Low Cost and Portable PCR Thermoelectric Cycle

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ABSTRACT

In this work, we develop a rapid polymerase chain reaction (PCR) device with thermoelectric cooling for the fast PCR thermo cycling process. The PCR system utilizes a 60W commercial thermoelectric module, the Tianjin TEC1-12710 model, which allows fast heating and rapid cooling in the PCR temperature cycles. In this study, the system performance has been tested with DNA of shigella spp bacteria. The results are compared to the results of a commercial PCR system (Thermo cycle) operated under the same PCR parameters including initial amount of DNA, the number of cycles, temperature and time in each PCR step. The experimental results show that DNA amplification is successful with expected amount of the amplified DNAs that is comparable to those from the commercial PCR system.

1. INTRODUCTION

The polymerase chain reaction (PCR) is one of the most widely used techniques in molecular biology and other life sciences. The PCR is the process for DNA amplification in which DNAs are thermally treated in cycles between three different temperatures: denaturation at 94 °C, annealing at 54 °C, and extension at 72 °C [1]. The number of DNAs is double in every successful PCR cycle. Hence, the number of DNA amplification factor is 2n, where n is the number of PCR cycle [6]. Conventional PCR system is slow, inefficient, and still expensive due to a large size and cooling inability. Therefore, a number of researches have been focusing on development of small, fast and efficient PCR system which will enable rapid DNA processing and analysis. In thermo cycling PCR, the DNA is filled in a single chamber and the chamber temperature is cycling by varying heating power. This kind of system is inexpensive but relatively slow due to long natural cooling time. In order to increasing the thermo cycling speed, a cooling scheme is needed and thermoelectric (TE) cooling is a potential solution.

2. SYSTEMS DESIGN

A thermoelectric device is a completely solid-state heat pump that is operated based on Peltier effect in which electrons carry energy to transfer heat from hot to cold junction as shown in Fig. 1. At the cold junction, the energy (heat) is absorbed by electrons as they pass from a low energy level in the p-type semiconductor element to a higher energy level in the n-type semiconductor element. The power supply provides the energy to move the electrons through the system. At the hot junction, energy is expelled to a heat sink as electrons move from a high energy level element (n-type) to a lower energy level element (p-type). Heat absorbed at the cold junction is pumped to the hot junction at a rate proportional to current passing through the circuit and the number of p-n junctions [3]. The use of TE device needs a careful design and consideration including installation of fan for heat transfer. A proper design will allow TE device to be an effective heating and cooling source.

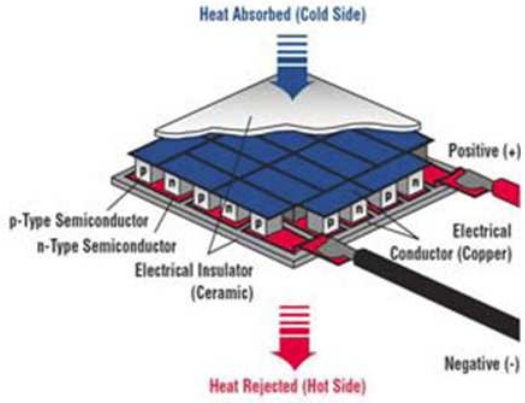

Fig.1: *Structure of thermoelectric module [3]*

In this PCR device, there are three 500 μL microchambers which are capillary Pyrex tubes fitted on a 5 mm thick aluminum holder. Two TE modules are attached on both sides of the aluminum holder. The

Manuscript received on January 28, 2008 ; revised on August 20, 2008.

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other sides of both TE modules are installed with fins and fans for heat transfer. The fans have the dimension of $40 \times 40 \times 10$ mm running at the speed of 2800 rpm and the fin sheets have dimension of $50 \times 50 \times 10$ mm. The all components are glued together by silicone wax as shown in Fig.2.

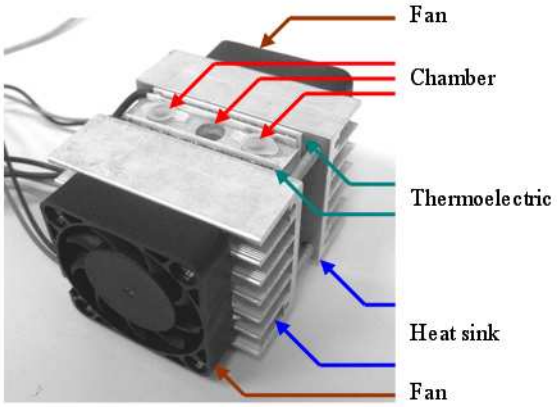

Fig.2: *PCR chamber with the thermoelectric module.*

The temperature control has 2 type, i.e., ON-OFF control and PID control. The ON-OFF control uses the principle ON-OFF switch when the temperature reaches a set-point. This produces a ripple characteristic of temperature around the set point and the ON-OFF control is defective inside a band called dead band [4].

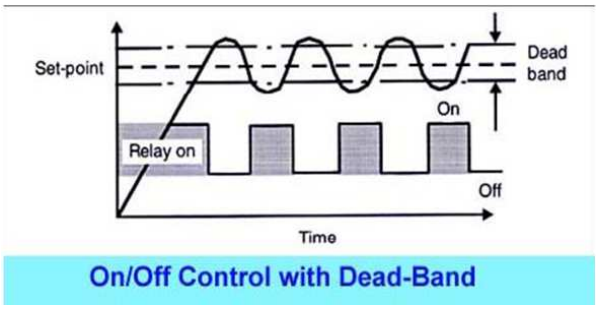

Fig.3: *Temperature characteristic of the ON-OFF control*

The parameters for PID control are:

- Proportional control action (P-Action);
- Integral control action (I-Action); and
- Derivative control action (D-Action).

P-Action

The proportional term makes a change to the output that is proportional to the current error value. The proportional response can be adjusted by multiplying the error by a constant K_p, called the proportional gain [10].

I-Action

The contribution from the integral term is proportional to both the magnitude of the error and the duration of the error. Summing the instantaneous error over time (integrating the error) gives the accumulated offset that should have been corrected previously. The accumulated error is then multiplied by the integral gain and added to the controller output. The magnitude of the contribution of the integral term to the overall control action is determined by the integral gain, K_i [10]

D-Action

The rate of change of the process error is calculated by determining the slope of the error over time and multiplying this rate of change by the derivative gain K_d. The magnitude of the contribution of the derivative term to the overall action is called the derivative gain, K_d.[10]

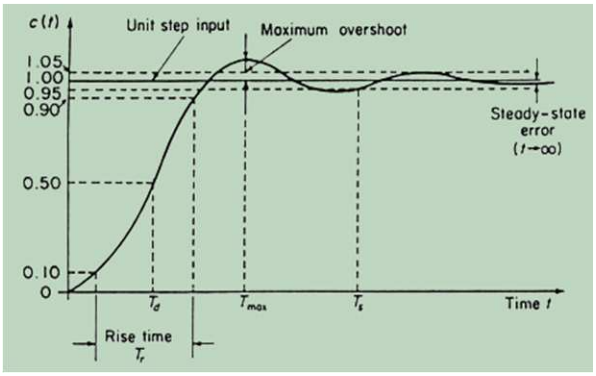

Fig.4: *Temperature characteristic of PID control [4].*

The low cost and portable PCR Thermoelectric cycle has a switching power supply with a current of 10 ampere and a voltage of 12 volt for thermoelectric module. For the developed PID temperature control, the temperature is fed back from LM35, a temperature sensor IC shown in Fig. 5. This sensor gives 10 mV output voltage change for each 1 C temperature increment [5]. The output from PID controller is applied to control a dual full-bridge driver IC L298N.

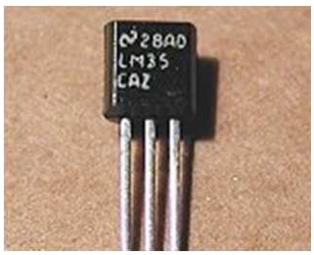

Fig.5: *Temperature sensor IC, LM35 [5].*

The diagram of portable PCR system is shown in Fig.6. The system consists of four parts: external control by a personal computer (PC), a microcontroller system with TE driving circuit, a PCR chamber with TE heater/cooler, and a standard 10A power

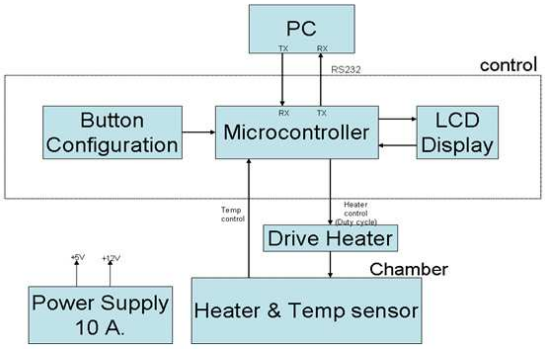

Fig.6: *Low cost and portable PCR thermoelectric cycle*

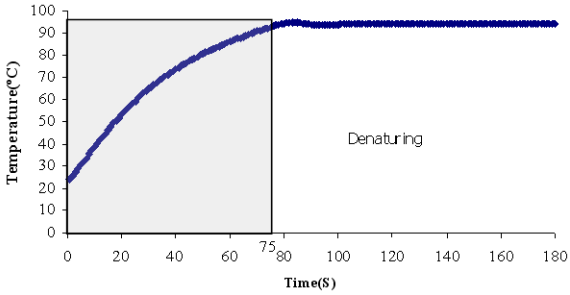

Fig.7: *Heating dynamic of the PCR chamber with the PID control and thermoelectric heating at the temperature of 94° C.*

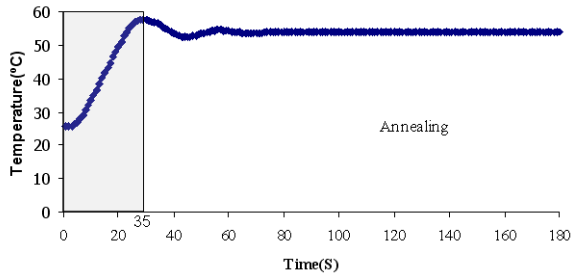

Fig.8: *Typical heating dynamic of PCR chamber with PID control and thermoelectric heating at temperature 54?C.*

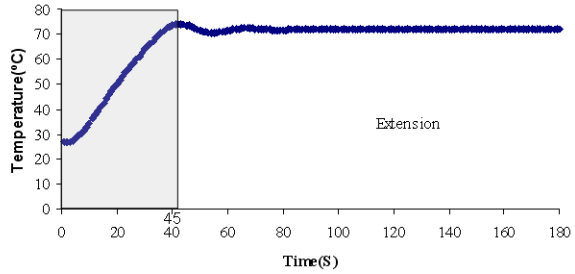

Fig.9: *Typical heating dynamic of PCR chamber with PID control and thermoelectric heating at temperature 72?C.*

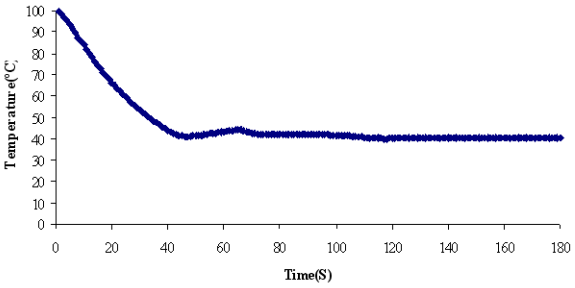

Fig.10: *Cooling dynamic of the PCR chamber with the PID control and thermoelectric cooling.*

supply.

The system is controlled externally via a PC. A PC program for the PCR interfacing is implemented using Visual Basic code. In the program, the user can set various PCR parameters including the temperature set point, duration, and the number of cycles. Next, the program sends the parameters to the microcontroller via a serial port. The program also reads the temperature signal back from the microcontroller.

3. EXPERIMENTS

DNA of *Shigella spp.* bacteria is used to test the performance of the developed PCR system. DNAs were extracted from bacteria source by an extract solution and treated at 90 C [10]. The extracted DNAs were then mixed with the DNA polymerase $3' \rightarrow 5'$ and $5' \rightarrow 3'$ primers which has a desired base sequence. The DNAs were then amplified by the PCR process for 30 cycles. In each cycle, DNAs were thermo cycled at 94 °C (denaturation) for 30 seconds, 53 °C (annealing) for 30 seconds, and 72 °C (extension) for 60 seconds, respectively.

The amplified DNAs were then detected by gel electrophoresis process. The gel was prepared at 0.3% concentration and placed on electrophoresis plate with a dimension of 6×8 cm consisting of 8 columns. In this experiment, only four columns are used. Columns 1 to 4 contain DNA marker, unamplified DNA (negative sample), first amplified DNAs and second amplified DNAs. Under applied electric field, negatively charged DNA will move toward positive electrode by a distance according to its own molecular weight. The band position of DNA indicates the base-pair position of DNA that can be identified by comparison with the DNA marker

4. EXPERIMENTAL RESULTS

The PCR system was first tested for heating and cooling performance. Fig.7 shows the typical heating cycle from 25 to 94 °C. The result shows that the PID controller can control constant temperature within 70 seconds. Fig.8 shows typical heating cycle from 25 to

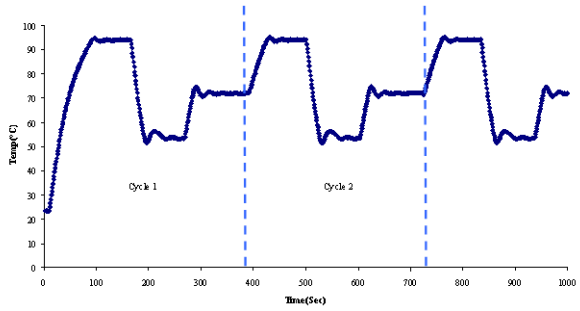

Fig.11: *Typical temperature response of full PCR thermo cycling of the PCR system.*

54 °C. The result shows that the PID controller can control constant temperature within 35 second. Fig.9 shows typical heating cycle from 25 to 72 °C. The result shows that PID controller can control constant temperature within 45 seconds.

Fig.10 shows typical cooling cycle from 100 to 40 °C. The result shows that the PID controller with thermoelectric cooling can reduce temperature rapidly with the cooling time of approximately 40 seconds. The cooling time is reduced by the factor of two compared to the cooling time without the thermoelectric cooling.

The developed PCR system was then experimented for the full PCR thermo cycling for 30 cycles and the temperature response of the first two cycles are shown in Fig. 11. In each cycle, DNAs were thermo cycled at 94 °C (denaturation) for 30 seconds, 53 °C (annealing) for 30 seconds and 72 °C (extension) for 60 seconds, respectively. The total cycle time including heating and cooling time is approximately 350 seconds which is as fast as the commercial PCR device.

Fig. 12 shows a picture of gel electrophoresis: the marker as shown in the column 1, the unamplified DNA as shown in the column 2, and the PCR amplified DNA as shown in the columns 3 and 4. The marker is the reference DNA of *Shigella spp.* bacteria.

It is shown that there is not a band of DNA in the column 2 as anticipated while in the columns 3 and 4 there are bands of the amplified DNAs at the 100 bp position. In addition, the thickness of band of DNA is correlated with the initial amount of DNA. The experimental results show that the developed PCR system can be used effectively for the DNA amplification.

5. CONCLUSION

In conclusion, we have developed a portable polymerase chain reaction (PCR) device with thermoelectric cooling for the fast PCR thermo cycling process. The PCR system utilizes 60W commercial thermoelectric module, i.e., the Tianjin TEC1-12710 model, that allows fast heating and rapid cooling in the

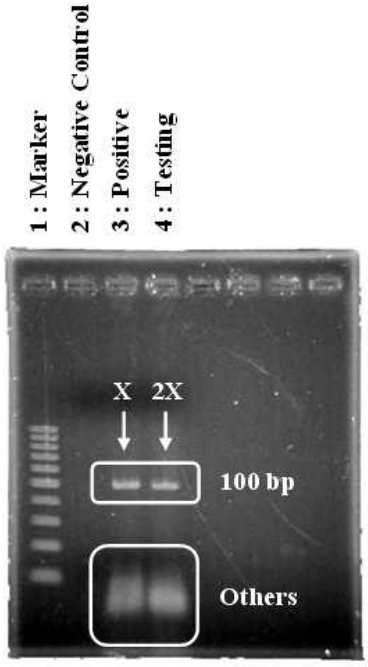

Fig.12: *A picture of gel electrophoresis: marker (column 1), unamplified DNA (column 2) and PCR amplified DNA (columns 3 and 4).*

PCR temperature cycles. Further, the PID temperature control has been designed using the microcontroller with computer interface. In this study, the performance of the developed system has been tested with DNA of shigella spp bacteria. The results were compared to the products using the commercial PCR system (Thermo cycle) by operating under the same PCR parameters including initial amount of DNA, the number of cycles, temperature and duration in each PCR step. The experimental results show that DNA amplification is successful with expected amount of amplified DNAs. This is equivalent to the commercial PCR system.

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