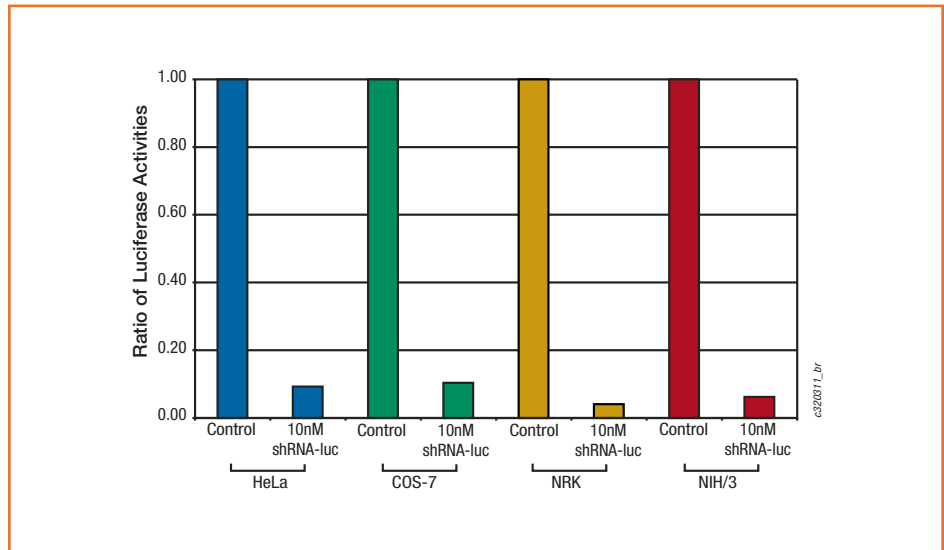


Figure 2. shRNA-luc, produced with the MessageMuter™ shRNAi Production Kit, effectively induced sequence-specific silencing of firefly luciferase in a variety of cultured mammalian cells. Transfections were performed in triplicate with firefly and *Renilla* (sea pansy) luciferase expression plasmids and 10 nM shRNA-luc, as described.³ Cells tested were HeLa (blue), COS-7 (green), Normal Rat Kidney (NRK; yellow) and mouse embryo fibroblast (NIH/3T3; red). Controls for all experiments are shown as a ratio of firefly luciferase activity to *Renilla* luciferase.

RNAi

FASTER, EASIER & at LOWER COST

Introducing the new
MessageMuter™ shRNAi Production Kit
from EPICENTRE

- Produce transfection-ready shRNA in 4 hours.
- Easier than other *in vitro* siRNA construction methods. . . no need to anneal RNA strands.
- Much lower cost than chemical synthesis.

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