Paraffin Wax as a Vapor Barrier for the PCR

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The PCR is a highly efficient method for the amplification of specific DNA sequences.⁽¹⁾ The use of thermostable DNA polymerases allows the reaction to proceed after repeated exposures to the elevated temperatures that are required for strand separation.⁽²⁾ To prevent the evaporation of the reaction at these elevated temperatures, the reaction is overlaid with mineral oil to form a vapor barrier. Mineral oil, also known as paraffin oil, is a liquid at room temperature and requires post-PCR chloroform extractions for its removal. We describe the use of reagent-grade paraffin wax as a vapor barrier for PCR reactions in place of the more costly commercial paraffin wax preparations that are marketed specifically as vapor barriers for PCR. Paraffin wax allows the separation of the reaction components prior to heating and upon cooling solidifies to allow easy removal of the reaction mixture from the tube without the need for chloroform extractions. The paraffin can also be melted again to form a barrier for prolonged storage at 4°C.

PCR reactions were carried out in a total volume of 100 µl and consisted of 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl₂, 0.1% Triton X-100, 0.2 mM dNTPs, 1.3 µM of each primer, and 50 µl of mineral oil (Cat. No. M5904, Sigma) or 50 µl of melted paraffin wax. The template DNA, a pGEM3 plasmid containing a 1-kb insert, was isolated from *Escherichia coli* HB101 by centrifuging 0.1 ml of an overnight culture, resuspending the pellet in the original volume of 0.1% Tween 20, and then boiling for 10 min. Cellular debris was pelleted by centrifugation for 5 min in a microfuge, and 3 µl of the supernatant was added to each reaction. The primers used were complementary to the T7 (5'-AATACGACTCAC-TATAG-3') and the SP6 (5'-ATTTAGGT-GACACTATAG-3') promoters. The reactions were thermally denatured at 85°C for 5 min and cooled to 25°C to allow the wax to solidify; 1.5 units of *Taq* DNA polymerase was added directly to the reaction that used mineral oil or was added directly onto the solidified wax. The reactions were conducted according to the "touchdown" PCR procedure,⁽³⁾ where the primers are annealed for 1 min at 52°C for the first cycle, then decreased 1°C each cycle until reaching 42°C. The PCR was allowed to proceed for 40 cycles, then held at 4°C. The wax solidified allowing the reaction to be re-

moved by punching through the wax layer with the pipette tip and removing the reaction mixture. The reaction with mineral oil was extracted twice with chloroform. The products were analyzed by agarose gel electrophoresis.⁽⁴⁾

We tested several types of paraffin wax: Aldrich (Cat. No. 32,721-2, m.p. 59–63°C), Surgipath (Cat. No. EM400, m.p. 55–57°C), and Baxter (Cat. No. M7346-1A, m.p. 55–57°C). All paraffin waxes tested worked as a vapor barrier, but the Aldrich paraffin proved superior as it provided the most product with the least background (Fig. 1). The Aldrich paraffin was a reagent-grade paraffin that contained no additives. The other two paraffins were for tissue embedding and contained additives. Also, the Aldrich paraffin had a slightly higher melting point, which allowed the reaction to reach a higher temperature before melting and allowing the *Taq* DNA polymerase to enter the reaction. The use of wax as a vapor barrier provides two significant advantages over mineral oil: (1) no extractions with organic solvents are necessary, and (2) reaction components

FIGURE 1 Analysis of PCR products from recombinant plasmid using several vapor barriers. (Lane 1) λ DNA restricted with *Hind*III; (lane 2) ϕ X 174 DNA digested with *Hae*III; (lane 3) mineral oil overlay; (lane 4) Aldrich paraffin; (lane 5) Surgipath paraffin; (lane 6) Baxter paraffin.

can be separated prior to initiation of the reaction, thereby decreasing many false priming events that occur at lower temperatures. The latter advantage is especially significant because events occurring early in the cycling reaction are critical to specificity and yield. The use of reagent-grade paraffin wax as a vapor barrier is both an efficient and economical alternative.

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