EPICENTRE Forum
Tools & Techniques for Genomics, Proteomics & RNA Research

Rapid, High Yield Transcription of Short Hairpin RNA (shRNA) for RNAi-Mediated Gene Silencing

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RNA interference (RNAi) is a powerful technique for elucidation of gene function.[1] Typically, the RNAi response in mammalian cells is mediated by short double-stranded RNA (dsRNA). Recently, short hairpin RNAs (shRNA), single-strand RNAs containing a high degree of secondary structure, have been shown to result in gene silencing as effectively as short dsRNA produced by methods such as chemical synthesis.[2,3,4] Here we demonstrate that EPICENTRE's new AmpliScribe™ T7-*Flash*™ Transcription Kit is ideal for rapid, high yield production of shRNA for RNAi studies.*

Methods

Design of the in vitro transcription template for shRNA production

Figure 1 provides an overview of the process for producing and using shRNA for RNAi. Two complementary 87-base DNA oligonucleotides were synthesized to serve as the *in vitro* transcription template for producing shRNA. The first oligo contained a T7 RNA polymerase promoter sequence followed by a 29-base sequence complementary to firefly luciferase mRNA, an 8-base non-complementary sequence (the "hairpin") and the complement to the 29-base firefly luciferase sequence.[3] The second oligo was the complement of the first. The 29-base sequence, complementary to firefly luciferase mRNA, was chosen to silence expression of firefly luciferase without affecting the expression of *Renilla* luciferase, which was used as a control in these experiments.

The 87-base DNA oligos were resuspended in TNE (10 mM Tris-HCl, pH 7.5; 100 mM NaCl; 1 mM EDTA), denatured at 95ºC for 5 minutes, and annealed at 65ºC for 10 minutes, followed by 37ºC for 10 minutes.

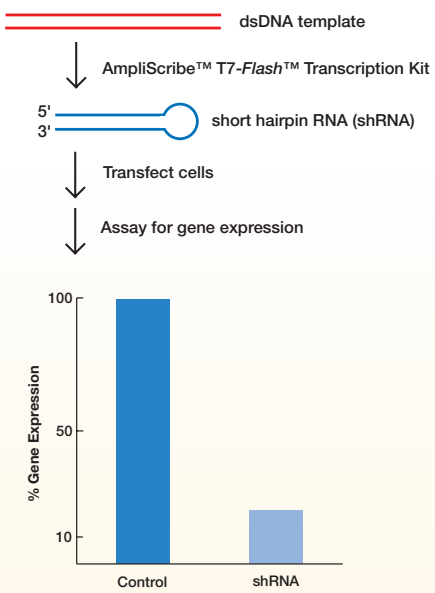

Figure 1. Overview of shRNA production, *in vitro*, and use for RNAi. Two complementary DNA oligos, containing a T7 transcription promoter and sequences of the targeted mRNA are synthesized, annealed, and transcribed *in vitro* using the AmpliScribe™ T7-*Flash*™ Transcription Kit. The short, single-stranded RNA transcript spontaneously forms a hairpin structure (shRNA). Transfection of the shRNA into cells mediates silencing of the targeted gene.

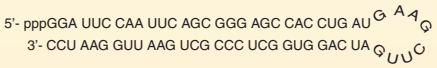

Figure 2. The 66-base, single-stranded shRNA-*luc* was transcribed from an 87-bp oligo DNA that contained a T7 transcription promoter, a 29-base sequence complementary to firefly luciferase, and an 8-base "hairpin" sequence.

In vitro transcription of shRNA using the AmpliScribe T7-Flash Transcription Kit

A 66-base shRNA (Figure 2), specific for silencing firefly luciferase and designated shRNA-*luc*, was transcribed from 1 µg of the 87-bp DNA template for 30

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minutes in a 20-µl AmpliScribe T7-*Flash* transcription reaction. Following transcription, the reaction was treated with DNase I, phenol:chloroform extracted, and the shRNA-*luc* was ethanol precipitated. Unincorporated nucleotides were removed by spin column chromatography and the yield of shRNA-*luc* was quantified by spectrophotometry.

Cell transfection and assay of shRNA-luc mediated RNAi activity

HeLa cells and Cos-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 100 µg/ml penicillin/ streptomycin (DMEM +10% FBS). Adherent cells were plated at $1x10^5$ cells per well in 24-well plates and grown in DMEM + 10% FBS for 24 hours. Media were aspirated and replaced with 100 µl of serum-free DMEM. Cells were then transfected in triplicate by adding 2 µl of Oligofectamine™ Reagent (Invitrogen), 250 ng of pGL3-Control firefly luciferase (*luc*) expression vector (Promega), and 10 ng of pRL-SV40 *Renilla* (sea pansy) luciferase expression vector (Promega) as a control, together with 9.2 to 36.8 pmoles of shRNA-*luc*. Transfected cells were incubated for 24 hours, lysed, and assayed for both firefly and *Renilla* luciferase activities (Promega–Dual Luciferase® Reporter Assay System). The ratio of firefly luciferase to *Renilla* luciferase was normalized to replicates that were transfected with the firefly and *Renilla* luciferase expression vectors without shRNA.

Results

Typically, >2500 pmoles (>60 µg) of shRNA-*luc* were produced in a 30 minute AmpliScribe T7-*Flash* transcription reaction from 1 µg of the 87-bp DNA template. The effect of shRNA-*luc* on firefly luciferase expression in HeLa cells is presented in Figure 3A as a ratio of firefly luciferase activity to *Renilla* luciferase activity. As shown in Figure 3A, 92nM shRNA-*luc* (9.2 pmoles; ≈200 ng) suppressed expression of firefly luciferase by more than 80% without affecting expression of *Renilla* luciferase. Firefly luciferase expression was reduced by 90% by 276 nM shRNA-*luc* (27.6 pmoles; ≈600 ng). Similar results were seen using Cos-7 cells (Figure 3B).

Based on the gene silencing data of Figure 3A and 3B, a single AmpliScribe T7-*Flash* transcription reaction produced enough shRNA-*luc* for at least 100 RNAi experiments.

Discussion

Short, hairpin RNA (shRNA), prepared by *in vitro* transcription was effective in targeted gene silencing (RNAi) in HeLa and Cos-7 cells. The advantages of shRNA for RNAi studies, compared to dsRNA, include:

1. Lower cost than chemical synthesis of double-stranded RNA.
2. No need for DNA cloning of the transcription template.
3. Eliminates the need for post-transcriptional purification and annealing of sense and anti-sense RNA strands.
4. Easier design of DNA templates because shRNA is processed by the cell.[3,4]
5. No need to digest long double stranded RNA transcripts *in vitro* with RNase III or Dicer prior to transfection.

The AmpliScribe T7-*Flash* Transcription Kit produces high yields of shRNA in 30 minutes (also see the article on page 6). A single 20-µl AmpliScribe T7-*Flash* reaction produces enough shRNA for at least 100 RNAi experiments.

References

1. Fire, A. *et al.* (1998) *Nature* **391**, 806.
2. Svoboda, P. *et al.* (2001) *Biochem. Biophys. Res. Comm.* **12**(5), 287.
3. Paddison, P.J. *et. al.* (2002) *Genes & Development* **16**, 948.
4. McManus, M.T., *et. al.* (2002) *RNA* **8**, 842.

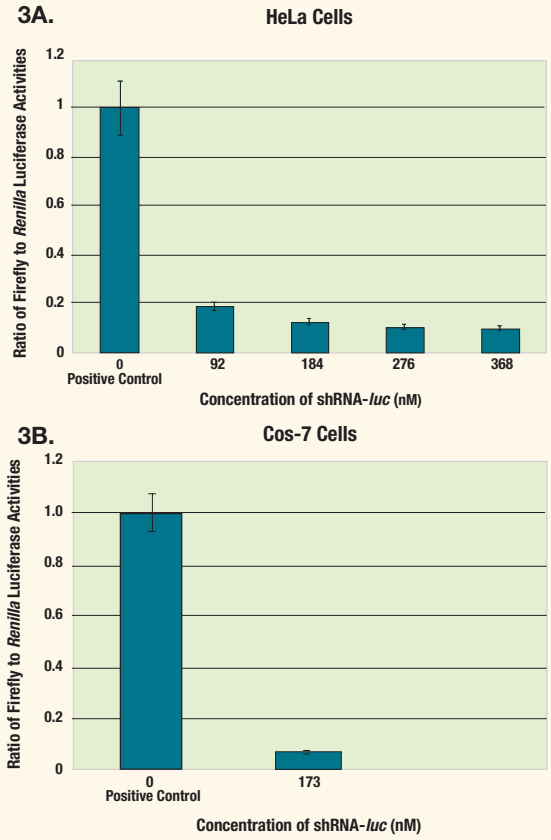

Figure 3. shRNA-*luc*, produced using the AmpliScribe™ T7-*Flash*™ Transcription Kit, effectively induced sequence-specific silencing of firefly luciferase in HeLa cells (3A) and in Cos-7 cells (3B). Transfections were performed in triplicate with firefly and *Renilla* (sea pansy) luciferase expression plasmids, as well as the indicated concentration of shRNA-*luc*, as described in the text.