Promega Notes Magazine **Number 60, 1996, p. 20**

In vitro Transcription from Biotinylated DNA Immobilized on Streptavidin MagneSphere® Paramagnetic Particles

By Gary Kobs
Promega Corporation

Rapid purification and in vitro *transcription of PCR products can be accomplished using Promega's Streptavidin MagneSphere® Paramagnetic Particles and RiboMAX™ RNA Production Systems[(a)]. The technique described in this update yields optimal amounts of high quality RNA, which can be used for further applications such as RNA probes or* in vitro *translation.*

[(a)]U.S. Pat. No. 5,552,302 has been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor (PRI). Inhibitors of Angiogenin, which comprises a segment of human PRI, is the subject of U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687 assigned to the President and Fellows of Harvard College and exclusively licensed to Promega Corporation.

Introduction

The PCR[(b)] technique allows the highly specific amplification of unique DNA segments defined by two surrounding primer sequences (1). This amplification process can be further enhanced by several variations in the standard PCR method. One alteration is to include the sequence of a bacteriophage RNA polymerase promoter (i.e., T7, SP6 or T3) within either one or both of the primers used in the amplification procedure. The resulting DNA fragment can then be used as a template for *in vitro* transcription for further downstream applications such as probes (2), *in vitro* translation (3) or mutagenesis (4).

[(b)]PCR is a patented process. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

A second useful primer modification is incorporation of a biotin molecule at the 5´-end of one of the primers. The interaction between biotin and streptavidin has an extremely high binding coefficient ($K_d = 10^{-15}$M) (5). Thus, the resulting biotinylated PCR fragment can be easily purified from the amplification reaction by use of streptavidin-coated magnetic particles such as Promega's Streptavidin MagneSphere® Paramagnetic Particles (SA-PMPs) in a magnetic separation procedure.

Combining these two primer modifications allows for rapid isolation of the PCR product and confers the ability to transcribe RNA from PCR products using the incorporated phage promoter and the appropriate RNA polymerase. Unfortunately, once the PCR product is immobilized on the SA-PMPs, it is very difficult to elute it for use in subsequent *in vitro* transcription reactions. In this update, we describe a convenient method for performing *in vitro* transcription of a captured DNA template still bound to the SA-PMPs (Figure 1).

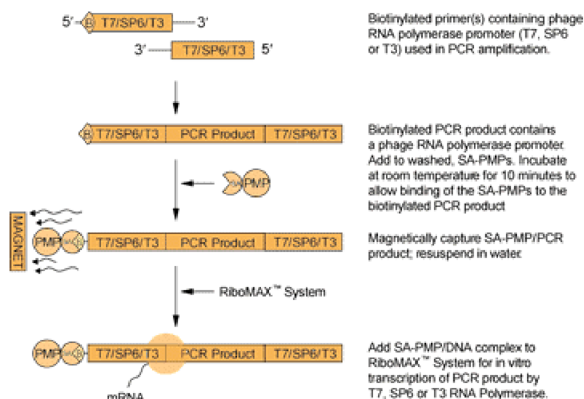

Figure 1. Outline of the procedure for direct *in vitro* transcription from SA-PMP-immobilized PCR products.

Experimental procedure

The above-mentioned modifications were performed on primers and these primers were used to amplify DNA by PCR. Illustration of this application is shown in Figure 1; highlights of a typical experiment are as follows. PCR amplification was performed using a

biotinylated T7 primer, a conventional SP6 primer and Promega's PinPoint™ Control Vector[c] (Cat.# V2041) to generate a 1,600bp PCR product as the template. The PinPoint™ Control Vector contains the CAT gene flanked by T7 and SP6 RNA polymerase promoters.

[c]For research purposes only. Not for diagnostic or therapeutic use. For nonresearch uses of the portion of the vector encoding the biotinylation sequence, please contact Promega Corporation for licensing information.

After amplification, the mineral oil was removed and the product was added directly to 0.6ml of SA-PMPs, prepared as recommended (6). The mixture was allowed to incubate at room temperature for 10 minutes. The SA-PMP/PCR product complex was magnetically captured, using the MagneSphere® Technology Magnetic Separation Stand (2-hole stand; Cat.# Z5332), and then resuspended in 50µl of water.

For transcription, a 5µl aliquot of the SA-PMP/PCR product complex was used directly in a RiboMAX™ SP6 (Cat.# P1280) or RiboMAX™ T7 (Cat.# P1300) RNA Production System. After transcription, the reactions were centrifuged to remove the SA-PMP/PCR product complex and the RNA was purified following the procedure recommended in the RiboMAX™ Systems Technical Bulletin (7). However, we omitted the RQ1 RNase-Free DNase treatment to remove the template after transcription. The DNase treatment step is not necessary in this procedure, as the DNA remains attached to the SA-PMPs via the biotinylated primer, and therefore does not pose a problem for subsequent uses of the transcribed RNA.

Yields of RNA corresponded well to those obtained by standard transcription methods. In addition, the correct size RNA product was observed (Figure 2) using either SP6 or T7 RNA Polymerase, indicating no steric hinderance by the particles themselves.

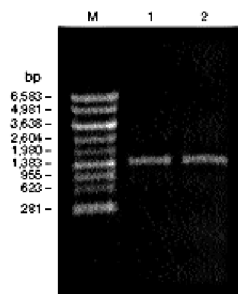

Figure 2. Gel analysis of RNA transcripts. The SA-PMP-immobilized PCR product (DNA) was transcribed to RNA using SP6 or T7 RNA Polymerase, and purified as noted in the text. The RNA was analyzed on a 1% agarose/formaldehyde gel. Lane M, Promega's RNA Markers (Cat.# G3191); Lane 1, RNA from SP6 reaction; Lane 2, RNA from T7 reaction.

Summary

We describe a convenient method by which one can capture a biotinylated PCR amplification product that contains the sequence for SP6, T7 or T3 phage RNA polymerase promoters, by use of Promega's MagneSphere® Paramagnetic Particle technology. *In vitro* transcription can be performed on the PCR products, still attached to the SA-PMPs, without further manipulations.

References

1. Saiki, R.K. *et al.* (1985) *Science* **230**, 1350.
2. Griffin, H.C. (1994) *PCR Technology, Current Innovations,* CRC Press.
3. Titlow, C. (1992) *PCR Meth. Appl.* **2,** 172.
4. Goldrick, M.M. *et al.* (1996) *BioTechniques* **21**, 106.
5. Green, N.M. (1975) *Adv. Protein Chem.* **29**, 85.
6. *PolyATtract® mRNA Isolation Systems Technical Manual* #TM021, Promega Corporation.
7. *RiboMAX™ Large Scale RNA Production Systems, SP6, T3, T7 Technical Bulletin* #TB166, Promega Corporation.

Ordering Information

Product	Size	Cat.#
Streptavidin MagneSphere® Paramagnetic Particles	9ml 25ml	Z5481 Z5482

RiboMAX^{TM} Large Scale RNA Production System-SP6	P1280
RiboMAX^{TM} Large Scale RNA Production System-T7	P1300
RiboMAX^{TM} Large Scale RNA Production System-T3	P1290
MagneSphere® Technology Magnetic Separation Stand (two-hole; 1.5ml)	Z5332

© 1996 Promega Corporation. All Rights Reserved.

MagneSphere and PolyATtract are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office. PinPoint and RiboMAX are trademarks of Promega Corporation.