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ORIGINAL PAPER

Development of Thermal Cycler Using Proportional-Integral Controller for Polymerase Chain Reaction

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ABSTRACT

The thermal cycler is an instrument that employs precise temperature control and rapid temperature changes to conduct the polymerase chain reaction, PCR. 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 causes incomplete reactions. In this work, the effect of the proportional gain of PI temperature controller on the performance of thermal cycler was investigated. The results show that low proportional gain value produces large overshoot and cause the PCR block temperature oscillated at the desired block's temperature. As the proportional gain value increase, the rise time decrease. The temperature ramp rate of developed system was equal to 5.5 °C/s with the PI temperature controller setting, Kp = 15 A/V and Ki = 1.8 A/Vs. The developed system was successfully amplified the DNA sample and benchmark with Illumina, Eco PCR.

INTRODUCTION

Polymerase chain reaction (PCR) has become an important tool for replication of DNA in biomedical research since 1985. PCR is a molecular method for the synthesis of specific deoxyribonucleic acid (DNA) fragments [R. Saiki et al., 1985] [K. Mullis et al., 1987]. It is performed by a thermal cycler in the presence of specific oligonucleotides (primers), four deoxynucleotides (dNTPs) and buffer solution [T. Powledge, 2004]. Each cycle doubles the amount of DNA, through the steps of denaturation at high temperature (95°C), annealing at low temperature (50°C) and extension at medium temperature (72°C), typically giving a billion copies after 30 – 40 cycles [T. Powledge, 2004]. For an effective PCR, the temperature of the vessel (PCR tube) with DNA mixture must be changed quickly, repeatedly and accurately between the three stages, which are characterized by high ramping temperature and short temperature holds [Q. H. Wang et al., 2003].

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 aluminium 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 proportional gain on the thermal cycler performance. The PI 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].

MATERIALS AND METHOD

Development of Thermal Cycler

Fig. 1 shows the block diagram of the thermal cycler system. The system was categorized into four subsystems which were the heating chamber, control, power management and personal computer. In heating chamber subsystem, DNA mixture was placed in the PCR block to perform DNA amplification. The PCR block was 6 samples well block and tight contact with standardized 0.1 µL PCR tubes. It was built by aluminium alloy (6082) material. Aluminium alloy was selected because it has high thermal conductivity and low heat capacity. Most of the PCR block in commercializing thermal cycler is using aluminium material. The PCR block was heated and cooled using a thermal electric module (APHC-12708-S). The thermal electric module acts as a heat pump and it can add or remove thermal capacity stored in the PCR block which results in increasing and decreasing of block temperature. The amount of heat transfer was determined by the temperature difference between the hot side and cool side of the thermal electric module and the current supply to the thermal electric module. The PCR block was placed on the cool side of the thermal electric module while a liquid cooler (Seidon 120V

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Plus) was placed on the hot side of the thermal electric module. A proportional-integral (PI) controller (PTC10K-CH) was used to control the thermal electric module and set the temperate of PCR block. It used an NTC thermistor (TCS651) as a temperature feedback system to measure and maintain the temperature of PCR block. The PI controller was controlled by a data acquisition (DAQ) device (NI USB-6009) with a sampling frequency equal to 1000 Hz and 16-bit resolution. A GUI was developed using LabVIEW to monitor the PCR block temperature. GUI allows the end user to define PCR protocol and record the data for further processing. Table 1 shows the PCR protocol which was used to amplify the DNA samples. The temperature controller controlled the current supply to the thermal electric module and monitor the temperature of PCR block based on the PCR protocol in the GUI.

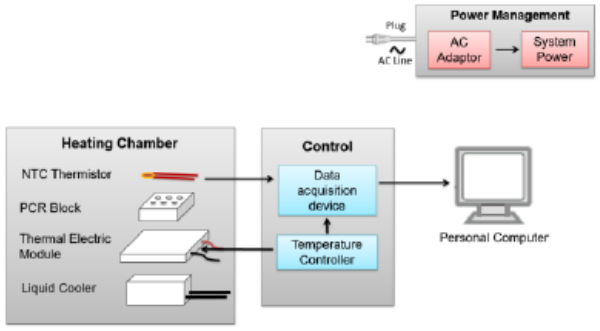

Fig. 1 Block diagram of developed thermal cycler system.

Table 1 PCR protocol for DNA amplification.

Component	Temperature (°C)	Dwell Time (s)
Pre-heat	95	910
Denaturation	94	70
Annealing	50	70
Extension	72	70
Final Extension	72	600

PI temperature controller

Fig. 2 shows the block diagram of PI temperature controller, where Ti (t) = input temperature, Vi (t) = input voltage, Vt (t) = thermistor voltage, e (t) = Vi (t) – Vt (t), I (t) = output current, QH (t) = thermal power and To (t) = output temperature. The Steinhart-hart equation converted the Ti into Vi as an input to PI controller. The PI controller generated I and supplied to the thermal electric module (Peltier). The Peltier output QH based on the input I supply to increase or decrease the PCR block temperature. The temperature of PCR block was defined as To. The To was monitored by NTC thermistor. The thermistor converted the To into Vt using Steinhart-hart equation and feedback to the PI control.

In this work, three different temperatures steps (50°C to 94°C, 50°C to 72°C and 72°C to 94°C) were used to study the performance of PI temperature controller. The temperature step was the PCR block's temperature changed from initial temperature state to final temperature state. The time taken to reach the defined temperature input and the overshoot were recorded. The integrator gain of the PI control fixed at 1.8 A/Vs while the proportional gain of PI control was changed from 0, 5, 10, … until to 40 A/V for each temperature step.

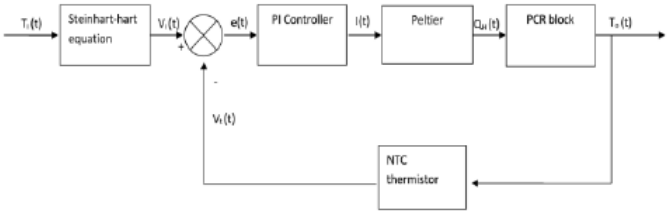

Fig. 2 Block diagram of PI temperature controller.

RESULTS AND DISCUSSION

A portable thermal cycler and GUI was successfully developed as shown in Fig. 3. The thermal cycler casing was built by using Perspex. The top part of the thermal cycler system was the DNA heating chamber. The heating chamber casing was built by using Teflon plastic. The melting point of Teflon plastic is 326.8°C. It can withstand the high temperature of PCR block (95°C) and heating lid (120°C). The interfacing between developed thermal cycler system and GUI was using universal serial bus (USB). 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 dwell time as shown in Table 1. The process of PCR started after completed the thermal cycler settings. The GUI monitored the PCR block temperature during PCR process. All the data was saved as text file in the personal computer for further analysis.

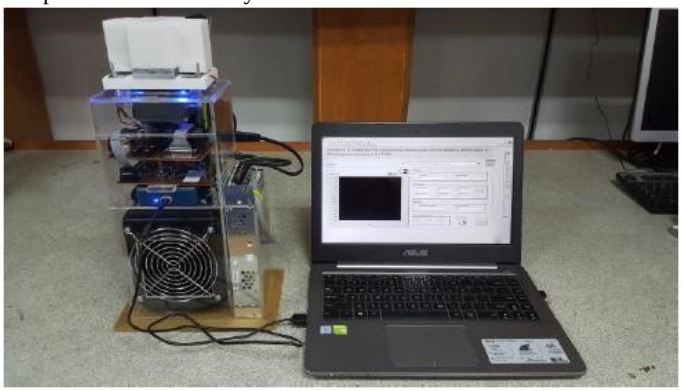

Fig. 3 The developed thermal cycler system and GUI.

Three differences temperature step were used to study the temperature ramp rate characteristic of the developed thermal cycler which were 50°C to 94°C, 50°C to 72°C and 72°C to 94°C with period equal to 100 s. Each temperature input step was tested in the thermal cycler under different proportional gain, Kp value, and a fixed integral gain, Ki value. Kp value was used to multiply e(t) and inverse of Ki value was used to multiply the integral of e(t) to obtain the To. The increase of PCR block temperature corresponding to the temperature input step was recorded and analysed by using MathWorks (MATLAB) software. Table 2 and 3 show the rise time and overshoot under different Kp value for each temperature input step. Rise Time is the amount of time the response signal takes to go from 10% to 90% of the steady-state value of temperature input step. Overshoot is the amount that the process variable overshoots the final value of temperature input step.

From the table 2 and 3, as the Kp value increase, the rise time and the overshoot of the temperature response decrease from Kp = 0 A/V to Kp = 10 A/V. The rise time and the overshoot value slightly unchanged from Kp = 15 A/V to 40 A/V. Fig. 4 shows the temperature response of the thermal cycler corresponding to different Kp value. The low Kp value produces large overshoot and causes the PCR block temperature oscillated at the final temperature step. The high Kp will decrease the rise time and took a longer time to reach the steady state temperature step. The decrease of rising time causes an increase the temperature ramp rate. The rise time was slightly unchanged after Kp = 15 A/V because the rise time was reached the time constant of the system as the Ki was fixed in this study. The temperature ramp rate was equal to 5.5 °C/s at the temperature step 50°C to 94°C when Kp = 15 A/V.

Six DNA mixtures were inserted into the developed thermal cycler to perform DNA amplification. The Kp = 15 A/V and Ki = 1.8 A/Vs were selected because it has low overshoot value and short rise time. Fig. 5 shows the evaluation of six amplified DNA samples using gel electrophoresis. The amplification result was compared to a

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commercial PCR instrument (Illumina, Eco PCR) and labelled it as HRM in the Fig. 5. The six DNA mixtures were successfully amplified as the six DNA mixtures show the six bright bands which were equal to the HRM result.

Table 2 Temperature raise time for each temperature input set under different proportional gain value.

Proportional gain, K_p (A/V)	Time taken from 50°C to 94°C (s)	Time taken from 50°C to 72°C	Time taken from 72°C to 94°C (s)
0	8.33	3.60	4.90
5	8.48	3.74	4.96
10	7.94	3.55	4.78
15	7.95	3.58	4.95
20	7.96	3.56	4.82
25	7.94	3.55	4.83
30	7.94	3.55	4.81
35	7.95	3.54	4.80
40	7.91	3.56	4.77

Table 3 Temperature overshoot for each temperature input set under different proportional gain value.

Proportional gain, K_p (A/V)	Peak temperature from 50°C to 94°C (°C)	Peak temperature from 50°C to 72°C (°C)	Peak temperature from 72°C to 94°C (°C)
0	112.05	80.89	111.59
5	105.83	75.87	107.78
10	98.75	74.00	100.26
15	94.81	73.64	95.14
20	94.79	73.70	94.81
25	94.82	73.88	94.81
30	94.75	74.01	94.78
35	94.76	73.98	94.76
40	94.73	74.07	94.71

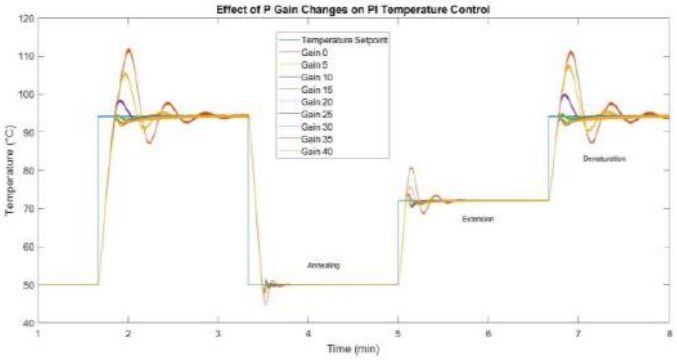

Fig. 4 The temperature response for each temperature input set under different proportional gain value

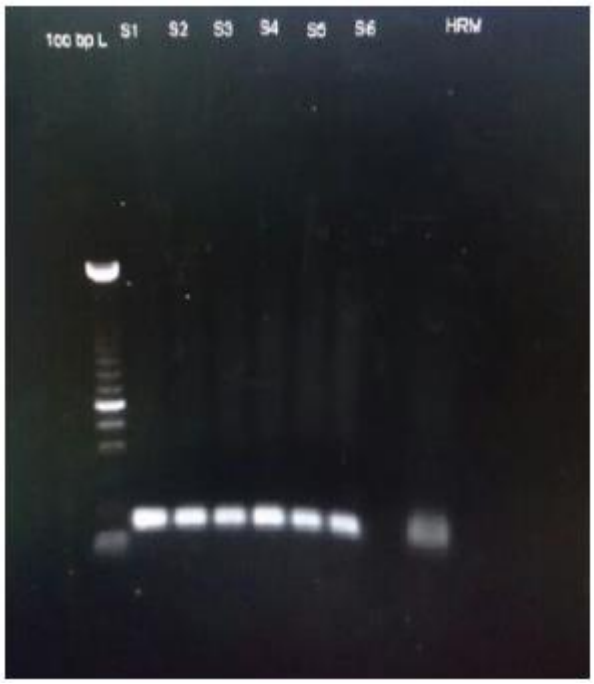

Fig. 5 The gel electrophoresis result shows the 6 DNA samples was successfully amplified by the developed thermal cycler with K_p = 15 A/V and K_i = 1.8 A/Vs.

CONCLUSION

A study on the effect of the proportional gain on the developed thermal cycler was successfully performed. A low 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 the temperature ramp rate of developed system was equal to 5.5 °C/s based on the temperature step input 50°C to 94°C. The developed thermal cycler was successfully amplified six DNA samples and benchmarked with Illumina, Eco PCR under Kp = 15 A/V and Ki = 1.8 A/Vs.

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