Long term temperature stability of thermal cycler developed using low profile microprocessor cooler

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Article Info

Article history:

Received Jan 22, 2022
Revised Jul 17, 2022
Accepted Aug 13, 2022

Keywords:

Bang-bang
Proportional integral derivative
Temperature stability
Thermal cycler
Thermoelectric

ABSTRACT

Developing a low-cost thermal cycler for a polymerase chain reaction (PCR) is becoming interested in the pandemic era caused by viruses. PCR is the standard gold for the diagnostic. However, in a low-income country, the availability of the device is limited. In this work, the development of a thermal cycler uses electronic modules available in the market. The central part is thermoelectric for heating and cooling, an embedded system to control, and a low-profile cooling fan. The system temperature control used a combination of feedforward, bang-bang, and proportional-integral-derivative (PID) control. The control parameter of the PID was successfully obtained by using Chien servo tuning. The feedforward and bang-bang control are used to optimize the cooling cycle and minimize the rise time. The system shows a well-suited temperature accuracy at the denaturation, annealing, and extension temperature with a temperature deviation of less than 0.5 °C. System performance is maintained even though the system has been running non-stop for 24 hours. The low-profile cooling fan, which is usually used for CPU cooling, shows good results in maintaining temperature stability.

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1. INTRODUCTION

In the Covid-19 pandemic, many low-income countries lack the polymerase chain reaction (PCR) machine or thermal cycler to detect the virus. There is a wide range of PCR machines or thermal cyclers available in the market [1]–[3], portable PCR with battery-operated [4]; even a mini thermal cycler is also available [5], [6]. Thermal cycler becomes an attractive tool during the Covid-19 pandemic and in the study of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The mini thermal cycler, which is considered low cost, limits the cycling speed due to using hardware with a passive cooling mechanism for the cooling process. Although some work is reported on the development of the thermal cycler [7]–[10], the simplicity of the design using a limited budget may open a wider possibility for the development and usage of the thermal cycler.

In the market, thermal cyclers are widely available. However, it is still interesting to explore and develop a simple, low-cost system that can be developed with a limited budget but with good performance. The heater, cooler, and control system are essential aspects of optimizing for a rapid temperature gradient and good temperature stability. From the conceptual design, the temperature of the DNA sample to be amplified in the PCR tube needs to be put at a specific temperature to enable DNA denaturation, primer annealing, and primer extension condition [11]. At the first cycle, the temperature is raised from the initial state to denaturation temperature, followed by a temperature change at annealing and extension. The temperature is maintained for a certain period (dwelling time) in each step. The denaturation, annealing, and extension cycle is repeated up to 40 cycles. After the extension, the temperature is pulled down to room temperature or low temperature (close to 0 °C) for further process. The denaturation temperature is around 92 °C, annealing at around 55 °C, and primer extension at 72 °C.

Temperature control in the heating and cooling process at this time can be done in several ways. Thermoelectric is the most widely used method because it conveniently controls the heating and cooling process temperature using a single system [12]–[15]. The direction of the current flow to the thermoelectric changes the thermoelectric element as a heater or cooler. Those make the electrical and mechanical design easier.

In general, the heating process does not cause many problems. However, the heat removal system requires attention to achieve a faster cooling process. A cooling system such as an aluminum block and a cooling fan with fins is commonly used. The cooling system selection is based on the given constraints. The selection of the system cooling system with an aluminum fin and fan has been used widely for a microprocessor cooler in a personal computer. The reliability and durability of such a cooler are proven [16], [17].

Operating a thermal cylinder during heating and cooling requires energy channeled to the thermoelectric and other electronic systems. Heat removal to the surrounding area is required to avoid the increasing temperature of the system. If the heat dissipation system to the environment does not work well, then continuous use for a long time will cause problems in the performance of the thermal cycler system.

The proportional integral derivative controller (PID) has been widely used because of its simplicity and effectiveness [18]. The PID controller is commonly used to control temperature, flow, level, pressure, and speed [15], [19]–[24]. Furthermore, the determination of the three parameters Kp, Ki, and Kd has been well developed, hence, it is easy to be implemented and tuned to obtain the best parameter related to the controlled plant. Some common methods are Ziegler–Nichols, Cohen-Conn, Relay method, and Chien-Hrones-Reswick method [25]–[28], PID and bang-bang control together have been used and show a better response compared to the PID only [29]–[31]. Feedforward and PID also show a better result than PID only [32].

This paper combines a control method using PID with feedforward and bang-bang control to increase the system's response speed. The control system is implemented in the Raspberry Pi. The use of the low-profile central processing units (CPU) cooler makes the system compact and light. The Chien servo method is the tuning method to determine the PID parameters used. This method was chosen as the target temperature conditions in the thermal cycler system change according to the cycle. With the design of the casing and the placement of the sub-components of the thermal cycler, it is expected that heat dissipation will not affect the performance of the thermal cycler system. The developed system was tested to work 24 hours to see the operating performance.

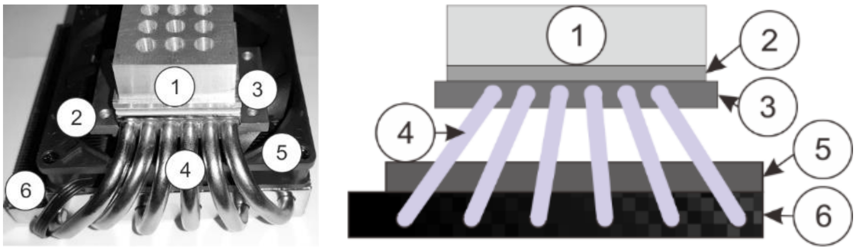
2. METHOD

The thermal cycler system and its control system can be developed from scratch. It requires a detailed circuit design and time for the electronic design and fabrication process. We develop the system by utilizing the available electronic system modules in this work. So, the development concept is a modular concept from available modules in the market. There is no need to design a low-level electronic circuit. The functional block of the system is realized with the available electronic module. For the cooling system, a low-profile cooling fan (cooler), commonly used for cooling a CPU, is used for compactness and durability.

Using available electronic modules in the market, we reduce the design step on the electronic circuit and lower production time and the entry barrier for developing the system. The selection also minimizes the skill for fine soldering or the use of any soldering station. The user with experience in microelectronics and control may improve the system's performance by using a more complex control algorithm and minimizing the electronic system by changing the electronic board into a single board containing the microcontroller, H-bridge, and metal oxide semiconductor field effect transistor (MOSFET) circuit.

The thermal cycler consists of a heating and cooling system implemented using a thermoelectric and electronic control system to perform the temperature cycle. The heating-cooling system consists of an aluminum block for placing the PCR tube, thermoelectric, and cooler. The cooler consists of a cooper block, heat pipe, fan, and aluminum fin. The heating-cooling system configuration is presented in Figure 1. Figure 1(a) shows the photograph of the heating cooling system and the figurative block diagram in Figure 1(b). The PCR tube (0.1 mL) is placed on the aluminum block. Under the aluminum block is thermoelectric and placed on top of the copper base of the cooler.

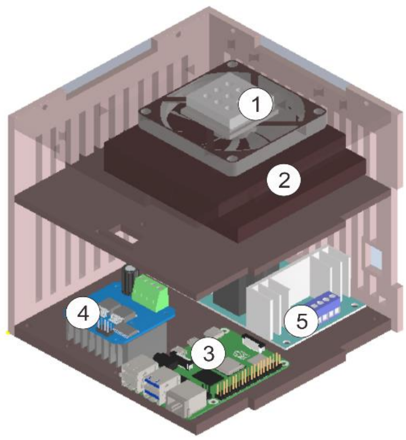
We developed a mini compact thermal cycler using an available electronic module that could be quickly developed in the laboratory without any complex electronic circuit design detail. We developed the system using the widely available electronic module to perform the required function. The system consists of an embedded system (Raspberry Pi) as the core of the control system, switching power supply (12 V, 10 A), buck converter steps down module for 5 V supply, H-bridge to control the thermoelectric cooling and heating direction, N-type MOSFET board for fan controller, thermoelectric, negative temperature coefficient (NTC) sensor, low profile cooling fan (cooler), analog digital converter (ADC) module, and voltage level shifter. The system's body was developed using acrylonitrile butadiene styrene (ABS) material made using a 3D printer, 8 mm acrylic parts cut using laser cutting, and the thermal block was made from aluminum 6061. The 3D design of the system is presented in Figure 2. The block diagram of the system is presented in Figure 3.



(a) (b)

Where:
(1) Aluminum block
(2) Thermoelectric
(3) Cooler cooper based
(4) Heat-pipe
(5) Fan
(6) Aluminum fin of the cooler

Figure 1. Heating-cooling system configuration (a) photo and (b) the block diagram



Where:
(1) Aluminum block for tube,
(2) The low-profile CPU cooler
(3) Raspberry Pi
(4) H-bridge
(5) Power supply

Figure 2. 3D design of the thermal cycler system

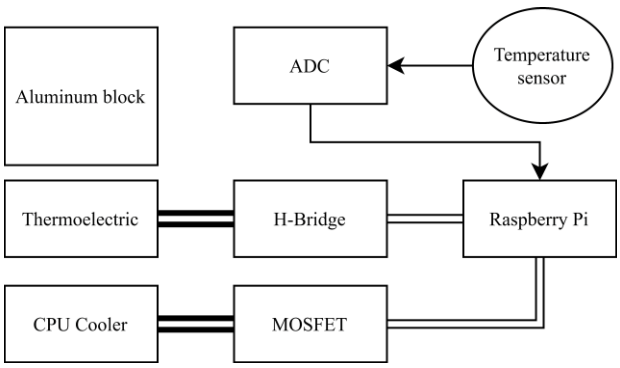


Figure 3. System block diagram

Changes in the target temperature and changes from the initial conditions to the target temperature in the temperature change cycle in the thermal cycler require attention in the design of the control system. Rapid temperature changes, small overshot, and demands for stability at high target temperatures require implementing a good control system. For this purpose, the control system design is implemented using a PID system combined with on-off control bang-bang (BB) and feedforward, as shown in Figure . The feedforward is used to minimize disturbance. The bang-bang control is intended to speed up the cooling and heating at the beginning of the target temperature change. It is a priory knowledge that the target temperature changes abruptly. With the ease of implementation in the device, each control system has a role in optimizing changes-temperature and temperature stability at the desired state. The PID control parameter values, namely *K*p, *K*i, and *K*d, are determined according to the specified temperature range using the tuning method.

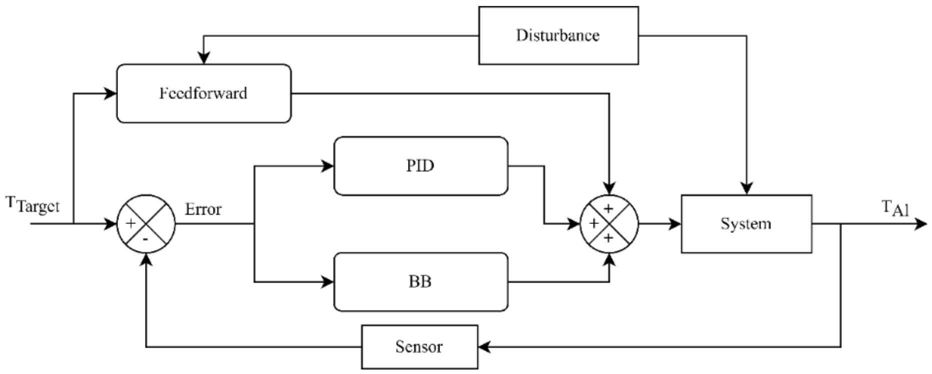


Figure 4. Control system block diagram

With the control parameters obtained from the tuning process, the temperature control process follows the flow chart in Figure 5. The target temperature (T_{Target}) is set as required in each part of the temperature cycle. The aluminum block temperature (T_{Al}) is compared to T_{Target}. If the difference exceeds the threshold, heating and cooling are carried out with bang-bang control through a 100% PWM duty cycle. If the temperature difference is smaller than the threshold, the control uses PID with the appropriate parameters. The PID control works until the end of the dwelling time in each cycle step. This process is executed for each part of the target temperature cycle.

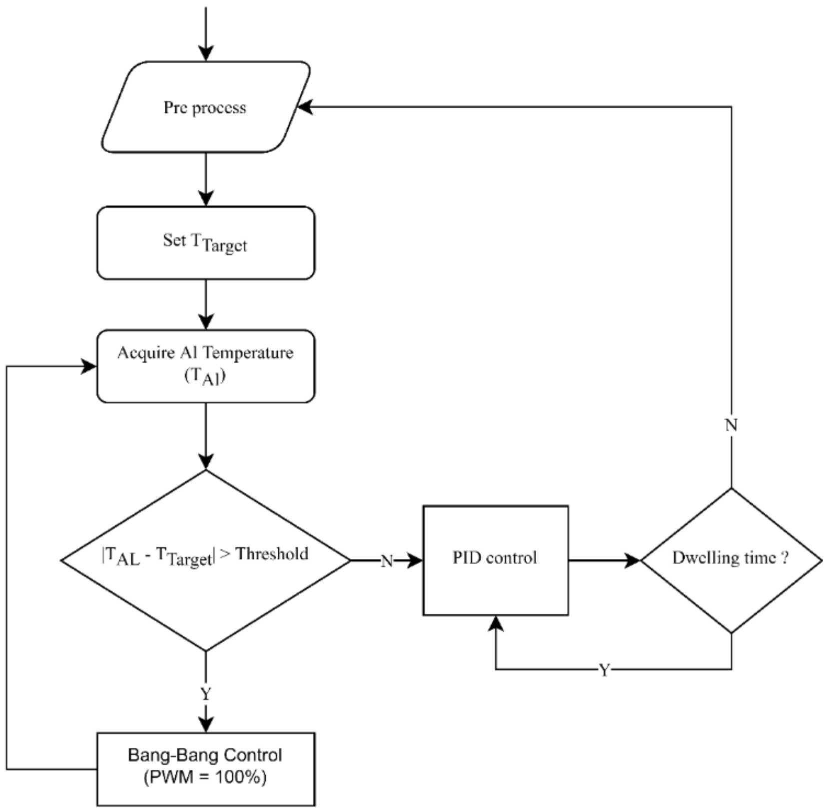


Figure 5. Control system flowchart

3. RESULT AND DISCUSSION

In this study, thermoelectric temperature control is carried out through a pulse-width modulation (PWM) pulse duty cycle to flow current to the thermoelectric through the H-bridge. PWM working frequency is 25 KHz. On average, the longer the duty cycle, the greater the current flowing into the thermoelectric for heating or cooling. At 100% duty cycle, thermoelectric works with maximum power.

In the control design, as shown in Figure , the feedforward control works at the beginning of the process and during the cooling process, namely, to control the thermal temperature of the aluminum block from room temperature to the denaturation temperature (round 94 °C). The use of feedforward is intended to reach the target temperature quickly. After the system is in the temperature cycle, the bang-bang control (BB) and PID work in the denaturation-annealing-extension control system. These methods are effective methods primarily to design a mono variable control. However, during the tuning process for the cooling stage from denaturation to annealing temperature, the PID control showed an error of more than 0.5 °C. By introducing the BB and feedforward, the response faster reaches the target temperature and has a less steady-state error.

The feedforward control value is determined by making a relationship between the duty cycle and the temperature achieved. Based on the test results, the magnitude of the $T_{disturbance}$ value of the system is 34.207. The duty cycle can be calculated using (1).

$$\%\ duty\ cycle = \frac{T_{steady} - T_{disturbance}}{1.7254} \tag{1}$$

The BB control works at the beginning when the target temperature changes until the temperature is less than the threshold. The thermoelectric is controlled with a 100% duty cycle to heat or cool the aluminum block when the BB is working. Compared to a pure PID control which is not working at full 100% duty cycle. Heating occurs when the temperature changes from annealing to extension and extension to denaturation. The cooling process is carried out when changing from denaturation to annealing temperature.

When the thermal block temperature approaches the target temperature, i.e., the temperature difference is less than the threshold, the control is transferred to the PID control. The PID control parameters are determined based on the desired target temperature conditions. For this purpose, parameters were determined using Chien-servo tuning with bump tests for various low-duty cycles. The temperature change of the aluminum block at a given duty cycle is presented in Figure 6.

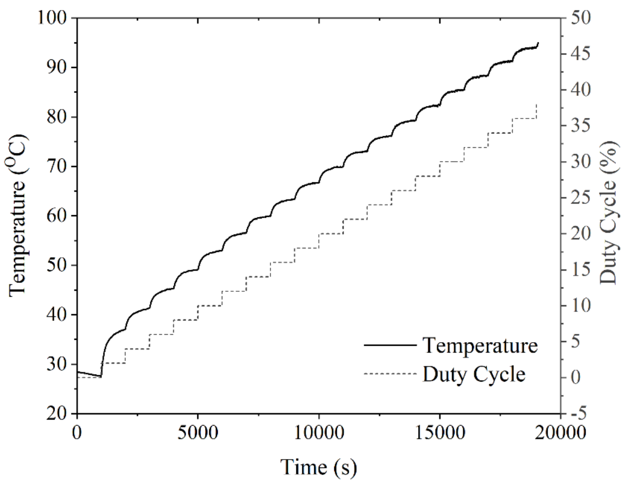


Figure 6. Temperature change at bump test to determine the PID parameters

The determination of PID control parameters is based on the equation in Table 1. The PID parameters for each temperature condition are obtained in Table 2 using the bump-test as presented in Figure 6. The proportional, derivative, and integral control parameter values are slightly different at different temperature setpoints. These different PID parameter values are used by storing the parameters in a look-up table in the Raspberry PI implementation for control. The system control parameters are implemented in the program on the Raspberry Pi with the system algorithm, as shown in Figure 5. The threshold value in control is set at 2 °C.

Table 1. PID parameter equation

Kp	Ki	Kd
$\frac{\mathbf{0.6T}}{\mathbf{KL}}$	$\frac{Kp}{T}$	$Kp\,.0.5L$

Table 2. PID parameter for temperature target from 45°C to 94°C

Setpoint (°C)	Kp	Ki	Kd
45	33.61163	0.114325	42.01453
49	31.25138	0.119737	39.06422
53	35.95431	0.118271	44.94289
57	31.82256	0.128836	39.77819
61	33.67367	0.126119	42.09209
65	32.11367	0.131613	40.14208
69	29.36100	0.129916	36.70125
72	33.22567	0.127301	41.53208
76	32.43461	0.126698	40.54326
80	22.88167	0.141245	28.60209
83	27.98795	0.130177	34.98494
87	28.84282	0.128190	36.05353
91	26.89011	0.136498	33.61264
94	21.81368	0.131408	27.26710

The temperature cycle change of the system at the start is shown in Figure 7. In the beginning, the temperature of the aluminum block and the cooler's temperature are at the same temperature of 30 °C. The initial setting of the target denaturation temperature is set at 94 °C. Thermoelectric works with maximum power to heat the aluminum block. In this condition, the cooler heat pipe temperature remains at 30 °C. When the aluminum temperature reaches 94 °C (the first denaturation temperature), the system keeps the temperature with a temperature fluctuation of less than 0.5 °C. This result is comparable with many well-known PCR systems [33] and better than the reported proportional controller using liquid cooling [34].

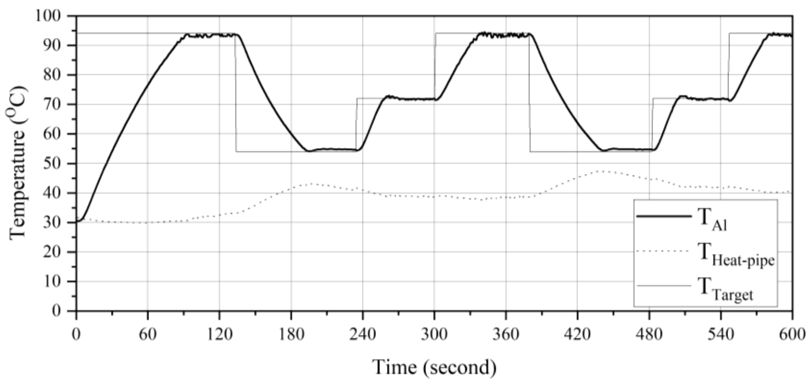


Figure 1. Detail temperature at the first two cycles

After reaching the denaturation temperature, the aluminum temperature is cooled to 54 °C (annealing temperature). On the graph, the temperature of aluminum is decreasing. Thermoelectric works to transfer heat from the aluminum and is discharged to the cooler. The discharge causes an increase in temperature in the heat pipe of the cooler. The heat pipe temperature changes up to a temperature of about 40 °C.

The aluminum block assembly process is carried out by changing the target temperature to 72 °C (extension temperature) and then to the denaturation temperature (94 °C). In this cycle, the thermoelectric heat is transferred from the copper block in the cooler to the aluminum. The thermoelectric side in contact with the copper block is at a low temperature. The cooling process of the cooler can be seen from a decrease in the temperature of the heat pipe.

In the next cycle, the thermoelectric cools the aluminum. The temperature of aluminum changes from denaturation (94 °C) to annealing (54 °C). The heat from the aluminum is discharged to the cooler. The heat pipe temperature of the cooler increased and reached a temperature close to 50 °C. This process is repeated. When the aluminum is heated, the heat pipe temperature cools down and vice versa.

Figure 8 shows the temperature cycle for aluminum, the heat pipe, and the temperature target for the aluminum temperature in the first hour of the thermal cycle. The temperature profile of T_{Al} from each cycle (denaturation-annealing–extension) in the first hour remains. The heat pipe temperature undergoes an up and down process following aluminum's heating and cooling cycle. The average heat pipe temperature increased up to the fifth cycle. In the next cycle, the heat pipe temperature fluctuations were relatively constant. An equilibrium condition has been reached in heat dissipation by the coolant to the environment. The ramp rates for heating reach 0.7 °C/second, and cooling is 0.5 °C/second, which is slower than the commercial PCR [1], [3], [35].

In an entire 24-hour operation, as shown in Figure 9, it can be seen that there is no change in the temperature cycle in the aluminum or heat pipe. Figure 10 shows the temperature conditions 24 hours after the thermal cycler runs. There is no change in the temperature profile or the aluminum or heat pipe temperature value as shown in Figure 8. It shows that the thermal cycler control system's design and the heat dissipation system to the environment run well.

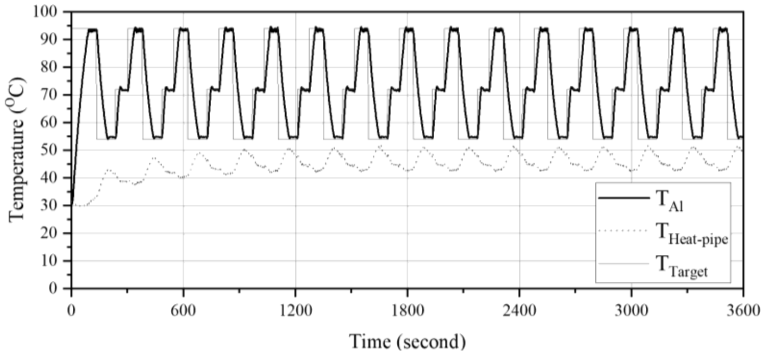


Figure 8. Thermal cycler temperature in the first hour

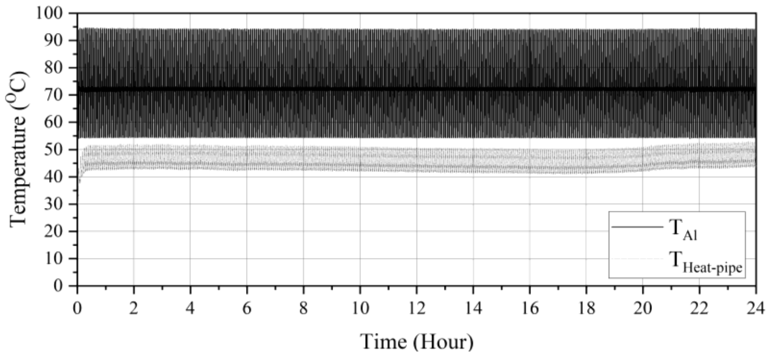


Figure 9. Thermal cycler temperature along 24 hours continuous operation

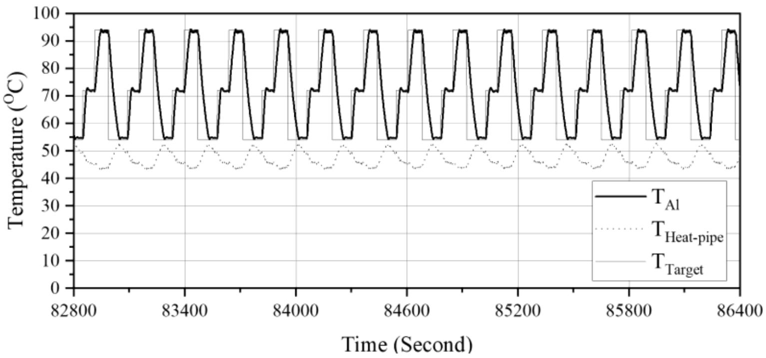


Figure 10. Thermal cycler temperature after 24 hours of continuous operation

4. CONCLUSION

Electronic modules available in the market to build a thermocycler provide convenience and speed to realize the system. A good temperature profile and temperature stability are obtained using a combined control model involving feedforward, bang-bang, and PID. PID control parameters optimized for specific temperature targets can efficiently be run using the Raspberry Pi embedded system by placing a list of control parameters in a look-up table. The target temperature, denaturation, annealing, and extension can be achieved quickly and precisely. The use of coolers by utilizing the low-profile cooling fan shows good cooling quality. The cooling system's temperature does not change significantly when the thermal cylinder is run for a full 24 hours. At each temperature of denaturation, annealing, and extension, the temperature difference between the aluminum block and the target temperature is less than 0.5 °C.

ACKNOWLEDGEMENTS

This work is supported by the Ministry of Research and Technology and the National Research and Innovation Agency (BRIN) of the Republic of Indonesia under PDUPT Research Grant.

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