

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 95°C for 5 minutes, and annealed at 65°C for 10 minutes, followed by 37°C for 10 minutes.

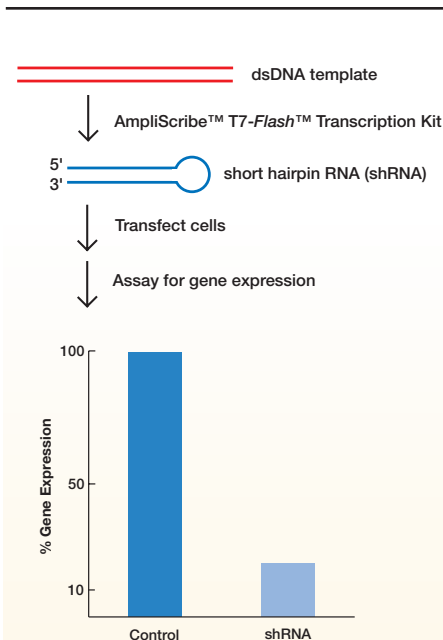


Figure 1. Overview of shRNA production, *in vitro*, and use for RNAi. Two complementary DNA oligos, containing a T7 transcription promoter and sequences of the targeted mRNA are synthesized, annealed, and transcribed *in vitro* using the AmpliScribe™ T7-Flash™ Transcription Kit. The short, single-stranded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

5'- pppGGA UUC CAA UUC AGC GGG AGC CAC CUG AU G A A G
3'- CCU AAG GUU AAG UCG CCC UCG GUG GAC UA G U U C

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

... continued on page 2

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In This Issue

- 1 Rapid, High Yield Transcription of Short Hairpin RNA (shRNA) for RNAi-Mediated Gene Silencing
- 3 High Quality RNA for Use with MicroArrays MasterPure™ Genomic DNA for *Bacillus anthracis* Analysis
- 4 DuraScript™ dsRNA Digested Using Human Dicer Enzyme Is As Effective and Specific in RNAi-Mediated Gene Silencing As Canonical "Diced" dsRNA
- 6 Transcribe More RNA in 30 Minutes Than Other *In Vitro* Transcription Kits Produce in 2 Hours
- 8 Obtain PCR-Ready Genomic DNA from Buccal Cells, HeLa Cells, Hair Follicles, Tail Snips, Bacterial Cells, or Feathers Using the QuickExtract™ DNA Extraction Solution
- 9 10 Reasons Why DNA Cloning Kits from EPICENTRE Enable the Fastest and Most Reliable Results
- 10 Efficient Cloning of Entire Mitochondrial Genomes in *Escherichia coli* by *In Vitro* Insertion of a Transposon
- 12 CopyControl™ Cloning Systems Allow Single-Copy Cloning, Then Controlled Induction for High-Copy DNA Production
- 14 The FailSafe™ Real-Time PCR System with SYBR® Green I Dye Provides Shorter Cycle Times and More Consistent PCR Quantitation with Every Template Every Time

minutes in a 20- μ l 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 μ g/ml penicillin/ streptomycin (DMEM +10% FBS). Adherent cells were plated at 1×10^5 cells per well in 24-well plates and grown in DMEM + 10% FBS for 24 hours. Media were aspirated and replaced with 100 μ l of serum-free DMEM. Cells were then transfected in triplicate by adding 2 μ l 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 μ g) of shRNA-*luc* were produced in a 30 minute AmpliScribe T7-Flash transcription reaction from 1 μ g 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; \approx 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; \approx 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- μ l 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.

www.epicentre.com/t7-flash.asp

AmpliScribe™ T7-Flash™ Transcription Kit

ASF3257	25 Reactions
ASF3507	50 Reactions

Contents:

AmpliScribe™ T7-Flash™ Enzyme Solution (with RNase inhibitor), AmpliScribe™ T7-Flash™ 10X Reaction Buffer, 100mM ATP, CTP, GTP, UTP Solutions, RNase-Free DNase I, DTT, RNase-Free Water, and Control DNA Template (linearized).

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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.

