Improved synthesis of full-length RNA probe at reduced incubation temperatures

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In vitro transcription systems utilizing bacteriophage RNA polymerases are routinely used to copy selected DNA sequences into single-stranded RNA probe. However, the synthesis of full-length RNA probe becomes more difficult as the length of the template sequence increases and as the concentration of the limiting nucleotide is reduced (1). Unfortunately, synthesis of high specific activity radioactive probes requires that the labeled nucleotide is present at a low final concentration and this may lead to problems in synthesizing full-length probe transcripts. Data presented in this report demonstrate that synthesis of full-length RNA transcripts is facilitated by reducing the temperature of incubation of the transcription reaction.

To examine the influence of temperature on transcription products, a 50 μ l reaction mix was assembled and 5 μ l aliquots were removed and incubated at temperatures ranging from 43°C down to 0°C. The template is a 1 kilobase fragment of *Xenopus laevis* genomic DNA and the 32 P-labeled nucleotide, (UTP), is present at a concentration of 5 μ M. The remainder of the reaction mix contains 40 mM Tris-HCl pH 7.5, 6 mM MgCl₂, 2 mM spermidine-HCl, 10 mM DTT, 100 μ g/ml BSA, ATP, CTP and GTP at 500 μ M, approximately 20 μ g/ml of template DNA and T3 RNA polymerase at 500 units/ml (2).

The results of the experiment are presented in Figure 1. Examination of the transcription products synthesized at 37°C shows the presence of two major incomplete transcripts with lengths of approximately 800 and 550 bases, (labeled A and B on the figure). As the temperature of incubation is lowered the amount of the incomplete transcripts decreases steadily until at about 5°C they are no longer detectable. The amount of full-length transcript, however, is approximately constant at all incubation temperatures and corresponds to about one RNA transcript per molecule of template DNA. Although it is known that the rate of RNA synthesis is much lower at 5°C than at 40°C (1), the amount of limiting nucleotide in a labeling reaction is so small that it is easily consumed during the 60 minute incubation, even at the lowest temperatures. While the experiment in Figure 1 was performed using T3 RNA polymerase, identical results have been obtained using SP6 and T7 RNA polymerases. The optimum transcription buffer contains spermidine (1), and this is also required for efficient transcription at low incubation

temperatures, (data not shown). To avoid possible precipitation of the template DNA by spermidine, the reaction mix should be assembled at room temperature and then adjusted to the desired reaction temperature before adding enzyme. Since the precise temperature of incubation is not critical it is often convenient to carry out the probe synthesis reaction in a cold room or in a laboratory refrigerator. In general, incubation of the reaction at 0°C, (e.g. on ice), is not recommended because very low incorporation of label is observed in some cases.

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Figure 1. Nine identical transcription mixes containing T3 RNA polymerase and UTP at a final concentration of 5 μ M, were incubated for 60 minutes at the temperatures shown at the top of the figure. The total products of each reaction were fractionated on a 5% denaturing acrylamide gel. Incomplete transcripts, present at high incubation temperatures, are marked A and B.