WAVELENGTH ELECTRONICS

Polymerase Chain Reaction Thermal Cycler with Proportional-Integral Temperature Controller

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ABSTRACT

Researchers have developed a polymerase chain reaction (PCR) thermal cycler using a proportional-integral (PI) temperature controller. The precise temperature controller is needed for stable temperature control and rapid temperature changes in the PCR process. The portable and low-cost thermal cycler allows better opportunities for low-resource areas compared to the bulky and expensive commercial devices available. The developed system produces a temperature ramp rate of 5.5ºC/s at a proportional gain value of 15 A/V and an integral gain value of 1.8 A/V. Using gel electrophoresis to analyze the amplified DNA, the samples were validated at the expected molecular weight of 150 base pair. The temperature controller, as well as other commercial off-the-shelf products, reduce prototyping costs and maintain accurate and precise reactions.

PCR BACKGROUND

Polymerase Chain Reaction (PCR) enables amplification of a specific deoxyribonucleic acid (DNA) region between two regions of known DNA sequence. Through precise heating and cooling steps, DNA can be replicated in an exponential manner. PCR can be used for genotyping, cloning, mutation detection, sequencing, microarrays, forensics, and paternity testing.

There are three stages to the PCR process: *Denaturing*, *Annealing*, and *Extending* (**Figure 1**). Before the cycle begins, the PCR reaction solution needs to contain: the DNA template (original DNA to replicate), DNA polymerase enzyme to pull molecules together for new strands of DNA, primers to initiate PCR reaction and bind to a side of the single-stranded DNA, deoxynucleoside triphosphates (dNTPs) - the building blocks of the new replicated DNA, and the buffer to ensure the correct conditions for the reaction.

In the first stage of PCR, the sample is heated to 94ºC - 98ºC. This denatures or destroys the characteristic properties of the DNA and splits it into two single-stranded DNA molecules. Each single strand of DNA will eventually create two double-stranded DNA replicates. The second stage decreases the temperature to 50ºC - 65ºC. In this stage, the primers anneal, or bind, to specific sequences of the DNA at each end of the target sequence of the DNA template. This step prepares and starts the extension of the DNA. The third stage increases the temperature to 72ºC to complete the cycle. The DNA polymerase extends the primers and copies the DNA by adding dNTPs to create a new strand. The cycle is finished when the quantity of DNA is doubled. These steps can be repeated for many cycles to create the exponential amplification of the template DNA.

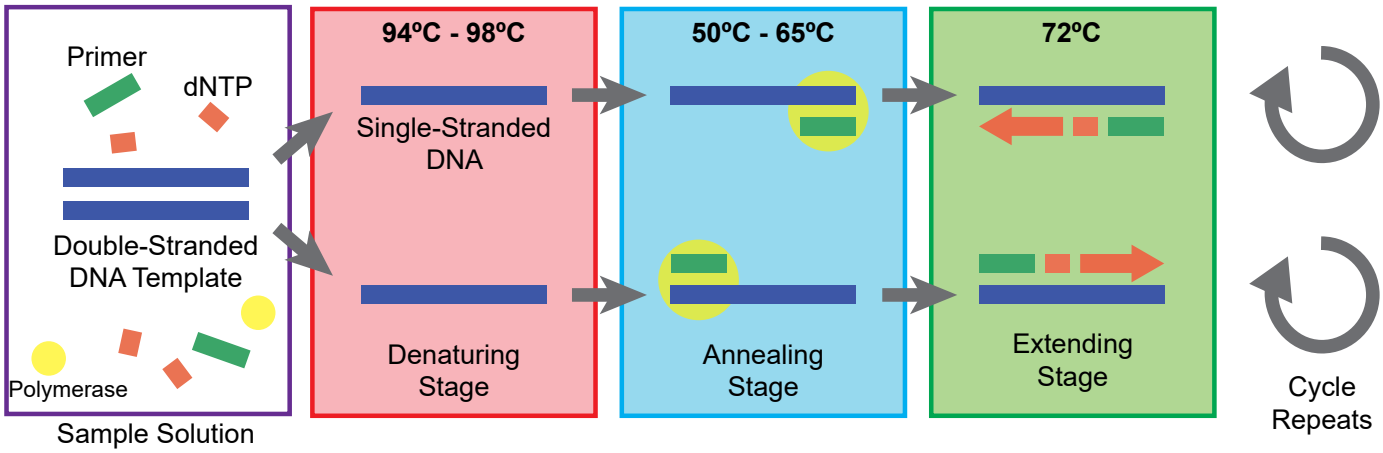

Figure 1. PCR Cycle: A solution of the template DNA, primer, and dNTPs are heated and cooled to control DNA replication. Primers initiate PCR reaction and bind to a side of the single DNA to copy. The dNTPs make up the construction of the new strand of DNA. The cycle is repeated to exponentially replicate the template DNA.

This process is not an infinite cycle of DNA amplification. The primers and dNTPs will eventually be totally consumed, and the cycling will reach a plateau phase without any additional amplification. The PCR process can be accomplished using a thermal cycler to precisely control the environment of the PCR reaction solution. Fast and accurate temperature control is critical to effective and efficient PCR cycles.

COMMERCIAL PROBLEMS

There are commercially available thermal cyclers to meet the increased demand for point-of-care or molecular diagnostic PCR systems. Using on-site designs and products involving DNA replication avoid the long and tedious process of sending DNA samples to a centralized facility for testing. Having centralized testing reduces time delays, enables faster diagnostics, treatments, and control of infectious diseases or adverse side effects.[1] This can be extremely effective in low resource settings or rural areas and would improve general healthcare for developing countries.

The problems with the current thermal cyclers are the size and the cost. Commercial systems can cost as much as $12,500 for a single unit and can be large and not portable. This limits the ease of diagnosis for developing or rural areas. Ideally, these systems would be inexpensive and portable to act as a bedside screening device.

A crucial aspect of the thermal cycler is the temperature controller. A low cost controller with high performance can contribute to a thermal cycler solution. The keys to high performance include fast temperature ramp rate, minimal temperature overshoot, and tight temperature stability.

SOLUTION & METHOD

Researchers from the Center for Advanced Electronics and Communication Engineering (PAKET) and from Universiti Kebangsaan Malaysia have developed a portable PCR system with high temperature ramp rate and low cost using off-the-shelf commercial products to reduce expenses. Included is a proportional - integral (PI) temperature controller to control and ramp the temperature of the sample. The temperature control is evaluated, in the next section, at three temperature steps (50ºC - 94ºC, 50ºC - 72ºC, and 72º - 94ºC) to determine optimal settings for fastest temperature ramp rate and smallest temperature overshoot. It is one of the most critical aspects of the thermal cycler.

The thermal cycler consists of four sections (**Figure 2**): *heating chamber* - thermistor, PCR block, thermoelectric module, and liquid cooling; *control chamber* - data acquisition (DAQ) device and temperature controller; *power management* - alternating current (AC) adapter and system power; and the *computer* - monitor values, data entry of temperature setpoints, number of cycles, timing, and more. The experimental setup can be seen in **Figure 3**.

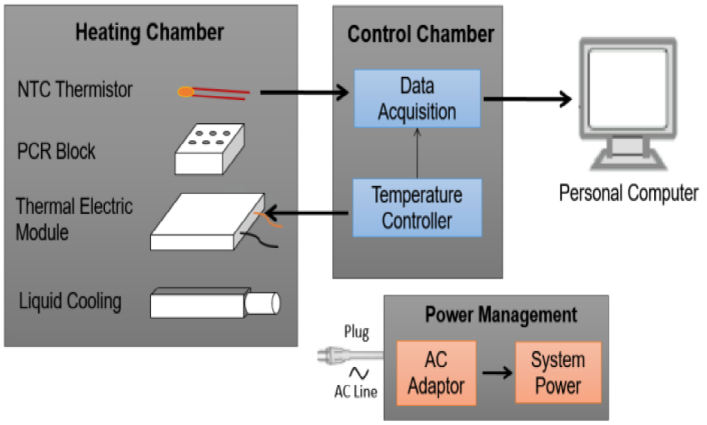

Figure 2. Block diagram of developed thermal cycler system. The system consisted of four section which are heating chamber, control chamber, power management and personal computer.[1]

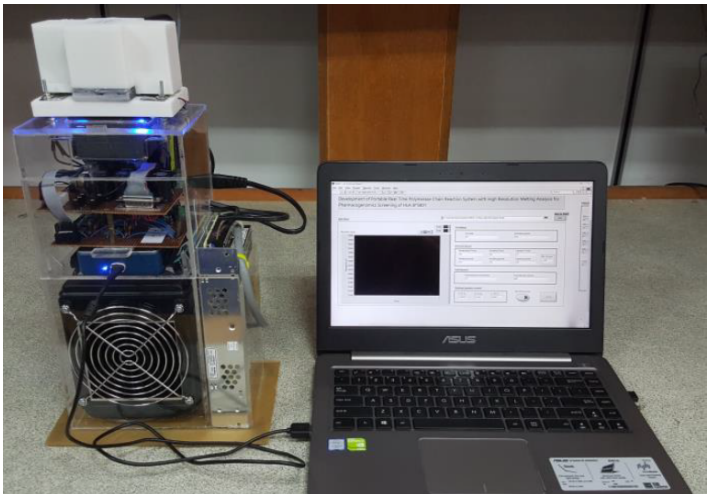

Figure 3. The developed thermal cycler and graphical user interface in the personal computer. The recorded temperature data was transferred to the personal computer via universal serial bus.[1]

When initiating the PCR cycle, the sample is pre-heated to 95ºC for 12 minutes. The solution contained both forward and reverse primers targeting a region of 150 base pair (bp) long, a mix comprising the DNA polymerase and buffer and the dNTPs, and 150 ng of the DNA template to make up 25 µL in volume. **Table 1** shows the five stages of the PCR cycle.

Table 1. PCR temperature cycling profile for five stages. Each stages has specific heating temperature and period.[1]

STAGES	TEMPERATURE (ºC)	PERIOD (MINUTES)
Pre-heat	95	12
Denaturation	94	1
Annealing	50	1
Extension	72	1
Final Extension	72	10

After the pre-heating, the sample goes through 35 cycles of denaturing to final extension to create sufficient DNA amplification. The PCR block holds six standardized 0.1 µL microcentrifuge tubes, and an aluminum alloy lid prevents condensation of the reaction solution. The thermoelectric module is placed below the PCR block to control the temperature. The temperature controller drives the current to the thermoelectric module based on the feedback from the thermistor that is place on the PCR block. The bottom side of the thermoelectric module is regulated by liquid cooling. The computer saves the data and uses the DAQ to control the temperature.

The amplified DNA is then analyzed using gel electrophoresis. This method was run at 80 V for 60 minutes with a 100dp DNA ladder for comparison. Each centrifuge tube was separately analyzed to confirm uniform amplification throughout the PCR block.

TEMPERATURE CONTROLLER EVALUATION

Wavelength Electronics' PTC10K-CH temperature controller was used to control the PCR block temperature. The PI feedback system uses the thermistor to compare the Steinhart-hart equation output, $V_i(t)$, against the negative temperature coefficient (NTC) thermistor output, $V_t(t)$. This system can be seen in **Figure 5**.

The peltier produces heat or thermal energy, $Q_H(t)$, for the PCR block temperature. The NTC thermistor measures the temperature of the PCR block, $T_O(t)$, and provides feedback to the temperature controller. The error, e(t), between the two inputs to the temperature controller, is used to change the current to the peltier to change the temperature accordingly. A stable temperature would indicate that the voltage error is zero or close to zero.

For the quickest temperature ramp rate, the PI constants can be manipulated to optimize operation. The integral gain, K_i, was fixed to a constant value of 1.8 A/Vs. The proportional gain, K_P, was changed to measure the effects on temperature ramp rate and temperature overshoot.

Gel Electrophoresis

Gel electrophoresis is a method to visualize DNA amplification results. The main goal is separation of DNA fragments in order of size, and fragments can also form "bands" or groups of same-sized DNA fragments.

For distinguishing sizes of DNA, samples are put into wells at one end of a gel substance. An electric current is applied through the gel with two electrodes on either end of the gel. Because the DNA fragments are negatively charged, they are pulled toward the positive electrode through the gel. The shorter fragments are able to move farther through the gel, separating the fragments by size.

A DNA ladder is used to determine the size of the DNA fragments. The DNA ladder contains multiple sizes to create a chart to help determine the lengths of the DNA replicated samples. **Figure 4** shows an example of the DNA ladder with DNA fragments forming bands in the gel.

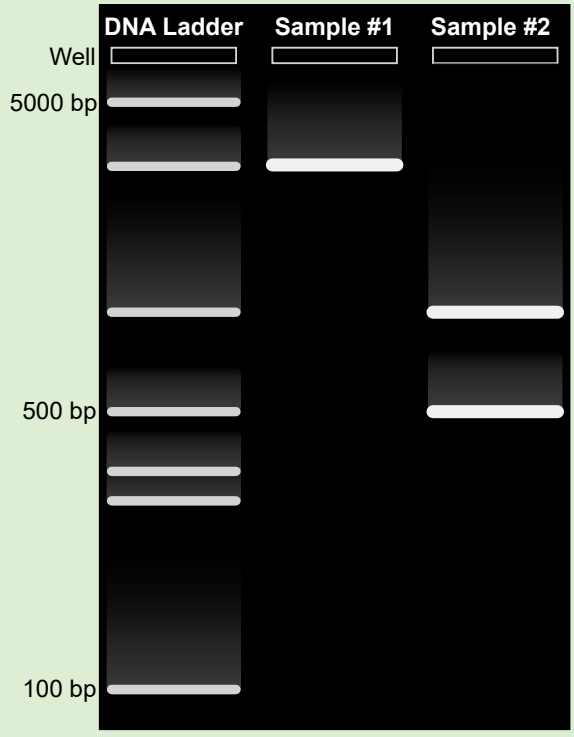

Figure 4. Gel Electrophoresis with DNA ladder and two samples.

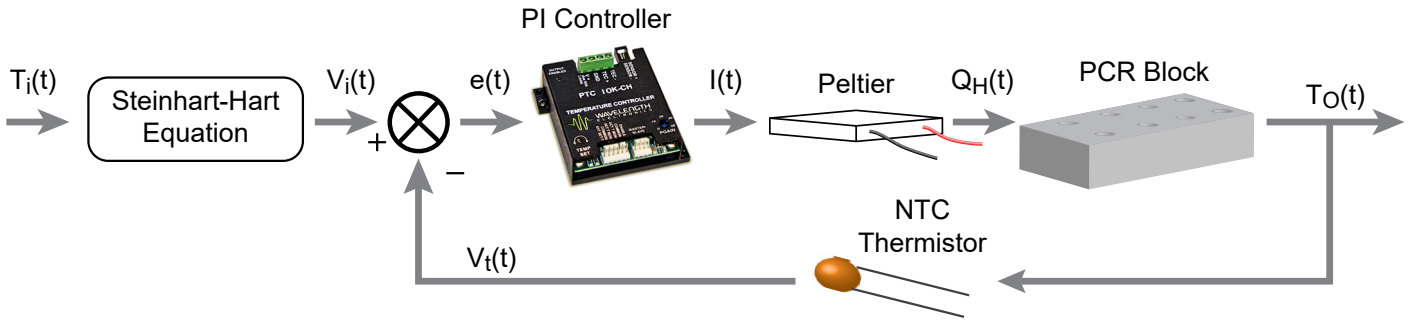

Figure 5. Block diagram of the PTC10K-CH temperature controller feedback control system.

The temperature controller was evaluated at the three different temperature steps shown in **Figure 6**. The K_P value was shifted from 0 to 40 A/V in 5 A/V steps. This affected the temperature overshoot and temperature ramp rate. The temperature ramp rate was the amount of time the response signal takes to go from 10% to 90% of steady-state value of the temperature step input or temperature setpoint. The temperature overshoot is the amplitude of the PCR block temperature that surpasses the final value of the temperature setpoint. A very high or low K_P could cause oscillations in the temperature.

When the K_P value increased from 0 to 10 A/V, the temperature ramp rate increased, improving the PCR system. The K_P value increase from 15 to 40 A/V did not further improve the temperature ramp rate.

When the K_P value increased from 0 to 10 A/V, the temperature overshoot decreased, improving the accuracy of the PCR system. Likewise, the K_P value increase from 15 to 40 A/V did not further improve the temperature overshoot.

The max temperature ramp rate was 5.5ºC/s (15 A/V from 50ºC to 94ºC), and the lowest temperature overshoot was less than 1ºC (greater than 10 A/V from 50ºC to 94ºC).

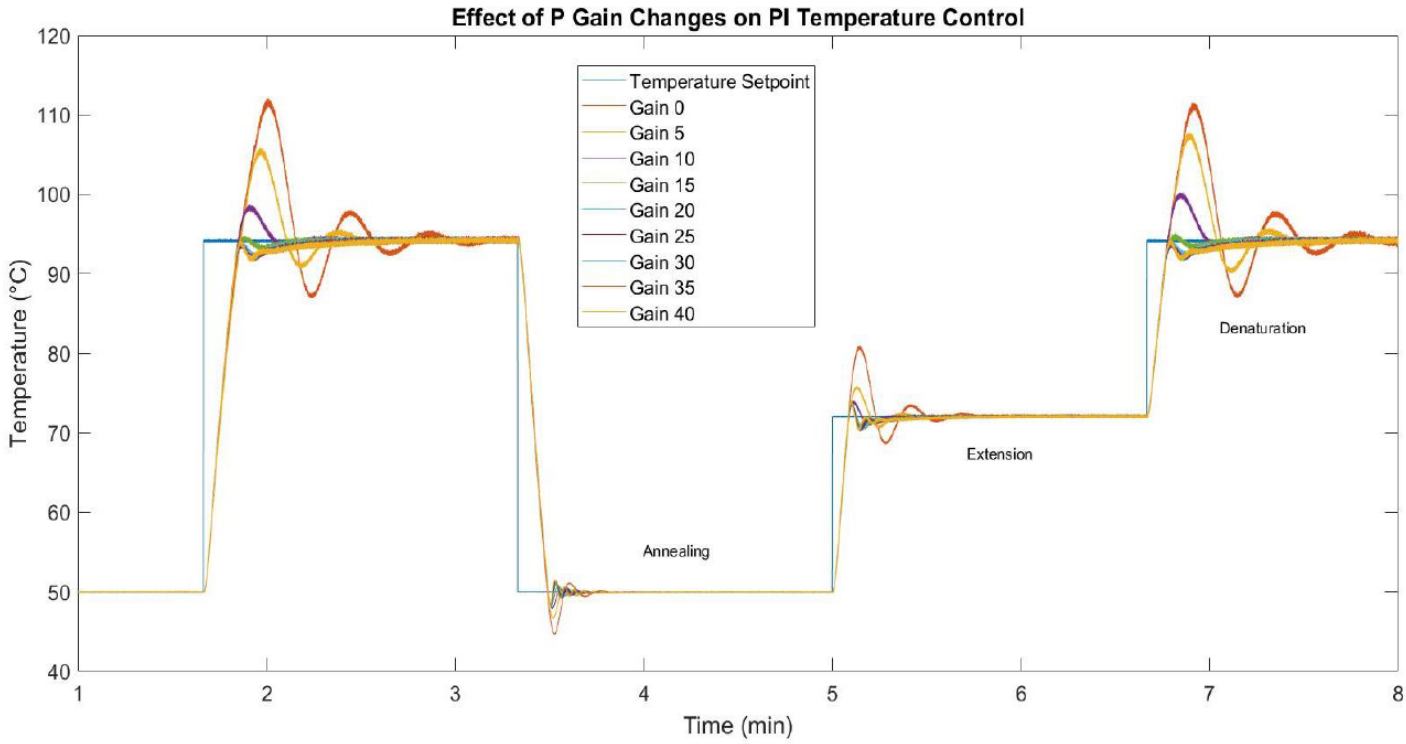

Figure 6. Comparison of the PCR block temperature step responses to the desired PCR block temperature (Temperature Setpoint). The integral gain of PI temperature controller was fixed at 1.8 A/Vs. The results show that as the proportional gain increased, the peak temperature overshoot decreased. The temperature stability of PCR block increased as the proportional gain increased.[1]

RESULTS

With the temperature controller configured for optimal results (K_P of 15 A/V and K_i of 1.8 A/V), the thermal cycler performed the DNA amplification. Six samples were compared to a commercial thermal cycler DNA amplification. Using gel electrophoresis, the results were analyzed from both PCR machines. A 100 bp DNA ladder was used as a reference and to determine the size of the DNA fragments in each sample. The commercial machine showed six bright bands at 150 bp as the commercial standard. The developed prototype also showed six, single, bright, and sharp bands at 150 bp, confirming the success of the amplification of the six DNA samples (**Figure 7**).

The developed portable thermal cycler is compared to four commercially available thermal cyclers in **Table 2**. While the dimensions, weight, sample format, and reaction volume are all comparable to the commercial devices, the cost of the developed device is the lowest among the five systems. The temperature ramp rate was also the highest compared to the commercially available devices, as a result of the optimized temperature controller. Although the dimensions of the prototype were similar to the commercial devices, it is much more portable.

Overall, the developed thermal cycler is portable, inexpensive, and successful for improving healthcare in low-resource environments by implementing modern, consumer grade electronics.

Figure 7. The gel electrophoresis results shows the six amplified DNA samples (S1, S2, S3, S4, S5, and S6) amplified by the developed prototype. This gel shows the 6 samples amplified at the correct target of 150 bp.[1]

Table 2. Performance comparison between the developed thermal cycler and four commercially available thermal cyclers.[1]

	Mini8 Plus real-time PCR system	Mic qPCR system	MyGo mini real-time PCR system	Chai real-time PCR system	Developed portable thermal cycler
DIMENSIONS (mm) LxWxH	205 x 190 x 98	150 x 150 x 130	120 x 120 x 160	280 x 240 x 190	220 x 150 x 400
WEIGHT (kg)	2.1	2.1	2.5	5	3.6
COST (USD)	6154	12500	8345	3499	2665
TEMPERATURE RAMP RATE (°C/s)	3	4 (Heating) 3 (Cooling)	3 (Heating) 1.5 (Cooling)	5	5.5
SAMPLE FORMAT	8	45	16	16	8
REACTION VOLUME (µL)	20	5 - 30	10 - 100	10 - 15	20
HEATING PLATFORM	Peltier	Magnetic Induction	Peltier	Dual Peltier	Peltier

WAVELENGTH'S ROLE

The improved temperature ramp rate of the thermal cycler can only come from a precision temperature controller. Using Wavelength Electronics' PTC10K-CH, researchers were able to develop a thermal cycler with a temperature ramp rate faster than the commercially available thermal cyclers. Overshoot was also limited to less than 1ºC using the precise temperature controller.

Because the PCR process is heavily temperature dependent, accurate and precise temperature control is needed. This enables the reactions to be fully complete and the many cycles to be precisely repeatable.

The PTC10K-CH provides up to ±10 A of current to the thermoelectric module using only a single supply of +5 V up to +30 V. The temperature stability of 0.0012ºC allows the experiment to have high repeatability and complete PCR cycle reactions.

The compact chassis mount design simplifies heatsinking and requires minimal space (3.0" x 3.2" x 1.1"). The PTC10K-CH can be found in systems in diverse applications such as particle and droplet measurement, biomolecular interaction analysis, manufacturing machine vision systems, and now DNA amplification.

REFERENCES

1. K.S. Chong, N.A. Devi, K.B. Gan, & S. Then, "Design and development of polymerase chain reaction thermal cycler using proportional-integral temperature controller," *MJFAS* (2018), 14 (2): 213-218. https://doi.org/10.11113/mjfas.v14n2.765

USEFUL LINKS

- PTC10K-CH Product Page

OPEN ACCESS

PRODUCT USED
PTC10K-CH

KEYWORDS
Polymerase chain reaction, PCR, proportional-integral temperature controller, thermal cycler, DNA, microcentrifuge tubes, temperature ramp rate

REVISION HISTORY

Document Number: CS-TC04

REVISION	DATE	NOTES
A	January 2021	Initial Release