

Transcribe High Yields of Active Ribozyme in 30 Minutes Using the AmpliScribe™ T7-Flash™ Transcription Kit

Judith E. Meis, EPICENTRE

Introduction

Ribozymes are catalytic RNAs with complex secondary structures. Hammerhead-type ribozymes have a specific structure with three stems and a 13-nucleotide active site which specifically cleaves the RNA substrate sequence GUX, where X=A, C, or U.¹ Here we demonstrate use of the AmpliScribe™ T7-Flash™ Transcription Kit for rapid, high-yield production of both an active hammerhead ribozyme, which specifically cleaves an RNA encoding chloramphenicol acetyl transferase (CAT), and the CAT RNA substrate.

High-Yield Synthesis of the Hammerhead Ribozyme and CAT RNA Substrate

Linearized plasmids containing a T7 promoter and either a 63-bp sequence encoding the CAT ribozyme or the 800-bp sequence encoding the CAT substrate were used as templates^{2,3} in an AmpliScribe T7-Flash Transcription reaction. More than 80 µg of the 63-base CAT ribozyme was obtained from only 2 µg (1 pmole) of template DNA in 30 minutes at 42°C (Figure 1). The CAT ribozyme was homogeneous and full-length based on denaturing polyacrylamide gel electrophoresis (Figure 2). Similarly, 190 µg of a 900-base CAT substrate (transcript included 100 bases of vector sequence upstream from the 800 bases of CAT substrate) was obtained from only 1 µg of the CAT RNA template in another AmpliScribe T7-Flash reaction in 1 hour at 37°C. The *in vitro*-transcribed CAT ribozyme was then evaluated for activity on the CAT RNA substrate.

Efficient Ribozyme Cleavage Reactions

Based on its known cleavage specificity, the CAT hammerhead ribozyme should cleave a CAT transcript 80 bases from the translation initiation codon,¹ resulting in predicted cleavage products of 320 and 580 nucleotides for the *in vitro*-transcribed CAT RNA substrate used here. Cleavage reactions containing 6.8 pmoles (2 µg) of CAT substrate transcript, 25 pmoles (0.5 µg) of CAT ribozyme, 50 mM Tris-HCl (pH 8.0), and 5 mM MgCl₂, were incubated for 5 to 30 cycles consisting of 37°C for 1 minute, to cleave the substrate, and 60°C for 30 seconds, to denature the cleavage complex. Reactions

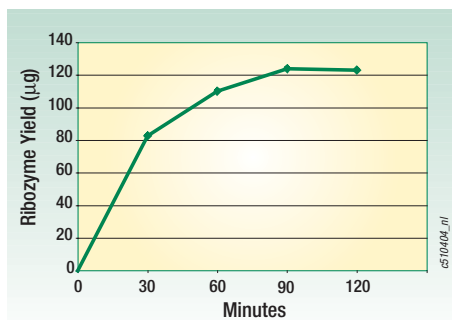


Figure 1. Yields of the CAT ribozyme transcript from an AmpliScribe™ T7-Flash™ Transcription reaction over time. The 63-base CAT ribozyme was transcribed from 1 pmole (2 µg) of a linearized 3235-bp plasmid template in a 20-µl AmpliScribe T7-Flash Transcription reaction at 42°C.

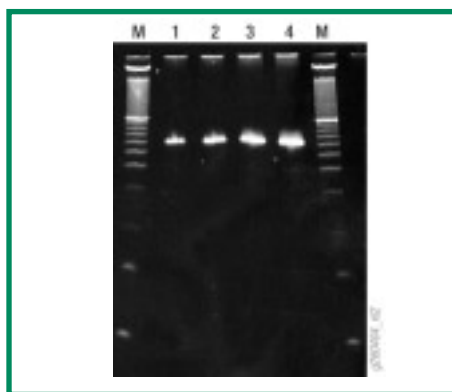


Figure 2. Integrity and homogeneity of the *in vitro*-synthesized CAT ribozyme transcript based on electrophoresis in a 15% denaturing polyacrylamide gel. Uniform transcripts of the expected size (63 bases) were produced from the ribozyme transcription reactions. Lane M, 10 bp DNA ladder; Lanes 1 to 4, 40 ng, 80 ng, 160 ng, 240 ng of ribozyme, respectively.

were stopped by the addition of a loading buffer containing formaldehyde, denatured at 65°C for 3 minutes, and separated by electrophoresis through a 1.5% denaturing agarose gel in formaldehyde/MOPS buffer.

Significant substrate cleavage was detected (Figure 3) in as little as 5 cycles (10 minutes). No specific-cleavage products were observed in a series of control reactions performed using the above conditions without ribozyme (data not shown), although some nonspecific CAT RNA substrate degradation was observed, presumably due to the 60°C denaturation step of the cycling profile.

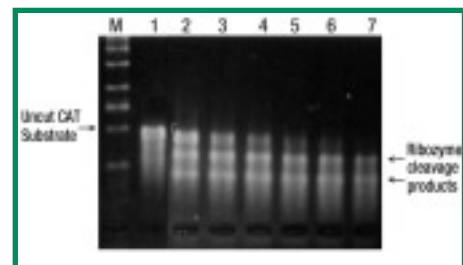


Figure 3. Ribozyme produced with the AmpliScribe™ T7-Flash™ Transcription Kit effectively cleaves CAT substrate RNA in 10 minutes. Lane M, denatured 100-bp DNA Ladder, Lane 1, control cleavage reaction containing no ribozyme RNA, Lanes 2 to 7, CAT ribozyme cleavage reactions after 5, 10, 15, 20, 25, and 30 cycles, respectively, of incubation and denaturation.

Conclusion

Recent improvements to *in vitro* transcription methods at EPICENTRE allow unprecedented yields of active ribozymes in as little as 30 minutes. Now *in vitro* transcription is a fast, economical, and easy alternative to chemical synthesis of ribozymes and RNA substrates.

References

1. Haseloff, J. and Gerlach, W.L. (1988) *Nature* **334**, 585.
2. Hoffman, L. and Johnson, M. (1994) *BioTechniques* **17**(2), 372.
3. Hoffman, L. *EPICENTRE Forum* **1**(1), 6.

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AmpliScribe™ T7-Flash™ Transcription Kit	
ASF3057	5 Reactions
ASF3257	25 Reactions
ASF3507	50 Reactions

AmpliScribe™ T3-Flash™ Transcription Kit	
ASF03725	25 Reactions
ASF03750	50 Reactions