

Technically Speaking

# Riboprobe® and RiboMAX(TM) *In Vitro* Transcription Systems

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Promega offers several systems for *in vitro* transcription reactions, which enable the user to synthesize RNA from a segment of DNA that has been cloned into a vector containing the appropriate RNA polymerase promoter. The synthesized RNA can be used for a variety of applications. This article is designed to help you choose a suitable system for your application, and addresses recent changes that were made to some of the systems.

## Q: What is *in vitro* transcription?

Transcription is the process by which RNA polymerase directs the synthesis of RNA from a DNA template under the control of a suitable promoter. *In vitro* transcription reactions use purified components to essentially mimic processes that occur *in vivo*. The principal components of a typical *in vitro* transcription reaction are the DNA template, RNA polymerase and ribonucleotide triphosphates (rNTPs).

## Q: Is this process the same in prokaryotic and eukaryotic systems?

Although some features of transcription are common to both prokaryotic and eukaryotic systems, the individual components of the reactions are substantially different. The Riboprobe® and RiboMAX(TM) Systems use specific phage RNA polymerases, (SP6, T7 or T3), which will not function in eukaryotic transcription reactions.

## Q: What is the difference between the Riboprobe® and RiboMAX(TM) Systems?

The Riboprobe® Systems are designed for the production of high specific activity, radiolabeled RNA probes. The RiboMAX (TM) Systems are large-scale RNA production systems and are not suitable for producing high specific activity radiolabeled transcripts.

## Q: Are the components in these systems interchangeable?

No, the reaction conditions used in the Riboprobe® Systems are substantially different from those in the RiboMAX(TM) Systems. Promega does not recommend using these systems interchangeably. The components in the Riboprobe® Systems are available separately. The components in the RiboMAX(TM) Systems cannot be purchased separately. Promega's High Concentration RNA Polymerases are not recommended for use in the RiboMAX(TM) Systems.

## Q: What are some features of the new Riboprobe® Systems?

The Riboprobe® Systems were recently reconfigured to provide added functionality and to facilitate the choice of a suitable system. These systems are available in two formats: single polymerase systems, which contain either SP6, T3 or T7 RNA polymerase, and combination systems, which include SP6 and T7 polymerases, or T3 and T7 polymerases. All of these systems now include Promega's RQ1 RNase-Free DNase, which may be used to remove the DNA template after performing the transcription reaction. These systems do not include vectors or bacterial strains, which may be purchased separately. For more details on the new systems, please see the New Products Section of this issue.

## Q: What do I need to use these systems?

In order to use these systems, the DNA sequence of interest must first be cloned into a vector that possesses a suitable promoter, such as the SP6, T3 or T7 promoters. Promega's pGEM®\* Vectors may be used for this purpose. Other vectors may also be used, provided they have an SP6, T3 or T7 promoter. These constructs can then be propagated and purified using standard plasmid preparation techniques. The DNA template is then added to a standard transcription mixture which contains the appropriate RNA polymerase, buffers and rNTPs.

\*U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

**Q: Does the DNA template need to be linearized for transcription to proceed?**

No, the plasmid can be transcribed even if it is supercoiled. However, this will often result in transcription through the insert and around the plasmid backbone if there is no transcription termination signal present in the plasmid. In order to produce transcripts of a defined size, and minimize background in hybridization applications, the plasmid should be linearized before it is added to the transcription reaction. In general, restriction enzymes that produce a 5' overhang will produce the best results. The use of enzymes that produce a 3' overhang may produce undesired transcripts (1).

**Q: How do I generate radiolabeled probes?**

To produce high specific activity RNA probes, the standard transcription reaction in the Riboprobe® Systems should include a radiolabeled rNTP, which is not provided with the system. Suitable isotopes are either 35S-labeled or 32P-labeled UTP, GTP or CTP. ATP is not recommended due to low incorporation kinetics.

**Q: What is the expected specific activity of the RNA transcripts?**

Typical reactions with the Riboprobe® Systems will yield probes with a specific activity of  $6-9 \times 10^8$  cpm/ $\mu$ g. If higher specific activity is desired, the amount of radiolabeled rNTP in the reaction mixture may be increased. Alternatively, more than one radiolabeled rNTP may be used. However, probes with very high specific activity may break down faster and thus affect further applications in which the probe is used.

**Q: How can I ensure that full-length transcripts are being produced?**

If the Riboprobe® System standard transcription protocol does not yield full-length transcripts, the reaction may be done at room temperature, which helps to increase the proportion of full-length transcripts in some cases. Some DNA sequences may contain termination signals for a particular RNA polymerase. Subcloning the DNA sequence into a vector that uses a different RNA polymerase promoter may alleviate this problem. The concentration of the limiting rNTP may also be increased to produce full-length transcripts, but this is at the expense of reducing the specific activity of the probe.

**Q: For what types of applications can the RNA probes be used?**

RNA probes generated with the Riboprobe® Systems can be used for Southern and Northern blot hybridizations (2,3). The greater thermal stability of RNA:DNA hybrids over DNA:DNA hybrids is advantageous for Southern blots because it allows conditions of higher stringency to be used, yielding increased signal to noise ratios. The same advantage applies when detecting RNA sequences in Northern blots, since RNA:RNA hybrids are more stable than RNA:DNA hybrids. RNA probes may also be used in plaque and colony lifts (4), and *in situ* hybridizations to tissue sections (5) and chromosome spreads (6). In addition, RNA probes may be used for a variety of RNA mapping applications which require solution hybridization techniques, such as Promega's RNase Protection Assay System.

**Q: Can the Riboprobe® Systems be used to generate unlabeled RNA transcripts?**

Yes, the Riboprobe® Systems can be used for this purpose. However, if large scale RNA production is desired, the RiboMAX (TM) Systems are recommended. Transcripts generated using the Riboprobe® Systems are suitable for *in vitro* translation reactions, using Promega's Rabbit Reticulocyte Lysate Systems or Wheat Germ Extracts. RNA produced using the RiboMAX (TM) Systems is recommended for *in vitro* translation as it exhibits enhanced "translatability" when compared to RNA from standard transcription reactions (7). In addition, this RNA may be used for studies of RNA structure or RNA-protein interactions.

**Q: Can I generate capped transcripts with these systems?**

Yes, however these systems do not include a cap analog. Suitable cap analogs may be purchased from other vendors. A protocol for the generation of capped transcripts is provided with both the Riboprobe® and RiboMAX(TM) Systems. The use of a cap analog may provide more efficient *in vitro* translation of some transcripts, and is also recommended for RNA that is used in microinjection experiments (8).

**References**

1. Schenborn, E.T. and Mierendorf, R.C. (1985) *Nucl. Acids Res.* **13**, 6223.
2. Melton, D. *et al.* (1984) *Nucl. Acids Res.* **12**, 7035.
3. Srivastava, R.A.K. and Schonfeld, G. (1991) *BioTechniques* **11**, 584.
4. Hanahan, D. (1983) *J. Mol. Biol.* **166**, 577.
5. Jorgensen, E.M. and Garber, R.L. (1987) *Promega Notes* **10**.
6. Matthaei, K.I. and Reed, K.C. (1986) *Promega Notes* **5**.
7. Beckler, G.S. (1992) *Promega Notes* **39**, 12.
8. Contreras, R. *et al.* (1982) *Nucl. Acids Res.* **10**, 6353.

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