Development of a PCR Thermal Cycling Instrument for Molecular Biology Applications]

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ABSTRACT

In this work, we designed and developed a rapid Thermal Cycler with thermoelectric cooling for the fast thermo cycling process. The thermal cycler is an instrument that employs precise temperature control and rapid temperature changes to conduct the polymerase chain reaction (PCR). The developed PCR system utilizes low power commercial thermoelectric module which allows fast heating and rapid cooling in the PCR temperature cycles. The experimental results of developed system show that efficiency of Thermal cycling of aluminum block. Unsuitable temperature controller setting will cause the block temperature overshooting or undershooting of the desired block's temperature which can result in damage to the DNA sample and cause incomplete reactions. The effect of Proportional Integral (PI) temperature controller on the performance of thermal cycler is investigated in the present study.

KEYWORDS: Thermal Cycler, Polymerase Chain Reaction, PID Controller, Tuning.

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. 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. 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 allied 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.

The thermal cycler is an instrument that employs precise temperature control and rapid temperature changes to conduct the PCR. Most of the commercial thermal cycler available in the market was using aluminum or silver material to build thermal cycler block (PCR block) [E. T. Lagally et al., 2000]. The PCR block holds the DNA mixtures to perform DNA amplification process. Temperature control in the PCR block is mostly performed with proportional integral-derivative (PID) control due its simple algorithm, robustness, and stability [Sadler D. J., 2003] [Chiou J., 2001]. The purpose of PID temperature controller is getting the PCR block to the correct temperature, and then maintaining them at that temperature. The performance of temperature controller depends on the values of proportional, integral, and derivative gains of a PID controller. Setting the unsuitable gain value in the PID temperature controller will cause the block temperature overshooting or undershooting of the desired block's temperature [Aidan O'Dwyer, 2009]. Undershooting or overshooting the temperature targets adds delays, and the overheating can result in damage to the DNA sample. Conversely, undershooting can lead to incomplete reactions, as the DNA mixture is not at the target temperature for long enough [Kim Y. H. et al., 2008]. The implementation of PID temperature controller will cause the system complexity and difficulty to tune the system. Understanding of the relationship between the PID parameters and the thermal cycler's ramping temperature and temperature stability will aid the PID tuning process to be less complicated. The objective of this paper is to study the effect of PI tuning parameters on the thermal cycler performance. The PID temperature controller is proposed in this work due to its simplicity and noise immunity compared to PID temperature controller. The derivative-term of temperature control will cause a fast change in the block's temperature due to noise response could destabilize the block temperature [Wavelength Electronic, 2005].

2. SYSTEM 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. 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. 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.

This PCR device consists of Heating metal block, having 25 microchambers fitted on a 9 mm thick aluminum holder and in tight contact with standardized 0.1μL PCR tubes. DNA mixture was placed in the PCR block to perform DNA amplification. The block was built with aluminum alloy (1060) material. Aluminum alloy was selected because it has high thermal conductivity and low heat capacity. Most of the PCR block in commercial thermal cycler is using aluminum material. Thermal contact enhancement can be achieved by using either thermal grease or mechanical pressing with another identical thermal cycler. The PCR block was heated and cooled using a 4V, 10A thermoelectric module. The thermoelectric module acts as a heat pump and it can add or remove thermal capacity stored in the PCR block which results in increasing or decreasing of block temperature respectively. The amount of heat transfer was determined by the temperature difference between the hot side and cool side of the thermoelectric module and the current supply to the thermoelectric module. The PCR block was placed on the cool side of the thermoelectric module while the other side of TE module is installed with Heat sink and fans for heat transfer. The fans have the dimensions of 120x120x38 mm running at the speed of 3600 rpm. All the components are fitted together by screws. A proportional-integral (PI) controller algorithm was used to control the thermal electric module and set the temperate of PCR block. The PI constants are tuned using Ziegler-Nichols closed loop method. The output from PI controller is applied as PWM to control a dual full-bridge driver. The full-bridge temperature controller controls the current supply to the thermoelectric module and the resistive heated lid of the thermal cycler. NTC thermistor is used as a temperature feedback system to measure and maintain the temperature of PCR block. Thermistor with a parallel linearizer resistor characteristic curve and equations are shown in Fig 2. The PI controller was controlled by a data acquisition using Analog to Digital Converter with a sampling frequency equal to 50k Hz and 24-bit resolution. A GUI was developed to monitor the PCR block temperature. GUI allows the end user to define PCR protocol and record the data for further processing. The Protocol of PCR is shown in Table 1 which was used to amplify the DNA samples.

Table 1: The PCR protocol for DNA amplification.

Steps in PCR	Temperature (°C)	Time (Sec)
Hot start: Initial Cycle	95	350
Denaturation	94	45
Annealing	55	30
Extension	72	60
Number of Cycles ~	30 Cycles	--
Final Extension: Last Cycle	72	600
Incubation	10	Forever

The system is controlled externally via a Personnel Computer (PC). The PC is connected to microcontroller unit (MCU) through UART/USB and 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. All these parameters are stored on EEPROM for future use.

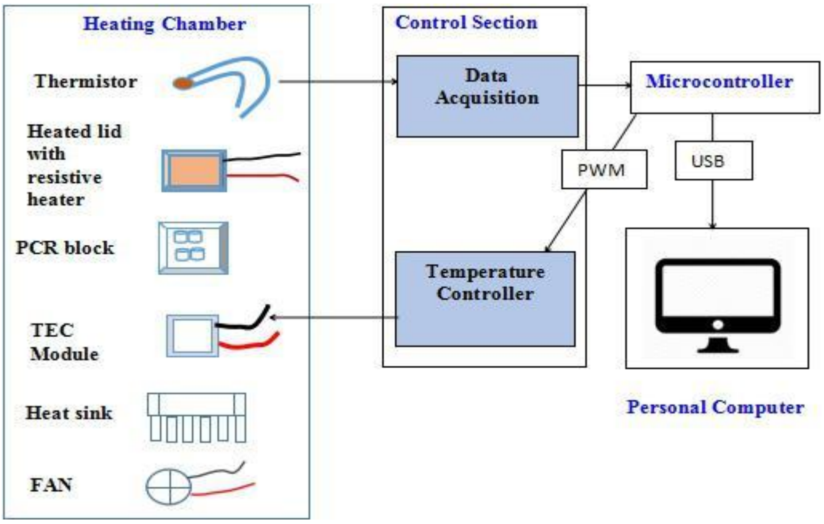

Figure 1: Block diagram of developed thermocycler

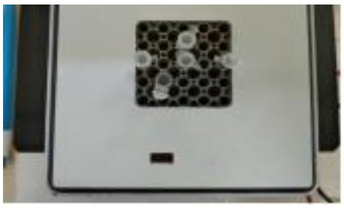

Figure 2: Sample holder and Heatsink setup.

The temperature can be controller in two ways 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.

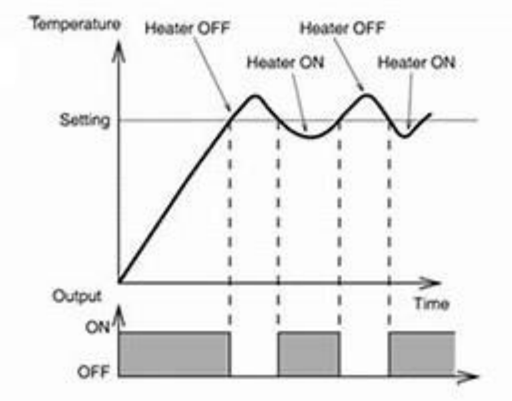

Figure 3: Temperature characteristic of the ON-OFF control

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 *Kp*, called the proportional gain.

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, *Ki.*

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 *Kd*. The magnitude of the contribution of the derivative term to the overall action is called the derivative gain, *Kd*.

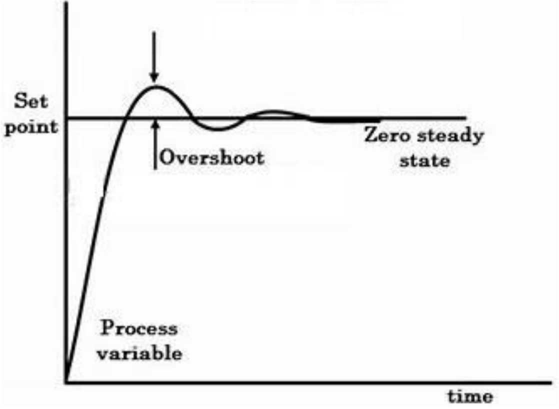

Figure 4: PID-Control Action

PI temperature controller

The Stein-hart equation for thermistor is used to convert the temperature to appropriate voltage as an input to PI controller. The PI controller generates equivalent PWM to drive full-bridge controller to generate Current (I Amp.) supplied to the thermoelectric module (Peltier). The Peltier output, based on input current supply increases or decreases the PCR block temperature. The temperature of PCR block is defined as PV (Process Value). The PV is monitored by NTC thermistor. In this work, three different temperatures steps (55°C to 94°C, 55°C to 72°C and 72°C to 94°C) were used to study the performance of PI temperature controller. The temperature step is the change from initial temperature state to final temperature state of the PCR block.

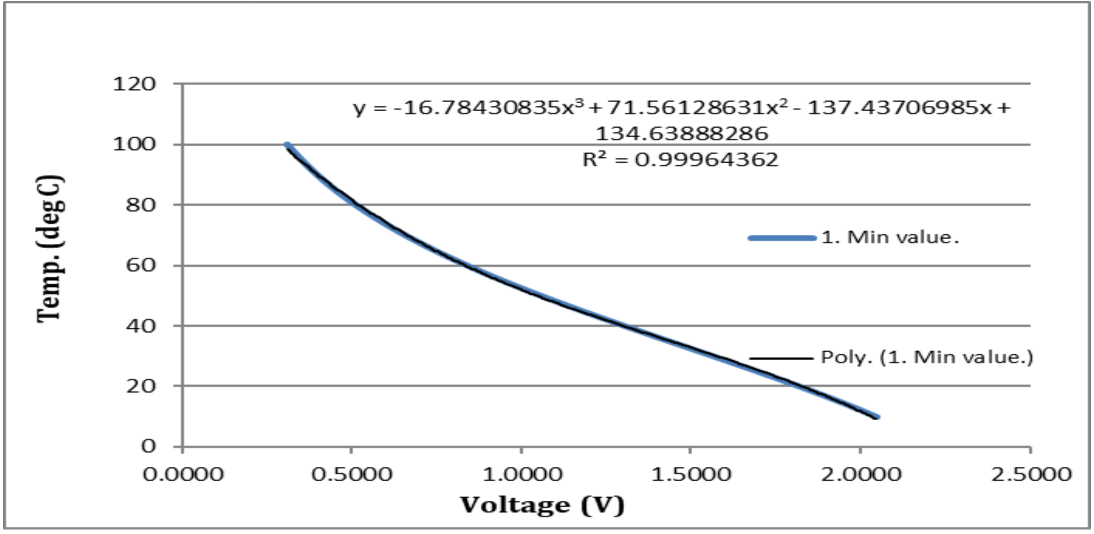

Figure 5: Thermistor Linearized graph and equation.

Table 2: Proportional Integral controller tuned Parameters for thermal cycler

Control type	Kp	Ti (min)	Td (min)
P	100	-	-
PI	90	0.038533333	-
PID	120	0.022666667	0.005893333

3. RESULTS AND DISCUSSION

A thermal cycler was successfully developed and tested. The PCR system was first tested for heating and cooling performance. The typical heating cycle is from 25°C to 94ºC. The result shows that the PI controller can maintain and control a constant temperature. The developed GUI was used to configure thermal cycler setting and monitor the PCR block temperature. The GUI allowed the end user to enter the PCR protocol such as the block temperature and time as shown in Table 1. The process of PCR started after completion of the thermal cycler settings. The GUI monitored the PCR block temperature during PCR process. All the data was sent to the personal computer for further analysis.

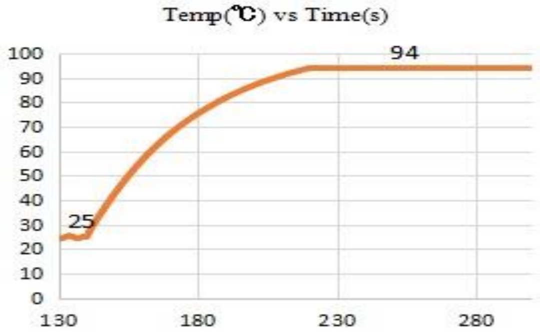

Figure 5: Denaturation Step - Typical heating cycle from 25ºC to the temperature of 94ºC.

The typical heating cycle from 25 to 95ºC is shown in figure 5 and figure 6. The cooling time is reduced by the factor of two compared to the cooling time without 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 Figure 8. In each cycle, DNA's were thermo cycled at 94ºC (denaturation) for 30 seconds, 54ºC (annealing) for 30 seconds and 72ºC (extension) for 60 seconds, respectively. The total cycle time including heating and cooling time is approximately 400 seconds which is as fast as commercial PCR device.

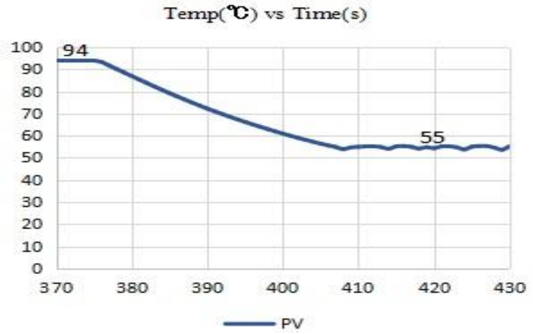

Figure 6: Annealing - Typical cooling cycle from 94ºC to temperature of 55ºC.

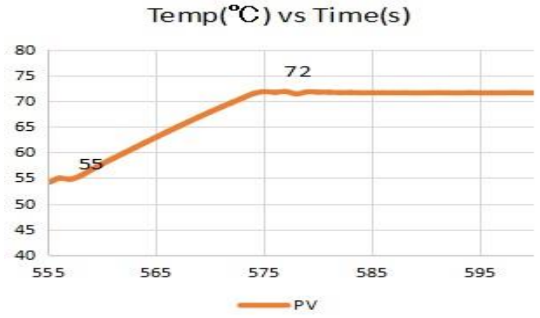

Figure 7: Extension - Typical cooling cycle from 55ºC to temperature of 72ºC

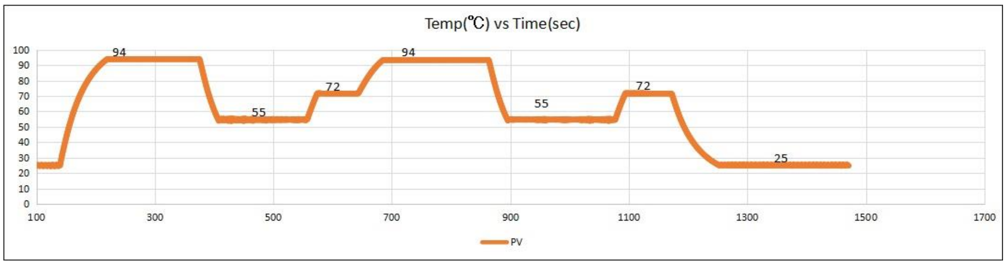

Figure 8: Typical temperature response of full PCR thermo cycling process.

4. CONCLUSION

A Thermal cycler (Polymerase Chain Reaction) device with thermoelectric cooling for the fast PCR thermo cycling process is designed, developed and characterized. The PCR system utilized low power commercial thermoelectric module, that allows fast heating and rapid cooling in the PCR temperature cycles. Further, the PID temperature control has been designed using the Ziegler-Nichols closed loop method. In the present study, the performance of developed system has been tested. A study on the effect of the PID parameters on the developed thermal cycler is successfully performed. A proportional gain will increase the rise time and overshoot of thermal cycler. The increase the rise time decrease the temperature ramp rate of the system. The experiment result shows that the temperature ramp rate of developed system was equal to 2.5 °C/s based on the temperature step input 72°C to 94°C. The further improvement in thermal cycling optimization is still ongoing.

5. ACKNOWLEDGEMENTS

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REFERENCES

[1] M. Hashimoto, F. Barany, and S. A. Soper, \Polymerase chain reaction/ligase detection reaction /hybridgization assays using flow-through microfluidic devices for the detection of low abundant DNA point mutations," *Biosensors and Bioelectronics 21*,pp.1915-1923, 2006.

[2] W.DhavepatanaCo.,Ltd.,2007,http://www.wdhave.inet.co.th/index/TempControl/TemperatureControl.pdf

[3] D. M. Rowe, \CRC Handbook of Thermoeletric",New York, CRC Press,1995.

[4] W.Dhavepatana Co., Ltd.,2007 TemperatureControl

[5] Q. Zhang, W. Wang H. Zhang, and Y. Wang,\Temperature analysis of continuous flow micro PCR based on FEA," *Sensors and Actuators B82*, 2002. reactors :Thermal and fluidic concepts," *Chemical Engineering Journal*, 2007.

[6] D. S. Lee, M. H. Wu, U.Ramesh, C. W. Lin,T. M. Lee, and P. H. Chen, \A novelreal-time PCR machine with a miniature spectrometer for fluorescence sensing inamicro liter volume glass capillary", *Sensors and Actuators 100*, pp. 401-410, 2004.

[7] R. Saiki, S. Scharf, F. Faloona, K. Mullis, G. Horn, H. Erlich and N. Arnheim, “Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia,” Science, vol. 230, no. 4732, pp. 1350-1354, 1985.

[8] K. Mullis and F. Faloona, “[21] Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction,” Methods in Enzymology, pp. 335350, 1987. T. Powledge, “The polymerase chain reaction,” AJP: Advances in Physiology Education, vol. 28, no. 2, pp. 44-50, 2004.

[9] Q. H. Wang, Y. Tan and H. Q. Gong, “An integrated system for real time PCR analysis based on microfluidc chip”, In. J. omput. Eng. Sci., 2 285-288, 2003.

[10] Sadler D. J., Changrani R., Robert P., Chou C. F., Zenhausem, F., “Thermal management of BioMEMS: Temperature control for ceramic-based PCR and DNA detection devices”, IEEE Trans. Comput. Pack. Tech., 26(2), 309-316 (2003).