

Minimizing the Time Required for DNA Amplification by Efficient Heat Transfer to Small Samples

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Hot-air temperature cycling of 1- to 10- μ l samples in glass capillary tubes can amplify DNA by the polymerase chain reaction in 15 min or less. A rapid temperature cycler of low thermal mass was constructed to change sample temperatures among denaturation, annealing, and elongation segments in a few seconds. After 30 cycles of 30 s each, a 536-bp β -globin fragment of human genomic DNA was easily visualized with ethidium bromide on agarose gels. With rapid cycling, amplification yield depended on polymerase concentration. The time required for DNA amplification can be markedly reduced from prevailing protocols if appropriate equipment and sample containers are used for rapid heat transfer to the sample. © 1990 Academic Press, Inc.

The minimum time required for DNA amplification by the polymerase chain reaction (1,2) has not been rigorously investigated. No systematic study of optimal times for annealing, elongation, and denaturation is available because no device has been able to change the sample temperature quickly enough to make such study meaningful. Commercial instruments spend a significant amount of time changing the sample temperature (3).

A number of commercial cyclers use aluminum blocks and microfuge tubes to cycle temperature for the polymerase chain reaction. Standard protocols for a 30-cycle amplification are usually 2–6 h in length, and a large fraction of this time is spent heating and cooling the sample. Time is required both to bring the sample block to temperature and to transfer heat to the sample through a microfuge tube (3). These systems have limited response times because of high heat capacity of the metal blocks and low heat transfer through thick plastic microfuge tubes.

An alternative approach for thermal cycling uses air for heat transfer (4–6) and contains samples in thin glass

capillary tubes (5,6). With forced-air heating and 100- μ l samples, temperature profiles similar to those obtained by transferring microfuge tubes between water baths can be obtained (6). None of the air-cycling systems have capitalized on the potential for even faster response times. Our objective was to see if amplification times could be significantly reduced by decreasing both the heat capacity of the air-cycling system and the sample volume.

MATERIALS AND METHODS

The rapid air cycler is based on a previously described design (6). Its thermal mass was reduced by using thin aluminum sheeting for the housing and placing the fan motor (3000 rpm, 1/40 HP, ball bearing C-frame motor No. 4M080, Grainger, Salt Lake City, UT) outside of the airstream (Fig. 1). The fan blades (3.5-in aluminum No. 2C951, Grainger) were placed downstream from the heating coil to mix the heated air before reaching the samples. Up to 30 capillary tubes could easily be placed in the sample compartment. The sensing thermocouple, proportional temperature controller, and solenoid-activated door (for cooling with ambient air) have been previously described (6).

The proportional controller was programmed to obtain desired cycle times of 20, 30, 60, 120, and 180 s. The temperature response of the sample was recorded from the analog output of a BAT-12 temperature monitor (Sensortek, Clifton, NJ) connected to a miniature thermocouple (IT-23, 0.005-s time constant, Sensortek) placed within a 10- μ l sample in a microcapillary tube (KIMAX 46485-1, Kimble, Vineland, NJ).

DNA amplification was performed with 50 mM Tris, pH 8.5 (at 25°C), 3 mM MgCl₂, 20 mM KCl, 500 μ g/ml bovine serum albumin, 5% DMSO,¹ 0.5 μ M each of the

¹ Abbreviations used: DMSO, dimethyl sulfoxide; dNTP, deoxyribonucleoside triphosphate.

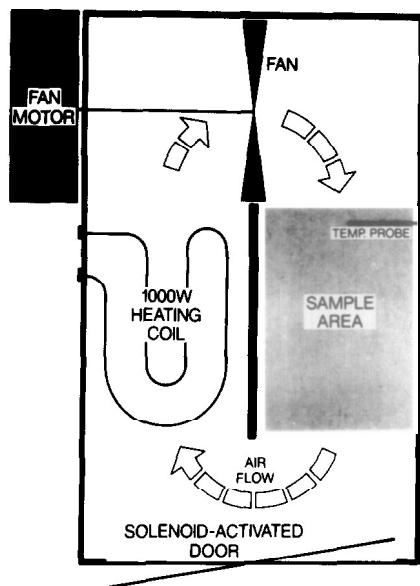


FIG. 1. Diagram of the rapid DNA amplifier. A horizontal section through the air cycler is shown. Recirculating air is heated by a 1000-W coil and mixed by fan blades while a thermocouple monitors the airstream temperature in the sample area and provides input to the proportional controller. The fan motor is mounted outside of the air-stream to decrease the thermal mass of the system. A solenoid-activated door opens for rapid cooling between denaturation and annealing stages. The air chamber is 10 cm in height, 10 cm in width, and 20 cm in depth. Samples are contained in glass capillary tubes that are placed vertically in the sample area of the cycler.

human β -globin genomic primers KM29 and RS42 (7), 0.5 mM of each dNTP, 50 ng of placental human genomic DNA, and 0.1–0.8 U of *Taq* polymerase/10 μ l. One unit (U) of polymerase activity was the amount of enzyme required to incorporate 10 nmol of [3 H]dTTP in 30 min at 80°C as defined by the manufacturer (Stratagene, La Jolla, CA). All other reagents were from Sigma (St. Louis, MO). The DMSO, KCl, albumin, and MgCl₂ concentrations were optimized by individual titrations for amplifying the KM29/RS42 primer pair region of genomic DNA. Samples (10 μ l) were placed in 8-cm capillary tubes (KIMAX 46485-1) and the ends fused with an oxygen–propane torch. Samples of 100 μ l were placed in larger diameter 10-cm tubes (KIMAX 34500). The capillary tubes were placed vertically in the sample area of the rapid air cycler. The temperature of the samples was cycled 30 or 40 times through denaturation, annealing, and elongation steps of 90–92°C, 50–55°C, and 71–73°C, respectively, for the times indicated in individual experiments. Amplification products (9 μ l unless indicated otherwise) were fractionated by electrophoresis on a 1.5% agarose gel and visualized with ethidium bromide and uv transillumination.

RESULTS

The temperature response of 10- μ l samples during 30- and 60-s cycles of the rapid air cycler is shown in Fig. 2

and Fig. 3, respectively. The annealing segment of each temperature profile is a spike corresponding to cooling of the sample with ambient air. The denaturation segment of the 30-s cycle is also a spike with very little time spent at the high temperature. The major difference between the 30- and the 60-s cycles is the length of the elongation segment. Some oscillation around the elongation temperature is evident in the 60-s cycle from the proportional controller. Temperature profiles for the 20-s cycle showed only a slight inflection at a sample elongation temperature of 72°C (not shown). The 120-s and 180-s cycles had elongation times twice their denaturation times (not shown).

Samples of 10 μ l can be amplified with a yield equivalent to 100- μ l samples in the air cycler (Fig. 4). In capillary tubes, the amplification volume can be reduced to 1 μ l with the product still detected by ethidium bromide staining in agarose gels.

Gels from rapid amplifications are shown in Figs. 5 and 6. In Fig. 5, the dependence of amplification on polymerase concentration is shown for 30-s cycles. Band intensity is strongly dependent on the amount of polymerase added. Figure 6 shows that although amplification efficiency is reduced with extremely rapid cycling, significant amplification still occurs after a total amplifi-

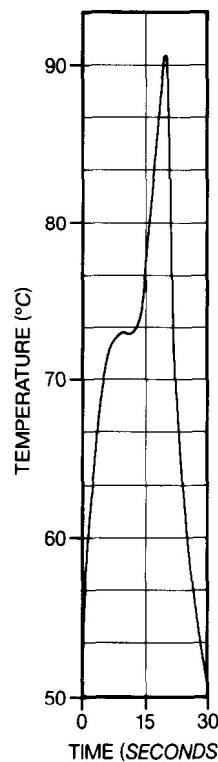


FIG. 2. Sample temperature during a 30-s cycle. Chart recording of the analog output of a BAT-12 temperature monitor with an IT-23 thermocouple probe (time constant 0.005 s, SensorTek, Clifton NJ). The thermocouple was placed in 10 μ l of water within a microcapillary tube.

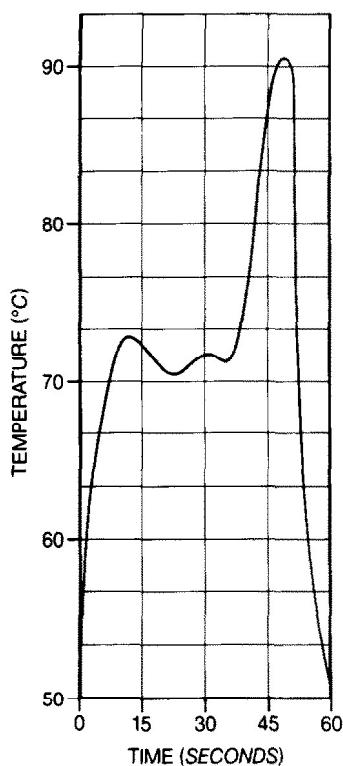


FIG. 3. Sample temperature during a 60-s cycle. Conditions of measurement were as described in Fig. 2.

cation time of only 10 min. Control samples without template DNA or polymerase did not show visible bands (not shown).

DISCUSSION

The advantages of an air-cycling system for DNA amplification include simplicity, low cost, and rapid temperature cycling. Air is an ideal heat transfer medium which can change temperature quickly because of its low density. Air can be rapidly mixed with baffles (6) or by a

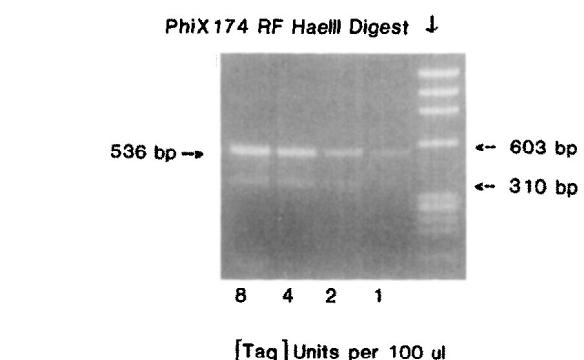


FIG. 5. Rapid DNA amplification is dependent on polymerase concentration. The total amplification time was 20 min and consisted of 40 30-s cycles as shown in Fig. 2. The amount of *Taq* polymerase varied from 0.1 to 0.8 U in each 10- μ l sample.

fan (Fig. 1) to provide homogeneous temperature exposure over the sample containers. The low thermal conductivity of air requires that air be rapidly blown past the heating coils and sample containers for efficient heat transfer.

Any temperature cycling protocol for DNA amplification can be divided into six segments: three endpoint temperatures and three temperature transitions. Time spent in transition is usually wasted, although theoretically a slow transition between annealing and elongation may be useful for a poorly annealing primer. Transition times after elongation and denaturation have no function; the faster the sample can be cooled after denaturation the better. Rapid cooling after denaturation favors the kinetic process (primer annealing to template/product) over the equilibrium process (product dimerization).

Annealing and denaturation are claimed to occur almost instantaneously once the sample has reached the

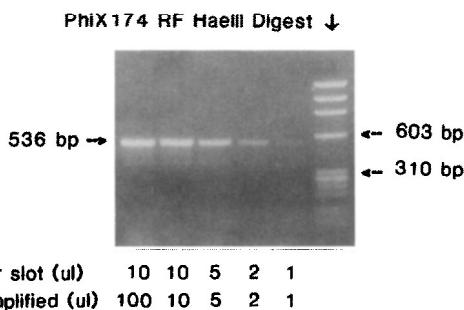


FIG. 4. Micro-DNA amplification. Ethidium bromide-stained amplification products of human genomic DNA delimited by the β -globin primers KM29 and RS42 (7). From 1- to 100- μ l samples were amplified in capillary tubes using 40 cycles of amplification (1 min at 90°C, 1 min at 55°C, 1 min at 72°C) and the air cycler previously described (6). The resulting product, 1 to 10 μ l, was applied to a 1.5% agarose gel.

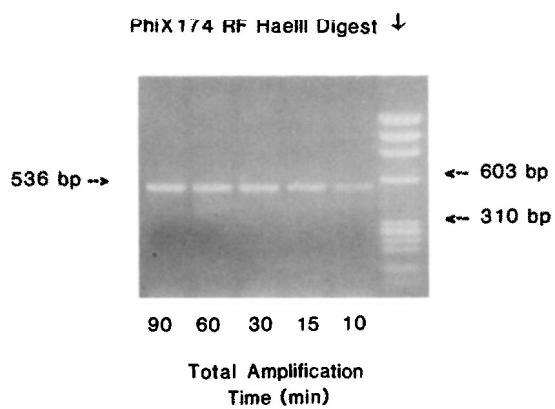


FIG. 6. Rapid DNA amplification. Each 10- μ l sample contained 0.8 U of *Taq* polymerase and 30 cycles of amplification were performed. Sample temperature profiles for the 15- and 30-min amplifications are given in Figs. 2 and 3, respectively. Other temperature profiles are described in the text.

appropriate temperature (3). Classical kinetic studies on DNA renaturation (8,9) also predict rapid annealing because of the high primer concentration used in DNA amplification. However, to our knowledge, this has not previously been tested. Our results suggest that denaturation and annealing do in fact occur very quickly in DNA amplification, with good amplification occurring even when the denaturation and annealing segments are reduced to spikes (Figs. 2, 5, and 6). The polymerase chain reaction need not take hours to perform; with appropriate temperature cycling equipment, DNA amplification can occur in minutes.

The ultimate limit of how fast DNA amplification can occur is not answered by this study. The times required for denaturation and annealing are apparently minimal. Primer extension is not instantaneous and the elongation time required depends on the length of the amplified product. *Taq* polymerase is highly processive with an extension rate of >60 nucleotides/s at 70°C (10). The large effect of polymerase concentration on band intensity with rapid cycling (Fig. 5) suggests that polymerization time becomes the limiting factor at very short cycle times (Fig. 6).

For rapid temperature cycling, the sample container is just as important as the thermal cycler. An optimal sample container should be water-vapor tight and have (i) low thermal mass, (ii) good thermal conductivity, (iii) minimal internal condensation, (iv) easy sample recovery without cross contamination, and (v) no inhibition of DNA amplification. Whatever the container, temperature equilibration will always be achieved faster if the sample volume is small, if the container wall is thin, and if the surface-to-volume ratio of the sample exposed to the container wall is high. Problems with condensation can be reduced by minimizing the free air space surrounding the sample.

Microfuge tubes are kept water-vapor tight by mechanical closure and, if necessary, overlaid mineral oil. Thermal conductivity is poor because of the material and its thickness (ca. 1 mm). Internal condensation can occur if mineral oil is not used and particularly if different parts of the tube are at different temperatures (which depends on the temperature cycler configuration).

In contrast, glass capillary tubes are made vapor tight by flame closure of the ends. They conduct heat to the

sample better than microfuge tubes because of decreased wall thickness (ca. 0.2 mm) and a better surface-to-volume ratio. Dead air space can be minimized to prevent significant condensation. Different diameter capillary tubes can be chosen for the sample volume desired.

Decreasing the heat capacity of the cycling system can markedly decrease the total time required for the polymerase chain reaction. In addition, air cycling and miniaturization significantly decrease the cost of DNA amplification. There may be other advantages of rapid cycling; decreased annealing and denaturation times should theoretically reduce nonspecific amplification and polymerase inactivation, respectively.

When the temperature response of the cycler and thermal equilibration of the sample are not limiting, questions about optimal temperatures and times for DNA amplification can be answered to much greater accuracy than before. The physical processes of denaturation and annealing and the enzymatic process of elongation can be specifically studied, without the confounding effects of long transitions between temperatures. This should lead to a more detailed understanding of DNA amplification and improved reaction efficiency and specificity.

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