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Noncontact Infrared-Mediated Thermocycling for Effective Polymerase Chain Reaction Amplification of DNA in Nanoliter Volumes

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We demonstrate that accurate thermocycling of nanoliter volumes is possible using infrared-mediated temperature control. Thermocycling in the presence of Taq polymerase and the appropriate primers for amplification of λ-DNA in a total volume of 160 nL is shown to result in the successful amplification of a 500-base pair fragment of λ-DNA. The efficiency of the amplification is sufficiently high so that as few as 10 cycles were required to amplify an adequate mass of DNA for analysis by capillary electrophoresis. This indicates that, as expected, PCR amplification of DNA in nanoliter volumes should not only require less Taq polymerase but require less cycling time to produce a detectable amount of product. This sets the stage for microchip integration of the PCR process in the nanoliter volumes routinely manipulated in electrophoretic microchips.

As the technology for fabricating microchips capable of rapid electrophoretic analysis continues to advance,[1−3] novel approaches for microminiaturizing chemical and biochemical processes become increasingly important. The effective application of ultrafast electrophoretic microchip separations to molecular diagnostics will ultimately require the integration of the PCR process for rapid on-microchip amplification of DNA. The PCR reaction introduced in the mid-1880s by Mullis and co-workers[4] has revolutionized the field of molecular biology. The PCR reaction has become one of the main laboratory tools for scientists in the life sciences, and countless PCR applications have been developed to amplify DNA sequences of interest with high specificity. An effective PCR reaction is indispensable in DNA sequencing applications, such as cycle sequencing or Sanger sequencing, and more importantly for a large number of assays in molecular biology and clinical diagnostics. The conventional format for PCR involves the cycling of the PCR components in thin-walled, plastic tubes in a temperature-controlled metal block between the desired temperatures needed to effect the amplification of a specific DNA sequence by the polymerase enzyme. While most of the instrumental improvements were initially focused on the accurate control of temperature, the aspects of instrumental advancement have changed, and the focus is now on increasing cycling speed. Progress in this area has been made, largely due to the work done by Wittwer and others,[5−7] who have demonstrated that DNA amplification could be improved dramatically if the PCR is carried out in thermocyclers that generate shorter cycle times. Using an air thermocycler, Wittwer et al.[8] demonstrated that an efficient heat transfer between the heat source and the sample during thermal cycling produced shorter cycling times and resulted in an increased specificity for the PCR amplification reaction. The cycle speed in conventional thermocyclers is typically limited by the total thermal mass and the heat-transfer rate between the heating/cooling medium and the sample. Cycling times can vary from 1 min to several minutes per temperature cycle. Faster thermocycling in a conventional thermocycler is, in principle, possible by reducing the thermal mass of the sample.[9] However, typical metal block heaters/coolers and large sample volumes do not allow a rapid temperature transition due to their high heat capacity. An even faster thermocycling can be obtained if, in addition to the reduced thermal mass, an optimal surface-to-volume ratio and a suitable material for the PCR reaction vessel increases the heat transfer to the sample. A cylindrically shaped glass capillary has a large surface area-to-volume ratio and allows for a fast exchange of heat between the sample and the heating/cooling medium. It is for this very reason that microbore capillaries are effective for electrophoresis in high fields without adverse heating effects on the separation.[10] The combination of reduced thermal mass and an efficient heat transfer to the sample in the capillary make rapid thermocycling feasible. Temperature cycles taking less than 1 min are typical for such instruments.[8]

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We have recently introduced a novel method for thermocycling, which exploits an inexpensive infrared heat source for accurate control of temperature with ultrafast temperature cycling capability.[11] A tungsten lamp allows for rapid heating and maintenance of the PCR reaction mixture temperature without contact between the heat source and the reaction vessel. The red part of the spectrum from a powerful tungsten lamp is focused on the optically transparent PCR reaction chamber where the sample itself and, to a lesser extent, the sample vessel are heated due to the absorption of radiation. Heat-transfer problems frequently encountered with the conventional heating methods are minimized with the noncontact method and allow ultrafast thermocycling in the PCR typical temperature range. Cooling is very effectively achieved by accelerated heat dissipation through a sample vessel surface (large in comparison to the volume). This noncontact method, which combines rapid heating with effective cooling (by compressed air, if necessary), lends itself to miniaturization, since light can be easily manipulated to focus on structures of small shape and size. Of course, the small size of the sample and vessel makes temperature sensing extremely challenging and will require some innovative development. In addition, there are a number of other potential problems that must be addressed, including the rapid thermocycling-induced expansion and contraction of the fluid in the confined space, bubble formation, and problems arising from nonuniform heating of the sample. However, our results with IR-mediated thermocycling in microchambers indicate that PCR in submicroliter volumes is challenging but not impossible. The total thermal mass that requires heating and cooling is essentially the mass of the sample and the sample vessel (i.e., not the heater). Therefore, ultrafast cycle times in the order of seconds should be possible.

In this report, we describe the application of the IR-mediated thermocycling to a capillary format using volumes as small as 160 nL. This method combines the advantages of IR-mediated sample heating with the advantages of fast cooling in capillaries providing a high surface-to-volume ratio to achieve ultrafast thermocycling for PCR applications. Temperature sensing is accomplished using a thermocouple introduced into a reference capillary, which allows temperature measurement with an accuracy of 1 °C and variations of less than 2 °C. We demonstrate that this method is capable of efficient DNA amplification with typical cycle times on the order of only a few seconds.

MATERIAL AND METHODS

Hardware. The hardware for performing the thermocycling experiments was constructed in-house and is similar to the one described previously.[11] The apparatus included a Pentium PC outfitted with an analog-to-digital (A/D) converter board (ComputerBoards, Inc, Mansfield, MA) with a 12-bit resolution. The desired temperature profile was programmed into the computer as a Labview application, which communicated through the A/D computer board with the analog devices. The temperature in the sample was maintained through the exact control of the heating and cooling intervals based upon feedback from the thermocouple. The infrared light was produced by a tungsten lamp (CXR, 8 V, 50 W, General Electric, Cleveland, OH) powered by a 5-V ac/dc transformer with an approximate color temperature of 3200 K. The lamp intensity and the cooling intervals were modulated by a digital signal (TTL-output) from the A/D board via activation of a solid-state relay (OACM-5, Potter-Brumfield). A proportional integral derivative (PID) controller was programmed into Labview to modulate the lamp intensity with time periods between 2 and 900 ms. Air pressure was kept constant at 20 psi. To prevent photolysis of vital PCR components, an optical long-wave pass filter with a 680-nm transmission cutoff was installed between the IR source and the PCR capillary.

Thermocycling in a Capillary. Thermocycling in nanoliter volumes was done in a 8-cm-long, 150-μm-i.d., and 375-o.d. sample capillary which was surface inactivated with bTMSTFA (Sigma, St. Louis, MO) and in a DB-17 capillary (J&W Inc., Folsom, CA). A 2-cm-long section of the polyimide coating on the capillary was removed in the middle of the capillary piece with respect to its ends to expose the bare glass surface to the radiation and avoid absorption of the radiation by the coating. The temperature was sensed in a reference capillary with a copper/constant thermocouple (t/c) with an outer diameter of 0.001 in. The original t/c of 0.005-in. diameter purchased (Omega, Stamford, CT) was reduced with fine sandpaper to the desired width of <150-μm o.d. The copper and constantan wires were inserted into a 150 μm i.d. × 8 cm long capillary from both sides. The wires were positioned in the middle of that piece of capillary so that the wires formed a thermocouple junction through intimate contact. The reference capillary was filled with the PCR buffer and sealed on both ends with epoxy glue. The reference capillary also had a 2-cm-long window where the polyimide was burned off in the center of the capillary to mimic the window in the sample capillary. The sample capillary was filled with the PCR reaction mixture, and both capillaries were horizontally aligned at equal distances in front of the tungsten lamp with a *xyz* translation stage so that the optical windows of both capillaries were optimally irradiated (Figure 1). The alignment of the IR radiation source with respect to both capillaries was tested by temporarily replacing the sample capillary with a second "reference capillary" containing a thermocouple. Optimal irradiation of sample and reference capillary was ensured when both capillaries containing a thermocouple indicated the same temperature. One of the capillaries containing a thermocouple was then replaced with the actual sample capillary containing PCR reaction mixture. A mirror placed behind the capillaries enhanced the effect of heating. A stream of compressed air at 20 °C was pointed at the capillary along the length axis to effect the cooling of the sample in the capillary.

PCR Using a λ-DNA Template. The thermocycling was tested using a GeneAmp PCR reagent kit with native Taq DNA polymerase (N801-0043, Perkin-Elmer, Norwalk, CT) containing the appropriate λ control primers and λ-DNA to amplify a specific DNA sequence of 500-base pair (bp) length. The PCR reaction solution was made as a 50-μL stock solution according to the manufacturers specifications except that the 0.75 unit of Taq DNA polymerase was substituted with 1.25 units of the same enzyme bound to the TaqStart antibody (Clontech, Palo Alto, CA) for the hot start. The 50-μL PCR stock solution was divided into two parts. One part (~2 μL) of the solution was partially used to fill the sample capillary, and the rest was used to conduct a control experiment using a conventional PCR thermocycler (Progene,

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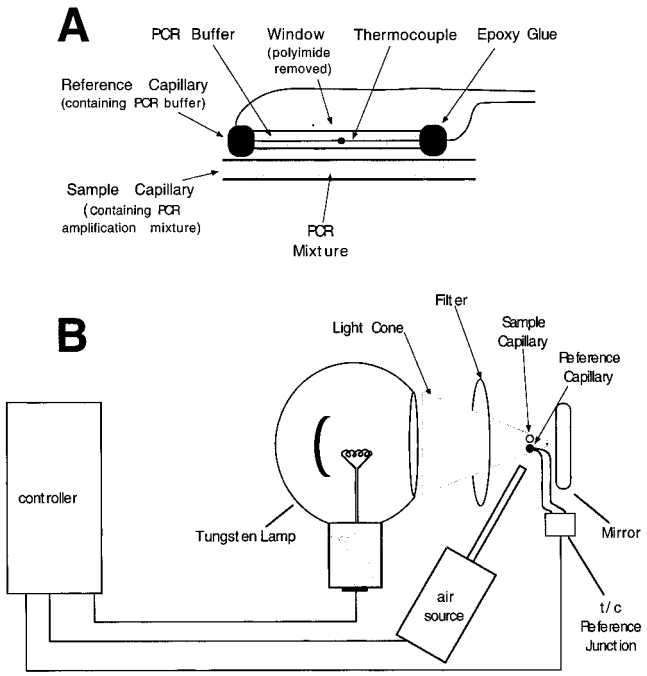

Figure 1. Instrumentation for IR-mediated thermocycling in capillaries: (A) creating a reference capillary via insertion of a "modified" thermocouple; (B) instrumental setup.

Techne Inc., Princeton, NJ). Thermocycling conditions for the PCR reagents in the conventional cycler were chosen as recommended by the kit manufacturer; 94 °C for 15 s and 68 °C for 60 s for 25 cycles with 5-min preincubation at 94 °C and a final extension of 10 min at 68 °C. Cycling conditions in the capillary varied and are specified below. The capillaries were prepared by flushing sequentially with MeOH, water, TE buffer, pH 8.5, and PCR reaction mixture using a 10-μL gastight syringe (Hamilton, Reno, NV) connected to the capillary with a 5-mm-long PE-10 tubing with 0.28-mm i.d. and 0.61-mm o.d. (Becton Dickinson Sparks, MD).

Analysis of the PCR Product by CE. The analysis of the PCR amplification product by capillary electrophoresis was conducted using a Beckman P/ACE model 5510 equipped with a laser-induced fluorescence detector with detection at 510 nm. Excitation of the fluorescent intercalator (YO-PRO-1, 1.5 μM final buffer concentration) was induced with a 488-nm argon laser (Beckman Instrument, Fullerton, CA). The separation was carried out at 7.5 kV from the inlet to the outlet of the capillary with an effective separation length of 20 cm. A 20-s electrokinetic injection of a 0.1 mg/mL pBR322 *Hae*III digest (Sigma) was used as a DNA size standard. The sample plug containing the PCR mixture, thermocycled in the capillary, was removed from the capillary using a syringe (see above), diluted with 9 μL of PCR reaction buffer (10 mM Tris, pH 8.6, 50 mM KCl, 2.5 mM $MgCl_2$, and 1 μM concentration of each dNTP), and injected electrokinetically at 6.1 kV for 20 s into 50 μm × 28 cm capillary for analysis. The sample injected was flanked with a 3-s, 3.5-kV electrokinetic injection of water. The control sample cycled in the conventional PCR thermocycler was diluted 50-fold with PCR reaction buffer and injected for analysis using the same injection conditions as the ones described above. The 500-bp amplification product was identified by comparing its migration with the migration time of a DNA size standard that was injected and separated subsequently. After each analysis, the capillary was flushed with CE separation buffer for 15 min to reduce the risk of carryover contamination.

RESULTS AND DISCUSSION

The advantage of IR-mediated heating using a tungsten lamp is the ability to carry out selective heating of aqueous solutions. The basis for this is an excellent overlap between the wavelength of light emitted from a tungsten filament lamp and the absorption properties of water. A standard tungsten lamp emits light in the visible and infrared part of the electromagnetic spectrum, in general covering the 350 nm−3 μm wavelength range. The specific IR-active absorption bands for water are at 2.66, 2.78, and 6.2−8.5 μm. Consequently, the use of a tungsten lamp as an IR source where the higher energy wavelengths of light (<600 nm) are filtered provides an effective energy source in the 1−4-μm range where water absorbs maximally and leads to a vibrational transition of the water molecules. In addition, if light in this region is absorbed less effectively by the vessel containing the solution, selective heating of the solution results. The 375-μm-o.d. vessel used in our experiments was a fused-silica capillary, which has major absorption bands at 3 and 4.5 μm. In this case, the vessel probably contributed partially to the heating of the PCR solution due to strong absorbance of silica in this IR region.

Rapid IR-Mediated Temperature Cycling with Nanoliter Volumes. Rapid temperature cycling of nanoliter volumes can be achieved in several ways. Most miniaturized and fast thermocycling methods described recently accomplish temperature cycling by attaching or incorporating heating elements to a small sample reactor.[12−14] These thermocycling systems invoke DNA amplification by carefully sensing and regulating the temperature of the heating/cooling element, e.g., indium−tin oxide, silicon. The concept of using an IR radiation source that specifically heats the sample without heating the medium surrounding the vessel distinguishes this rapid thermocycling approach from other methods.[12−14] Noncontact, IR-mediated thermocyling reduces the amount of thermal mass to that essentially of the sample and specifically heats that sample with the sample vessel heated secondarily. Therefore, direct control of the sample temperature, not the sample environment, during rapid thermocycling seems to be of advantage.

A variety of miniaturized temperature sensors are available for accurate temperature control of small sample volumes (e.g., resistors, thermocouples). However, the sensor must be in intimate contact with the sample solution to accurately measure the solution temperature. Even the use of the smallest possible temperature sensor to measure the sample temperature will add thermal mass to the nanoliter sample probe and modify the temperature behavior of the sample. Additionally, we have shown previously that the direct contact of a temperature-measuring device with the sample solution is problematic, leading to the inhibition of the PCR reaction.[11]

A workable solution is provided by the construction of a reference probe that exhibits the exact same thermal properties

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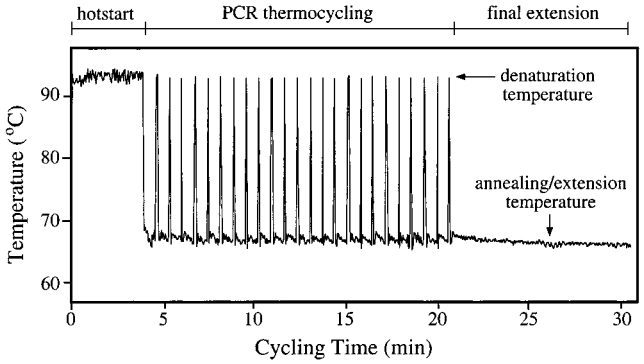

Figure 2. IR-mediated thermocycling. Sample temperature profile resulting from IR-mediated thermocycling in an 150 μm × 8 cm capillary. The controller was programmed for a two-temperature cycle procedure with dwell times of 94 °C for 2 s and 68 °C for 40 s for 25 cycles with a 240-s preincubation at 95 °C and a final extension time of 600 s at 68 °C. Cooling rates of 20 °C/s and heating rates of 65 °C/s were measured. Total cycling time was 30 min.

as the sample, i.e., a reference cell that contains the temperature-sensing element and a solution representative of the sample. Such a reference cell is constructed by bringing two thermocouple wires into intimate contact in a 150 μm × 8 cm capillary, filling the capillary with 1.4 μL of PCR buffer, and sealing the ends (Figure 1A). When placed adjacent to the sample capillary, the reference capillary experiences the same level of infrared radiation as the sample capillary, and therefore, the temperature sensed in the reference capillary should be equivalent to that of the sample (Figure 1B). Consequently, temperature maintenance is achieved by simply adjusting the lamp radiation output to balance the heat dissipation and the heat absorption. A more uniform heat exposure of the capillary is accomplished by reflecting the excess heat with a mirror onto the side of the capillary opposite to the radiation source. Cooling of the PCR sample through heat dissipation occurs as soon as the heat source (tungsten lamp) is turned off or the intensity of the heat source is reduced. However, more rapid cooling is achieved through the efficient removal of heat from the capillary by releasing air from a compressed air source and directing the stream along the length axis of both capillaries. Assuming that the sensor in the reference capillary represents the actual temperature of the PCR sample, the feedback from the thermocouple in the reference capillary can be utilized to control the temperature in the PCR sample. Due to the temperature-sensitive nature of the Taq enzyme used for PCR amplification, the ultimate proof that accurate temperature targeting is accomplished is provided by the successful amplification of a specific DNA fragment in the capillary.

Initial experiments sought to achieve rapid temperature transitioning between the desired target temperatures in a manner that reduced the overall cycling time. A two-temperature thermocycling protocol was initiated using a single 150 μm × 8 cm sealed reference capillary with dwell times at 94 °C for 2 s and 68 °C for 40 s for 25 cycles (240-s preincubation at 94 °C and a final extension of 600 s at 68 °C). Figure 2, shows a typical temperature profile as sensed by the thermocouple in the reference capillary. The effectiveness of IR for heating and rapid cooling through dissipation of the excess heat is evidenced by the fast transition from 68 to 94 °C at a rate of 65 °C/s (i.e., transitioned in less than 1 s), with cooling rates that were slightly slower but still

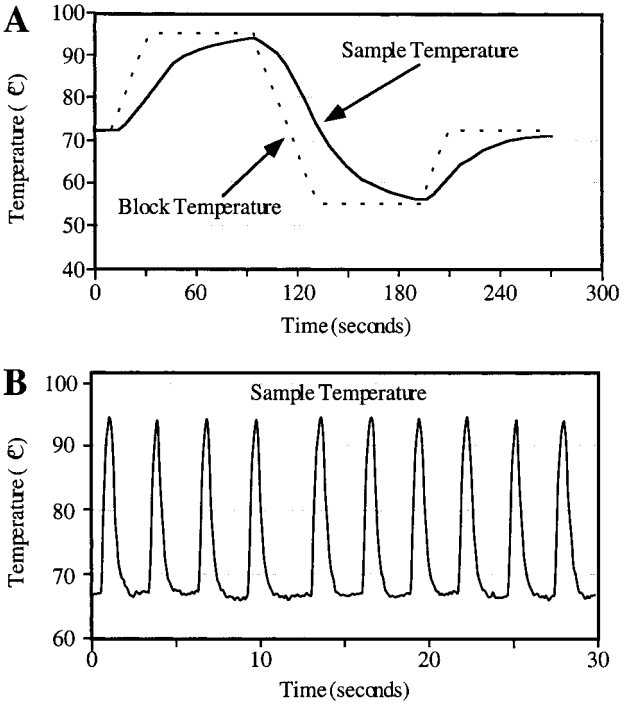

Figure 3. Ultrafast thermocycling in comparison with conventional heating block thermocycling. Ultrafast thermocycling improves the DNA amplification efficiency by minimizing temperature transitions times. (A) Conventional temperature cycling using a heating block and microliter volumes; (B) ultrafast temperature cycling using IR-mediated thermocycling in nanoliter volumes.

impressive at 20 °C/s. Consequently, ramping to the desired target temperature, e.g., 94 °C, is almost instantaneous (<1 s), with cooling to the target annealing temperature accurate and without substantial undershooting. From these results, it is clear that very sharp temperature transitions are attainable and that this method for nanoliter volume thermocycling should confer very high specificity to PCR-based DNA amplification.

Rapid Temperature Transitioning Key to Efficient Low-Volume PCR Amplification. A parameter that plays a key role in the efficiency of the PCR-based amplification of DNA is the transitioning to and from the annealing temperature. The slower the transition to the annealing temperature, the higher the chance for nonspecific annealing of the primer either with itself (primer dimer formation) or with sequences in the target DNA that are not complimentary. The opportunity for these interactions to occur with conventional thermocycling is exacerbated by a slow rate of transitioning inherent with having to heat and cool a relatively large thermal mass. Figure 3 A shows the temperature profiles for both the heating block and the PCR sample when a 50-μL volume is temperature cycled in a conventional thermocycler. Transitioning from 94 °C to an annealing temperature of 55 °C occurs at a maximum rate of 1 °C/s and contrasts the 20 °C/s transitioning of the IR-mediated temperature cycling in Figure 2.

In contrast, with heating and cooling rates of 65 and 20 °C/s, respectively, it is clear that the 42-s cycles shown in Figure 2 do not represent the limit of this technology. Figure 3B shows a temperature profile of IR-mediated thermocycling from 94 to 68 °C and back to 94 °C in less than 3 s—10 thermocycles are, therefore, completed in less than 30 s. It is noteworthy that, even with ultrafast cycling at this frequency, there is minimal over-

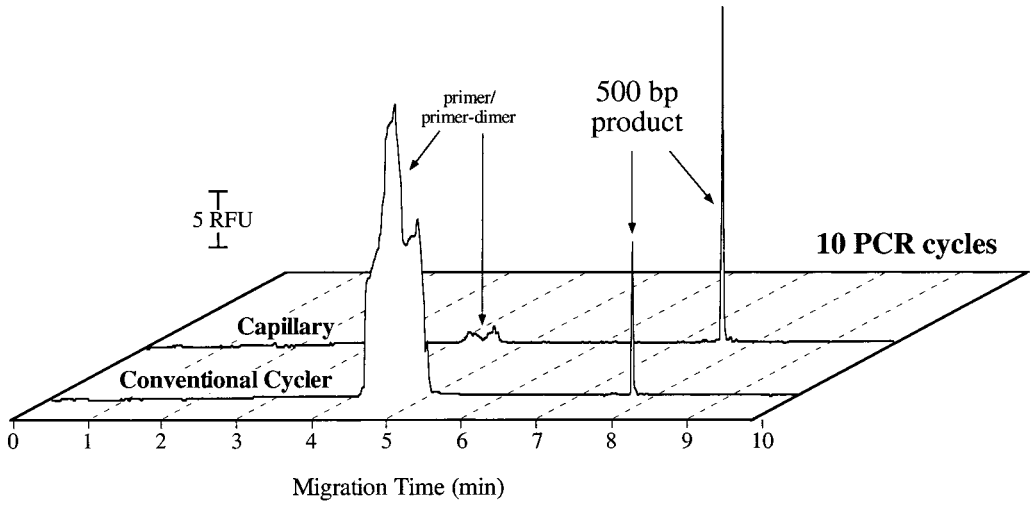

Figure 4. Analysis of PCR amplified λ-phage DNA. Capillary electrophoresis of a 500-base pair λ-phage DNA sequence PCR amplified in a conventional thermocycler starting with 0.1 ng (= 188 800 copies) of λ-phage DNA template in 48 μL of reaction mixture or in 150 μm $\times$ 8 cm capillary filled with 160 nL of PCR mixture and cycled for 10 cycles starting with 314 fg (= 593 copies) of λ-phage DNA template.

shooting of the 94 $\pm$ 0.3 °C denaturation temperature and reproducible realization of the 68 $\pm$ 0.45 °C annealing temperature. With the capability to accurately temperature cycle at this speed, it is probable that DNA amplification can be executed at the diffusion limit of its reaction partners.

Amplification of λ-Phage DNA in Nanoliter Volumes with IR-Mediated Thermocycling. The speed and accuracy with which the target temperature can be reached with IR-mediated heating is extremely important in PCR. Substantial overshooting of the DNA denaturation temperature (94 °C) leads to irreversible inactivation of the Taq polymerase enzyme, which will then be less effective in subsequent extension cycles. Undershooting the annealing temperature, which is defined by the primer sequence, can lead to problems with nonspecific amplification. A PCR performance kit commonly used to evaluate the robustness of the PCR protocol and temperature cycling conditions was utilized to probe the effectiveness of the thermocycling apparatus. The PCR performance kit contained a λ-bacteriophage template and a set of primers that allow for the characterization of the PCR amplification process. While optimal amplification results in the production of a 500-bp fragment, the primer sequence contains a GG/CC overlap that promotes the production of a 45−50-bp primer dimer side product *if thermocycling conditions are not optimal*. The detection of a DNA fragment equivalent in size to the primer dimer side product provides a signpost that significant primer−primer annealing instead of a primer−template adduct formation is occurring during the annealing step. The excess formation of a primer−primer adduct is usually associated with an annealing temperature that is either incorrect or too low, both of which favor the hybridization of shorter DNA fragments.

The efficiency and rapid cycling capabilities of the IR-mediated thermocycling were tested using this system. A 50-μL aliquot of the performance kit solution was prepared and split in two aliquots; one aliquot was used to fill a 150 μm $\times$ 8 cm capillary piece and mounted adjacent to the reference capillary on the IR-mediated thermocycling apparatus. The PCR reaction solution in the capillary ($\sim$160 nL) was thermocycled as a 1-cm-long sample plug at the focal distance of the tungsten lamp while flanked with PCR reaction buffer ($\sim$620 nL on each side). DNA amplification was initiated by cycling at 94 °C for 5 s and 68 °C for 20 s for a total of 10 cycles—this followed a 140-s preincubation at 94 °C and was trailed by a final extension of 300 s at 68 °C. The second aliquot of the PCR reaction solution was thermocycled in a conventional benchtop thermocycler for comparison. Amplification conditions were chosen as recommended by the manufacturer (see Experimental Section) and also performed for 10 cycles. Figure 4 shows the results of CE analysis of the amplified products, which indicates that an adequate amount of the 500-bp product was obtained in both experiments. With the conventional cycler, where the 48-μL volume contained 0.1 ng of λ-phage DNA (equivalent to 188 800 DNA template copies), the formation of the 500-bp product is evident but a substantial amount of primer dimer has been formed after 10 cycles. In contrast, with IR-mediated capillary-based amplification of a 160-nL sample volume containing only 314 fg of λ-phage DNA (equivalent to 593 copies), a large 500-bp product peak is detected by CE, and despite the low copy number of the starting template and only a 10-cycle amplification, virtually no formation of a primer dimer side product is apparent. The detection of an insignificant amount of primer dimer side product combined with the detection of a reasonable 500-bp product signal ($\sim$2 times that of the conventional thermocycling experiment based on peak height) indicates a highly efficient amplification.

The excellent efficiency of IR-mediated amplification conveyed by Figure 4 can be argued to result from the characteristics inherent to IR-mediated thermocycling. First, fast temperature transitioning provides a significant reduction in the time spent in transition between the target temperatures, which ultimately influences the overall yield of the reaction. Less time spent transitioning to and from the denaturation temperature reduces the extent of thermal inactivation of Taq enzyme, thus increasing the overall yield due to higher enzyme activity. Second, the rapid transition between the denaturation and annealing temperature favors the kinetics of specific primer annealing and reduces the undesired reassociation of template, therefore increasing the product yield and reducing the amount of nonspecific side products amplified.

Microbore Capillary as a Nanoliter Reaction Vessel. Capillaries with a cylindrical shape offer a large surface-to-volume

ratio that assists in the fast dissipation of heat during the cooling part of the thermocycle. However, the large interior surface of the capillary also provides a larger surface for nonspecific adsorption and subsequent inactivation of Taq enzyme. Problems with adsorption of vital PCR components on the large surface of capillaries have been reported previously.[5] Consequently, capillaries for use in the IR-mediated PCR experiments were surface inactivated. Both bTMSTFA-coated and commercially coated DB-17 capillaries were used as reaction vessels for thermocycling and both showed similar PCR product yields (data not shown). The coatings were heat stable, and the capillaries could be reused several times without significant change in performance. Problems, such as bubble formation due to buffer outgassing and loss of sample due to sudden thermal expansion, were expected but were not observed. Extreme overheating of small parts of the capillary occasionally lead to the ejection of the sample from the capillary. However, this was easily avoided by keeping the focal point of the radiation source parallel to the capillary.

CONCLUSIONS

We have demonstrated that accurate thermocycling of nanoliter volumes in capillaries using IR-mediated temperature control results in the successful amplification of a 500-bp fragment of λ-phage DNA. Heating rates that are 30 times faster with cooling rates 15 times faster than in conventional thermocycling methods can be achieved. The successful amplification of the 500-bp product in a 160-nL sample plug in a capillary with minimal production of primer dimer side product is proof that the thermocyling is not only extremely rapid but also efficient. The improved accuracy in transitioning between temperatures in this rapid thermocycling protocol promoted an increased specificity and sensitivity of the PCR amplification. The ability to execute PCR amplification in small sample volumes using an IR-mediated thermocycling approach that does not require direct heating of the vessel or the surrounding medium is an important finding and provides an alternative approach for realizing PCR in miniaturized format with nanoliter volumes. Consequently, it will be possible to execute the PCR reaction in structures that are an integral part of the electrophoretic chip, such as broadened channels and even the electrophoretic separation channel itself.

The amplification speeds possible with IR-mediated PCR provide an attractive alternative to approaches that thermocycle large reaction volumes with long reaction times. The integration of fast thermocycling technology provides a good match for the ultrafast analysis times possible on electrophoretic microdevices and moves the technology closer to a state where fast cycling and detection of specific DNA sequences may be possible. The accurate fabrication of a microchip-based temperature-sensing system will not be trivial but, when accomplished, will improve the accuracy of the temperature control and provide a more reliable sensor element. This will set the stage for a truly integrated device capable of accepting purified DNA and provided qualitative information about amplified target DNA sequences of interest to a variety of disciplines.

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Abbreviations: PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; dNTP, deoxyribonucleotide; IR, infrared; bTMSTFA, bis(trimethylsilyl)trifluoroacetamide; A/D, analog to digital; CE, capillary electrophoresis; YO-PRO-1, 1-(4-[3-methyl-2,3-dihydro(benzo-1,3-oxazole)-2-methylidene]-quinolinium)-3-trimethylammonium propoane diodide.

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