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Automated polymerase chain reaction in capillary tubes with hot air

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ABSTRACT

We describe a simple, compact, inexpensive thermal cycler that can be used for the polymerase chain reaction. Based on heat transfer with air to samples in sealed capillary tubes, the apparatus resembles a recirculating hair dryer. The temperature is regulated via thermocouple input to a programmable set-point process controller that provides proportional output to a solid state relay controlling a heating coil. For efficient cooling after the denaturation step, the controller activates a solenoid that opens a door to vent hot air and allows cool air to enter. Temperature-time profiles and amplification results approximate those obtained using water baths and microfuge tubes.

INTRODUCTION

Cyclic DNA amplification using a thermostable DNA polymerase allows automated amplification of primer specific DNA, widely known as the "polymerase chain reaction" (1,2). Automation requires repetitive temperature cycling. Commercial programmable heat blocks are available and low cost machines using water baths with fluidic switching (3) or mechanical transfer (4) have been described. Instead of heat transfer from metal blocks or water through high thermal resistance plastic microfuge tubes, we describe a device that uses hot air for temperature control of samples in thin glass capillary tubes.

MATERIALS AND METHODS

The device for temperature cycling is a closed-loop hot air chamber resembling a recirculating hair dryer (Fig. 1 and 2). The heating element is a 1000 W (125 VAC) nichrome wire coil (Johnstone Supply, Portland Oregon) wound around a mica support. The heating coil is activated via a 25 A, 125 VAC solid state relay (Crydom D1225, available as Omega SSR 240 D25 through Omega Engineering Inc, Stamford, CT), connected to a Partlow MIC-6000 proportional temperature controller (available through Omega as the CN8600 process controller) with thermocouple input and at least one SSR driver and one relay output. The relay output controls a solenoid (Dormeyer 2A173, Chicago, IL) mechanically coupled to open a door on activation

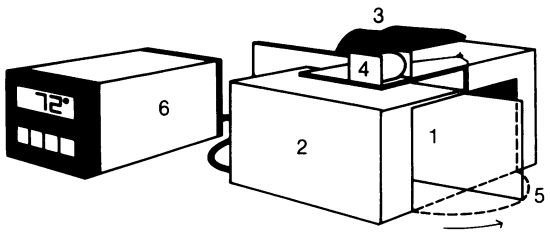

Fig. 1 Drawing of the capillary tube, hot air DNA amplifier. 1) reaction chamber where a removable stand for capillary tubes can be placed, 2) aluminum housing, 3) air blower, 4) solenoid mechanically coupled to open door on activation, 5) door, normally held closed with a spring, 6) temperature controller.

that interrupts the recirculating hot air and introduces ambient-temperature air during the cool-down portion of each cycle. The door pivots on a central axis and is normally held shut with a spring attached to a cam along the central axis. Baffles are placed downstream of the heating coil to mix the air efficiently before it reaches the sample compartment. Air is circulated through the system with an "in-line" 75 cubic feet per minute air blower (Fasco B75, Cassville, MO). Temperature monitoring during routine operation of the cycler is achieved by a 30-gauge iron-constantan "J-type" thermocouple placed just before the sample compartment in the air stream and connected to the temperature controller. The sample compartment is a 5 cm wide x 5 cm long x 10 cm high chamber accessible by manually opening the solenoid-controlled door. The housing of the apparatus is formed from aluminum sheeting.

The polymerase chain reaction was run in a 100 ul volume with 1 ug template DNA, 1.5 mM of each deoxynucleotide, 50 pmol of each oligonucleotide primer and 10% dimethyl sulfoxide in a reaction buffer consisting of 17 mM ammonium sulfate, 67 mM

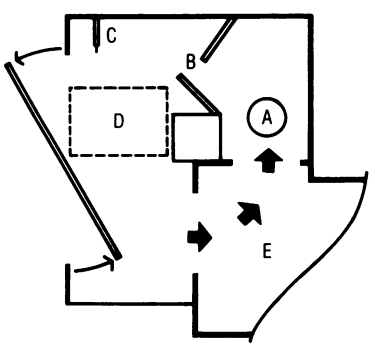

Fig. 2 Scale diagram of the amplifier. A) heating coil connected to the controller via a solid state relay, B) baffles to uniformly mix the hot air, C) thermocouple leads connected to controller, D) reaction chamber, E) air blower.

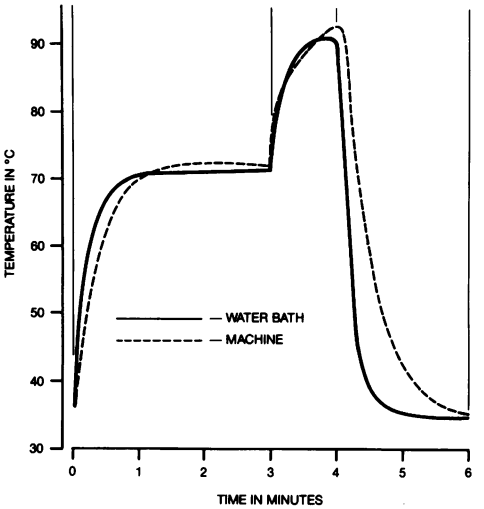

Fig. 3 Representative temperature profile of 100 ul deionized water, either in a 0.5 ml microfuge tube manually transferred between water baths, or in capillary tubes within the hot air amplifier.

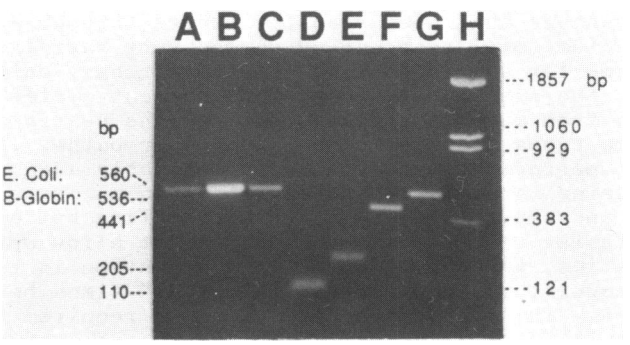

Fig. 4 Ethidium bromide stained amplification products. Lane A shows the product of amplification in microfuge tubes manually transferred between water baths for comparison to the hot air amplifier in lanes B-G. Lanes A-C) 560 bp fragment of E. coli DNA defined by primers TGAATCTGTACTCTGATGTAAC and CACTAATAGCAAGAGGGTACTCAG covering a portion of the regulatory region for pyelonephritis-associated pili (6). An asymmetric amplification (50 pmol of one primer and 0.5 pmol of the other) is shown in lane C. Lanes D-G) Amplification products of 4 different combinations of the human beta-globin gene primers PC03, PC04 (7), KM29, and RS42 (8). Lane H) BstN I digest of pBR322 DNA size markers (0.5 ug).

Tris-HCl (pH 8.8 at 25° C), 6.7 mM magnesium chloride, 10 mM beta-mercaptoethanol, 6.7 uM EDTA, and 170 ug/ml bovine serum albumin (5). After denaturing the reaction mixture at 94° C for 5 minutes, 1 unit of Thermus aquaticus polymerase (Taq polymerase - Stratagene, La Jolla, CA) was added, the samples placed in 10 cm long, thin-walled capillary tubes (Kimble, Kimax 34500), and the ends fused with an oxygen-propane torch so that an air bubble was present on both sides of the sample. The capillary tubes were placed vertically in a holder constructed of 1 mm thick "prepunched perfboard" (Radio Shack, Fort Worth TX). The mixture was cycled 30 times through denaturation (94° C - 1 min), annealing (37° C - 2 min) and elongation (70° C - 3 min) steps. Temperature monitoring within the capillary tubes was done with 30-gauge J-type thermocouple wire placed in 100 ul of deionized water and connected to a thermocouple meter (Precision Digital PD710, Watertown, MA). Amplification products (5/100 ul) were fractionated by electrophoresis on a 1.5% agarose gel.

RESULTS AND DISCUSSION

The temperature profile of a sample in the cycling apparatus was compared to that obtained by manually transferring microfuge tubes between water baths. Response times at each phase of the thermal cycle were roughly equivalent (Fig. 3). The temperature response of samples transferred between water baths is limited only by the heat conduction properties of the microfuge tube. The response times of commercial machines is also limited by the heat capacity of their metal heating/cooling blocks. The air cycler has the advantage that heat transfer occurs through a low heat capacity medium (air) that can be warmed very rapidly. The response time for sample cooling depends strongly on the heat capacity of the system materials. The current cycler was constructed from materials to approximate the heating response of microfuge tubes transferred between water baths. Although the current performance profile seems perfectly adequate, thinner housing material and an external fan motor (with only the blades and shaft exposed to the circulating hot air) could give even faster response times. This might allow optimization of denaturation, annealing, and elongation steps in terms of time and temperature, and shorten the "ramp" times between temperatures. This could decrease the time required for a complete amplification, as well as allow specific study of annealing, denaturation and enzyme kinetics within a polymerase chain reaction protocol.

Because of the low heat capacity of air, thin glass capillary tubes were used to contain the samples rather than plastic tubes. Attempts to amplify DNA in various plastic tubes with the air cycler were unsuccessful and temperature profiles were sluggish. Capillary tubes require a torch to seal the ends, but this can be readily achieved with only minimal practice. In order to obtain adequate temperature homogeneity within the sample compartment, baffles were installed between the heating coil and the samples. With the cycler set at a constant temperature (from 70 to 95° C), simple structural baffles decreased the temperature variation observed

throughout the sample compartment from about 10° C, to 2° C. This can be improved further by more complicated baffles if necessary.

Amplification products obtained with the device are qualitatively and quantitatively similar to those observed after manual water bath cycling (Fig. 4). We have used the apparatus to amplify both bacterial and human genomic DNA. Best results have been obtained with denaturation temperatures between 90 and 94° C. At temperatures above 94° C, amplifications are often not successful, apparently due to enzyme denaturation. This may result from faster equilibration of the sample at high temperature with the air cycler compared to other machines. This would effectively expose the polymerase to the high denaturation temperature for a longer period of time.

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