CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Publications of the IAS Fellows

A power-efficient thermocycler based on induction heating for DNA amplification by polymerase chain reaction

Debjani Pal[a] and V. Venkataraman
Department of Physics, Indian Institute of Science, Bangalore 560 012, India

K. Naga Mohan and H. Sharat Chandra
Centre for Human Genetics, G04, International Technology Park, Whitefield Road, Bangalore 560 066, India

Vasant Natarajan
Department of Physics, Indian Institute of Science, Bangalore 560 012, India

We have built a thermocycler based on the principles of induction heating for polymerase chain reaction (PCR) of target sequences in DNA samples of interest. The cycler has an average heating rate of ~0.8 °C/s and a cooling rate of ~0.5 °C/s, and typically takes ~4 h to complete a 40-cycle PCR protocol. It is power-efficient (~6 W per reaction tube), micro-processor controlled, and can be adapted for battery operation. Using this instrument, we have successfully amplified a 350 bp segment from a plasmid and *SRY*, the human sex determining gene, which occurs as a single-copy sequence in genomic DNA of human males. The PCR products from this thermocycler are comparable to those obtained by the use of commercially available machines. Its easy front-end operation, low-power design, portability and low cost makes it suitable for diagnostic field applications of PCR.

I. INTRODUCTION

An important tool in molecular biology is the polymerase chain reaction (PCR), which results in the recovery of millions of copies of a DNA sequence(s) of interest from small to minute amounts of template.[1,2] In PCR, DNA is thermally cycled ~30–40 times through three temperatures: Denaturation (usually ~95 °C), primer (oligonucleotide) annealing (~50–72 °C), and extension (~60–72 °C). This procedure results in a step-wise and geometric increase in the copy number of the target sequence, resulting in as many as a billion copies after 30–40 cycles. Other than amplifying DNA for research applications, PCR also has wide clinical and forensic applications such as in diagnosis of infectious diseases, blood product screening, identity determination, paternity testing, etc. A need for a low cost, power-efficient and portable thermocycler for field work, specially in remote or rural areas, prompted us to undertake this project.

Some of the important design parameters of a thermocycler are heating–cooling rates, temperature stability, power efficiency, ability to program into the cycler the required temperature profile, etc.[3,4] A high heating–cooling rate reduces the total reaction time. It also prevents nonspecific amplification. Therefore, a considerable amount of research in thermo-cycler design has involved exploring various heating and cooling methods.[5,6] Some of these methods are hot-air cycling,[7] water cycling,[8,9] Peltier heat pumping,[10] etc. Previously[11] we have reported the advantages of using induction heating for thermal cycling in silicon–glass chips. Although chip cyclers are faster, their application for PCR requires elaborate optimization due to PCR-compatibility issues.[12–15] In this work, we have extended the use of induction heating to demonstrate PCR in conventional polypropylene tubes. The advantage of this thermocycler over others lies in its power-efficiency, portability and low cost, while achieving comparable levels of DNA amplification.

II. EXPERIMENTAL DETAILS

A block diagram of the various parts of the cycler is shown in Fig. 1. It consists of the induction heater and its driver circuit, a fan for cooling the reaction tubes, a temperature probe and control circuit, and a micro-controller with a keypad and a LCD display for programming the cycling parameters.

A. Induction heating arrangement

The details of the induction heating arrangement have been described in an earlier paper.[11] However, since the circuit for induction heating was primarily designed for carrying out reactions in PCR chips, several modifications were necessary for use in polypropylene tubes in our setup. These modifications included optimizing the secondary coil design for holding the tubes, adding forced air cooling arrangements and replacement of analog temperature control with microprocessor-control.

The secondary coil of the induction heating circuit (Fig. 2) consists of a ~10 g copper block brazed to a 0.2 mm thick ring made of ferrous metal. The copper block holds two 0.2 ml thin-walled polypropylene PCR tubes. One of the tubes contains the reaction mixture and the other, a "dummy," contains an equal volume of water and houses the temperature-probe. The current due to electromagnetic in-

[a]Author to whom correspondence should be addressed; electronic mail: debjani@physics.iisc.ernet.in

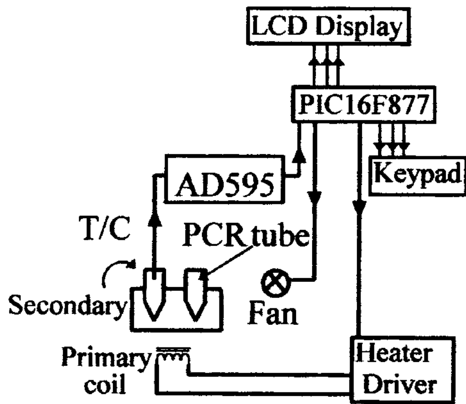

FIG. 1. Block diagram of the thermocycler showing its various components.

duction remains confined to the ring itself, and the copper block is heated by thermal conduction from the ring. The ring is made of a ferrous metal to ensure a high induced current in the secondary and the block is made of copper to ensure good thermal conduction. The outer and inner diameters of the ring are 14 and 5 mm, respectively. An optimum ring-thickness of 0.2 mm was chosen so that its thermal mass remains minimized, but at the same time it can withstand brazing to the copper block. The excess metal from the copper block is removed to minimize its thermal mass and improve the heating–cooling rates.

In contrast with our chip design,[11] the PCR tubes needed active cooling due to the increased mass of the secondary. Our earlier attempts at passive cooling had led to a cooling rate of only 0.1 °C/s. Therefore, cooling was carried out by blowing air from a 4 in. ~31 W fan, powered from a 220 V ac source, and controlled by a relay. A thermal compound (Part # 120-2, Wakefield Engineering Inc., USA) was used to ensure good thermal contact between the reaction tubes and the copper block during PCR.

B. PCR tube: Temperature calibration and thermal profile

A type-K teflon-insulated thermocouple is inserted into the dummy tube containing a volume of water, equal to the volume of the reaction mixture for measuring the temperature of the reaction. The thermocouple is connected to a complete instrumentation amplifier and thermocouple cold junction compensator chip (AD595 from Analog Devices)

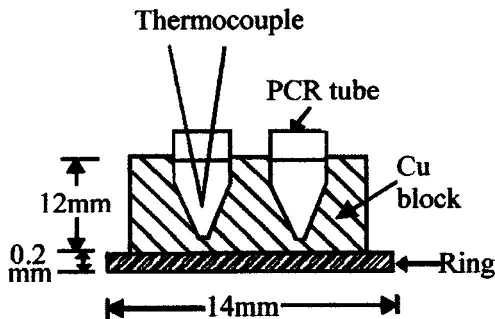

FIG. 2. Diagram of the secondary coil showing both reaction and "dummy" tubes.

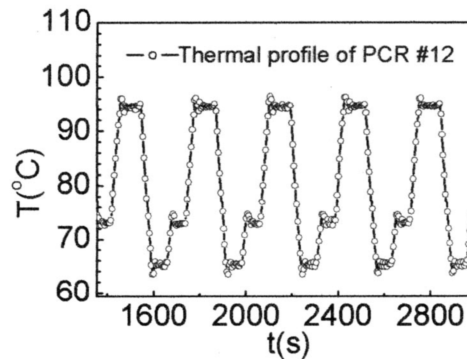

FIG. 3. Typical temperature profile during a PCR in our thermocycler.

with a voltage output of 10 mV/°C. The temperature, as well as the cycling information, can be read directly from the LCD display.

The thermocouple cannot be inserted directly into the reaction mixture during PCR as it inhibits the reaction.[15] Hence, the temperatures of the two tubes (reaction and "dummy") need to be calibrated against each other prior to the reaction. A thermocouple was inserted in each of the two tubes containing equal volumes of water and their temperatures were measured independently to ensure that both of them reach the same temperature simultaneously. This check was particularly important because the material of the tube (polypropylene) has a low thermal conductivity ($0.12\ Wm^{-1}\ K^{-1}$).[16]

We also calibrated the AD595 chip independently to compensate for any chip error. This was done by inserting a thermocouple and a platinum resistance thermometer into the same tube and comparing their readings. The thermocouple output was read simultaneously with the AD595 chip and a Keithley 2000 digital voltmeter, using its in-built module for reading type-K thermocouple.

The readings of the platinum resistance thermometer are almost the same as those registered by the thermocouple and the Keithley 2000 digital voltmeter. However, the thermocouple and AD595 registered a value that is lower by 2.8 °C. This error (within the specified ±3 °C calibration error of the chip) is compensated by programming the microcontroller.

The amplified output from AD595 is directly fed to the micro-controller (PIC16F877) to constitute the on–off electronic control. The cycling parameters (e.g., set-points, incubation times and the number of cycles) are entered into the micro-controller through a keyboard and can be monitored on the display during cycling. When the temperature of the tube exceeds the set-point, the micro-controller switches off the heating. One of the micro-controller pins is connected to the fan through a relay. The fan is switched on during cooling from denaturation temperature (~95 °C) to annealing temperature (~50 °C).

We also recorded the thermal profile during PCR to eliminate possible failure of the reaction due to an incorrect profile. A section of the temperature profile for the PCR tube is shown in Fig. 3. The maximum temperature variation at any incubation temperature (calculated as standard deviation with typically 20 data points, excluding the initial overshoot) is ±0.4 °C. There is an initial overshoot of temperature on

reaching any set-point, which was absent in the thermal profile of the PCR chip.[11] The overshoot in the present case results from the effect of the on–off control on the large thermal mass of the copper block. Since it dies down after one or two oscillations, it can be neglected while calculating the average temperature variation at the set-points. The average heating rate is ~0.8 °C/s and the average cooling rate is ~0.55 °C/s.

C. Power efficiency of the thermocycler

The power requirement for heating the PCR tubes is low in our thermocycler due to the switching mode design of the drive circuit. In this design, the power transistors are either on or off and never operated in the active region. Therefore, their power dissipation is minimized.

To calculate the power-efficiency, we first estimated the fraction of the total power dissipated in the secondary coil during open-loop operation (i.e., full heating without any set-point). This same fraction was used to estimate the power going into the secondary coil alone during the actual cycling. The total power drawn from the supply during open-loop operation is measured to be ~90 watts (6 A at 15 V). This was found to be mostly dissipated across the transistors and the primary coil. Taking into account the measured primary current and the FET, diode, and coil resistances, the average power dissipated in the primary circuit per cycle was estimated to be ~67 W. Therefore, only 25% (~23 watts) of the total power drawn from the supply is required to heat the secondary coil under open-loop conditions.

During closed-loop operation (actual cycling), on an average, the power dissipated in the secondary coil is only ~12 watts (including both heating and cooling), assuming only ~25% heating efficiency. Since there are two tubes, the power required per tube is ~6 watts. On the other hand, a commercial thermocycler[20] requires about 9 watts of power per tube (220 watts for 25 tubes). This shows that our switching power design for the induction heating setup is more power-efficient than the typical commercial cycler. Furthermore, the power loss in the drive circuit can be minimized by paralleling more transistors, and hence reducing their effective resistance, as well as by more efficient thermal design of the circuit. Since the aim of the present work was to demonstrate PCR in this new thermocycler design, the drive circuit was not optimized for its lowest-power operation.

III. DNA AMPLIFICATION BY PCR

We tested the thermocycler described above by amplification of the *SRY* gene (840 bp) from human male genomic DNA. For this purpose, genomic DNA was prepared from peripheral blood of a normal male by the proteinase K-phenol method.[17] Twenty nanograms of this DNA were mixed with forward (5′-GTACCCGGATCCAT GCAATCATATGCTTCTGCTATG-3′) and reverse (5′ - TGCAGAAGAATTCTAGGTCTTTGTAGCCAATGTT ACC-3′) primers (final concentration 50 pmols each, Sigma Genosys, UK), dNTPs (final concentration 125 μmol, MBI Fermentas, Lithuania) and 1.25 units of *Taq* DNA polymerase (Invitrogen, USA). The reaction mixture was overlaid with 40 μL of mineral oil (M-5904 from Sigma) and PCR was carried out as follows: Initial denaturation at 95 °C for 2 min, followed by 40 cycles at 95 °C for 1 min, 65 °C for 1 min and 72 °C for 1 min, and a final extension at 72 °C for 10 min.

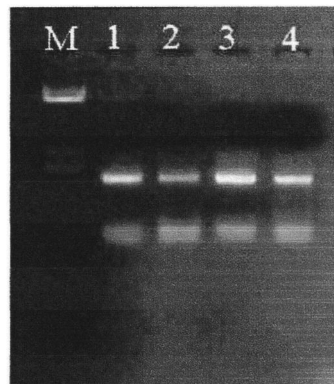

FIG. 4. Amplification of *SRY* from human male DNA. Lane M: λ DNA digested with the enzyme *Hin*dIII (molecular size DNA marker), Lane 1 and 3: PCR products obtained from PTC-150 (MJ Research), Lanes 2 and 4: PCR products obtained from the induction heating-based thermocycler (present study). The size of the PCR products is 840 bp.

The reaction products were resolved on 0.8% agarose gel and stained with ethidium bromide (Fig. 4). For comparison, duplicate reactions were carried out in a commercially available thermocycler (PTC-150, MJ Research), and the products obtained with this machine were also resolved in the same gel. These results show that in specificity and amount, the PCR products obtained by using the thermocycler described here are comparable to those from the commercial cycler (PTC-150).

IV. DETECTION OF PCR PRODUCT BY FLUORESCENCE FROM SYBR GREEN I

Sybr Green I is an intercalating dye which binds to double-stranded (ds) DNA and emits a bright green fluorescence upon binding. As PCR proceeds, the copy number of dsDNA increases, resulting in an increase in fluorescence. The increase in fluorescence is ~100-fold at the end of PCR, making this a very sensitive detection technique for the amount of PCR product synthesized.

We carried out PCR of *Prostatin C*1 (350 bp) cloned in the plasmid BlueScript (Stratagene, USA) with an initial denaturation at 95 °C for 3 min, followed by 40 cycles at 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min and a final extension at 72 °C for 5 min. The reaction mixture contained 10 ng of template DNA, 10 pmols each of T3 (5′-ATTAACCCTCACTAAAGGGA-3′) and T7 (5′-TAATACGACTCACTATAGGG-3′ primers, and 40 μL of Dynamo Sybr Green qPCR Mastermix. The PCR product was checked by means of increased fluorescence due to binding of amplified double-stranded DNA to Sybr Green I. The fluorescence images were recorded before and after PCR by a CCD camera.

In our detection system, light from a 100-watt halogen lamp passes through a 450–490 nm bandpass filter and falls on the sample through a 5*X* microscope objective. The emitted fluorescence is passed through a dichroic beam-splitter (510 nm), followed by a 515 nm filter to block the incident light. Sybr Green I has an excitation maxima at 494 nm and an emission maxima at 521 nm.[18] The filters were chosen to match the excitation and emission wavelengths of this dye, and the beam-splitter was appropriately chosen to increase the sensitivity of the detection system.

As in case of *SRY*, control reactions were setup in PTC-150, and fluorescence from the reactions performed in the commercial cycler and our thermocycler were compared. We checked for background fluorescence, if any, when no template DNA is present (data not shown). The average number of green pixels in each CCD image was used to obtain a quantitative measure of the fluorescence intensity from the respective samples. On a scale of 0–255, both the PCR images give a pixel value of ~254, as opposed to a pixel value of ~99 from the nonamplified sample. These results show that the amount of PCR products obtained from PTC-150 (254.58) and our cycler (254.76) are similar.

V. DISCUSSIONS

At present our thermocycler works at about ~25% power efficiency. It should be possible to improve this efficiency by the use of better drive transistors and by reducing the primary coil resistance. The heating and cooling rates of our instrument are lower than those of commercial cyclers which use Peltier heat pumps.[21] Although use of Peltier cooling in our instrument would have reduced the total reaction time, it is expensive and consumes more power. Since the total reaction time (~4 h) did not affect the outcome of PCR, and since we wanted to build a low-power and low cost instrument, we did not use Peltier cooling. The temperature stability at the set-points can be improved upon by using PID control. But this would increase the complexity of programming the microcontroller. In any case, the temperature stability (±0.4 °C) obtained with the present instrument with on–off control did not affect any of the PCR reactions reported here. Finally, this thermocycler design has the potential of being made into a real-time thermocycler[19] by integrating the fluorescence detection system into the cycler itself.

ACKNOWLEDGMENTS

The authors thank Sachin Barthwal and Joji Joykutty for help in the fluorescence experiments, and Professor P. Kondaiah and Ms. Prathibha Ranganathan for a few reagents. This work was supported by BRNS, Department of Atomic Energy, and the Life Sciences Research Board (LSRB), Government of India, and partly by a Programme Support grant from DBT, Government of India to the Centre for Human Genetics.

[1] E. Harris, *A Low-cost Approach to PCR: Appropriate Transfer of Biomolecular Techniques* (Oxford University Press, Oxford, 1998).
[2] R. A. Gibbs, Anal. Chem. **62**, 1202 (1998).
[3] The PTC-0150 minicycler operations manual, version 4.0, MJ Research, USA.
[4] MJ Research 2002/03 product catalogue, MJ Research, USA.
[5] P. K. Upadhyay and D. V. Gadre, Meas. Sci. Technol. **6**, 588 (1995).
[6] P. Upadhyay, Curr. Sci. **77**, 515 (1999).
[7] P. Denton and H. Reisner, in *PCR Protocols: A Guide to Method and Applications*, edited by M. Innis *et al.* (Academic, New York, 1990), Chap. 52.
[8] N. S. Foulkes, P. P. Pandolfi de Rinaldis, J. Macdonnell, N. C. P. Cross, and L. Luzzatto, Nucleic Acids Res. **16**, 5687 (1988).
[9] F. Rollo, A. Amici, and R. Salvi, Nucleic Acids Res. **16**, 3105 (1988).
[10] M. Collasius, H. Falk, C. Ciesler, and G. Valet, Anal. Biochem. **181**, 163 (1989).
[11] D. Pal and V. Venkataraman, Sens. Actuators, A **102**, 151 (2001).
[12] T. B. Taylor, E. S. Winn-Deen, E. Picozaa, T. M. Woudenberg, and M. Albin, Nucleic Acids Res. **25**, 3164 (1997).
[13] I. Schneegass, R. Bräutigam, and J. M. Köhler, Lab Chip **1**, 42 (2001).
[14] M. A. Shoffner, J. Cheng, G. E. Hvichia, L. J. Kricka, and P. Wilding, Nucleic Acids Res. **24**, 375 (1996).
[15] R. P. Oda, M. A. Strausbauch, A. F. R. Huhmer, N. Borson, S. R. Jurrens, J. Craighead, P. J. Wettstein, B. Eckloff, B. Kline, and J. P. Landers, Anal. Chem. **70**, 4361 (1998).
[16] W. D. Callister, *Materials Science and Engineering* (Wiley, New York, 2002).
[17] J. Sambrook, E. F. Fritsch, and T. Manatis, *Molecular Cloning* (Cold Spring Harbor Laboratory Press, 1989).
[18] M. Seville, Electrophoresis **5**, 814 (2001).
[19] Y.-C. Lin, M. Li, M.-T. Chung, C.-Y. Wu, and K.-C. Young, Sens. Mater. **14**, 199 (2002).
[20] PTC-150 Minicycler from MJ Research, Inc., USA.
[21] PTC-150 has a maximum ramp rate of 2.4 °C/s.