

Simple, Efficient Production of Short Double-Stranded RNA Using RNase III

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Double-stranded RNA (dsRNA) has become a powerful tool for modifying cellular gene expression levels.¹⁻³ Current methods used to produce dsRNA for evaluation in gene silencing experiments include *in vitro* transcription of a PCR-amplified or cloned DNA template or chemical synthesis. Recently, Yang *et al.*⁴ reported that a heterogeneous population of short dsRNA can be efficiently generated by partial digestion of long dsRNA templates using *E. coli* ribonuclease III (RNase III). These researchers also demonstrated that dsRNA up to 30 bp in length effectively mediated RNA interference in cultured cells.

Here we report the rapid and simple production of short dsRNA using EPICENTRE's AmpliScribe™ High Yield Transcription Kits and RNase III.

Preparation of DNA templates for *in vitro* transcription

A 1.4-kb DNA fragment was cloned into plasmid vectors containing either a phage T7 or phage T3 promoter. Clones were chosen so that the insert in the T7 clone would produce the "sense" 1.4-kb RNA transcript and the T3 clone would produce the complementary "anti-sense" 1.4-kb RNA transcript. The T7 and T3 transcription templates were prepared by separately digesting each with a restriction endonuclease that linearized the DNA downstream from the 1.4-kb insert. After quantitation, equal amounts of the two linear templates were mixed together and simultaneously transcribed in a combined AmpliScribe T7 and AmpliScribe T3 High Yield *in vitro* transcription reaction.

In vitro transcription of dsRNA

AmpliScribe T7 and AmpliScribe T3 transcription reactions were performed simultaneously in one tube using the reagents provided in the AmpliScribe Kits as described in Table 1. The sense and anti-sense RNA strands that were produced in the 2-hour reaction annealed spontaneously to form a 1.4-kb dsRNA. Approximately 50 µg (1.25 mg/ml) of intact, full-length 1.4-kb dsRNA was produced by the combined AmpliScribe T7 and T3 reaction as judged by agarose electrophoresis gels (Figure 1). The simultaneous AmpliScribe T7 and T3 reaction

Table 1. Reaction conditions for a combined AmpliScribe™ T7 and AmpliScribe™ T3 *in vitro* transcription reaction.

1. At room temperature, combine:

T7 template DNA (linearized)	1 µg
T3 template DNA (linearized)	1 µg
AmpliScribe™ T7 10X Reaction Buffer	4 µl
100 mM each ATP, CTP, GTP, UTP	3 µl each
AmpliScribe™ T7 Enzyme Mix	1 µl
AmpliScribe™ T3 Enzyme Mix	1 µl
100 mM DTT	4 µl
RNase-Free Water	to 40 µl total volume

2. Incubate the reaction at 37°C for 2 hours.

3. Add 1 U of RNase-Free DNase I and incubate at 37°C for 15 minutes to remove the DNA templates.

can be scaled up to produce milligram amounts of dsRNA if desired.

Combined AmpliScribe T7 and T3 reactions can be performed in a single tube because their optimal reaction buffers are very similar. However, the optimal AmpliScribe SP6 reaction buffer is significantly different from both the AmpliScribe T7 and AmpliScribe T3 buffer. Therefore, to produce dsRNA from templates with both SP6 and T7 promoters or both SP6 and T3 promoters, we recommend that the reactions be done separately and then the single-stranded RNA (ssRNA) reaction products be combined in equivalent amounts, followed by incubation for 1 hour to overnight at room temperature to allow the ssRNAs to anneal.

Production of short dsRNA fragments by partial digestion of long dsRNA with RNase III

The 1.4-kb dsRNA transcript was purified by precipitation with 2.5 M ammonium acetate and then resuspended in TA Buffer (33 mM Tris acetate (pH 7.8), 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM dithiothreitol) to a concentration of 0.5 µg/ml. Five micrograms of the 1.4-kb dsRNA were digested

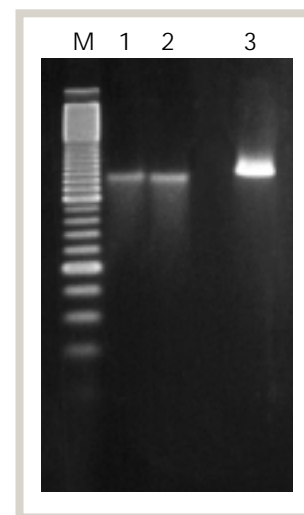


Figure 1. Synthesis of dsRNA in a single tube from simultaneous AmpliScribe™ T7 and AmpliScribe™ T3 High Yield Transcription reactions. Lane 1, 1.4-kb ssRNA produced from an AmpliScribe T7 High Yield Transcription; Lane 2, 1.4-kb ssRNA produced from an AmpliScribe T3 High Yield Transcription reaction; Lane 3, 1.4-kb dsRNA produced from single combined AmpliScribe T7 and AmpliScribe T3 reaction. All reactions were treated with RNase-Free DNase I to remove the DNA template. M, RNA size standards.

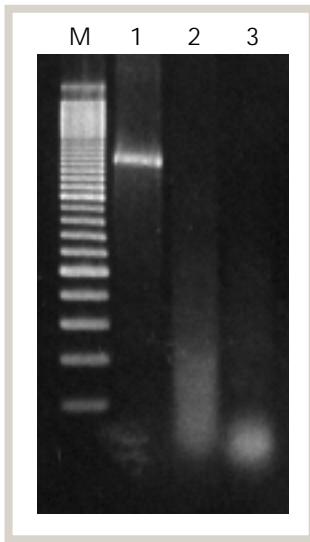


Figure 2. RNase III rapidly digests long dsRNA into short dsRNA. Five micrograms of a 1.4-kb dsRNA were digested as described in the text. Reaction aliquots were removed after 1 minute and 2 minutes of digestion and analyzed by 12% PAGE. Lane 1, 1.4-kb dsRNA; Lane 2, 1.4-kb dsRNA after 1 minute digestion with RNase III; Lane 3, 1.4-kb dsRNA after 2 minute digestion with RNase III.

with 1 Unit of RNase III (EPICENTRE) in 50 μ l of TA Buffer at 37°C. Aliquots of the RNase III digestion reaction were taken at 1 minute and 2 minutes and the RNA analyzed by electrophoresis on 12% polyacrylamide gels. Double-stranded

RNA fragments of approximately 15–30 bp were produced in 1 minute and complete digestion of the 1.4-kb dsRNA to dsRNA fragments of 12–15 bp was observed after 2 minutes (Figure 2).

Conclusion

Short double-stranded RNA fragments can be rapidly and inexpensively produced by partial RNase III digestion of long dsRNA obtained from reactions using AmpliScribe T7, T3 and SP6 High Yield Transcription Kits.

References

1. Fire, A. *et al.* (1998) *Nature* **391**, 806.
2. Misquitta, L. and B.M. Paterson (1999) *Proc. Natl. Acad. Sci. USA* **96**, 1451.
3. Sanchez-Alvarado, A. and P.A. Newmark (1999) *Proc. Natl. Acad. Sci. USA* **96**, 5049.
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AmpliScribe™ High Yield Transcription Kits

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AS2607	25 Reactions
AS3107	50 Reactions
SP6	
AS2606	25 Reactions
AS3106	50 Reactions
T3	
AS2603	25 Reactions
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RNase III (*E. coli*) Now Available from EPICENTRE

RNase III, from *E. coli*, is an endoribonuclease that specifically digests dsRNA to short dsRNA fragments containing 2-base, 3'-overhangs.^{1,2} Complete digestion results in dsRNA fragments of 12-15 bp.

Applications:

- Produce short dsRNA fragments from long dsRNA.
- RNA structure studies.
- RNA processing and maturation studies.
- Produce oligo RNA for *in situ* hybridization or other probe applications.

Unit Definition: One unit of RNase III is the amount of enzyme that solubilizes one nmole of ribonucleotide per hour using PolyA-PolyU as substrate.

Quality Control: RNase III is tested to specifically digest double-stranded RNA transcripts in a mixture containing double-stranded RNA, single-stranded RNA and double-stranded DNA.

Specific Activity: Approximately 1000 U/ mg protein.

1. Robertson, H.D. *et al.* (1968) *J. Biol. Chem.* **243**, 82.
2. Lamontagne, B. *et al.* (2001) *Curr. Issues Mol. Biol.* **V.3**, 71, Academic Press.

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