Designing a Polymerase Chain Reaction Device Working with Radiation and Convection Heat Transfer

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Abstract. Gene proliferation is vital for infectious and genetic diseases diagnosis from a blood sample, even before birth. In addition, DNA sequencing, genetic finger-print analyzing, and genetic mutation detecting can be mentioned as other procedures requiring gene reproduction. Polymerase chain reaction, briefly known as PCR, is a convenient and effective way to accomplish this task; where the DNA containing sample faces three temperature phases alternatively. These phases are known as denaturation, annealing, and elongation/extension which in this study -regarding the type of the primers and the target DNA sequence- are set to occur at 95, 58, and 72 degrees of Celsius. In this study, a PCR device has been designed and fabricated which uses radiation and convection heat transfer at the same time to set and control the mentioned thermal sections. A 300W incandescent light bulb able to immediately turn off and on along with two 8×8 cm DC fans, controlled by a microcontroller as well as PID and PD controller codes are used to monitor the applied thermal cycles. In designing the controller codes it has been concerned that they not only control the temperature over the set-points as well as possible, but also increase the temperature variation rate between each two phases. The temperature data were plotted and DNA samples were used to assess the device function.

1. Introduction

In 2001 Celera Corporation along with scientists from China, USA, Japan, Germany, France, and Britain published a draft of human genome [1], which was a landmark in anthropology science. Their results demonstrated that 99.99% of the genes were identical among all humans and only one-in-millions differences made the vast variety of people. Thus, discovering these tiny differences required vast investigation in medical and biological fields.

PCR, or polymerase chain reaction, is a technique through which a specific sequence of DNA can multiply for millions of times during a short period. Inventing this method in 1983 brought the Nobel Prize for Kary Mullis [2] because of being cheaper and more effective than the former expensive and time consuming conventional methods. Nowadays, PCR is widely used in laboratories for diverse applications such as genes identifying, separating, and cloning as well as living beings classification and identification, genetic diseases diagnosis, even criminal cases. PCR comprises three main sections namely denaturation, annealing, and elongation/extension occurring at 90-95, 50-65, and 70-74 degrees of Celsius, respectively. During denaturation stage, the hydrogen bonds between complementary bases

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break; consequently, the double-stranded DNA turns into two separated strands. In the next step, annealing, the primers stick to the single-stranded DNA templates. The next temperature stage, called elongation or extension, is the stage in which Taq-polymerase enzyme synthesizes new complementary strands to the already present and separated strands using free nucleotides suspended inside the sample. Stationary PCR - a well-known, conventional, effective, easy, and cheap sort of the PCR devices - is now widely being used and studies have been done to improve its functionality [3-7]. In this class of PCR devices, the DNA containing sample is steady and by utilizing equipment, temperature varies among the set-points. Therefore, the sample warms up and cools down without moving. Cho et al. [8] developed a rapid real-time micro-scale chip-based PCR system consisting of six individual thermal cycling modules capable of independent control of PCR protocols. They also conducted large-scale clinical evaluation study in order to test utility of a chip-based PCR system as a molecular diagnostic device. Consolandi et al. [9] tested a silicon biochip designed for PCR analysis of complex biological samples. They recorded a cooling rate of 3.2 °C/s and a heating rate of 11 °C/s during thermal cycling. Their device amplified the 2060-bp cyanobacterial 16S rRNA gene and the 330-bp human anti-α1-chymotrypsin gene. Kim et al. [10] designed a system to measure the temperature distribution inside a silicon PCR micro-channel, where they used a Pt film heater and a Pt film sensor. Bustin [11] investigated on the possibility of reducing PCR cycling time and denaturation temperatures. His results interestingly indicated that it is feasible to obtain good PCR results with short denaturation and annealing times and at low denaturation temperatures, which drastically improves the overall time of the reaction. In this paper, a low cost, light and portable stationary PCR device has been designed and fabricated which, applies radiative and convective heat transfer to control the thermal cycles. Its functionality has been assessed by testing a 324-bp DNA sample.

2. Experimental setup
A 300w incandescent light bulb capable of immediately turning off and on along with two 8×8 cm DC fans were chosen as heating and cooling sources, respectively. A micro-controller was coded by PID and PD codes to control the sources. The micro-controller controlled the system by turning two relays off and on, one connected to the lamp and the other to the fans. A small container with the shape of a truncated square pyramid with top(larger) and base surfaces of 17×17 and 5×5 cm, respectively, was designed and fabricated to hold and fix the lamp and the sample-holder chip. The burner of the lamp was bolted to the base section of the container and the chip was located on the top section. By doing so, the shape of the container helped conducting the reflected light from the walls to the chip; thereby, increasing the radiative power transmitted to the chip. Additionally, a special polished steel plate was opted as the material of the container. This kind of steel, similar to a mirror, reflected the light very well rather than storing it as its internal energy. Devising these arrangements, we maximized the radiative power transmitted from the lamp to the chip. A 0.5 mm thick polycarbonate plate with a surface area of 17×17 was located on the top section of the container, on which, the sample container vial was fixed. Two fans were placed on a fixture which itself was placed on the top of the container. Hence, the chip was located between the light bulb and the fans, in an open interaction with them. Since a great deal of the output energy of the light bulb is transferred in convection mode and in order to prevent this energy from running out through the walls, an elastomeric insulator was wound around the container. Figure 1 shows a schematic and real view of the temperature control system.
An infra-red temperature sensor module with an accuracy of ±0.02 degree of Celsius was placed on the chip to read the temperature. In order to evaluate the function of this sensor, a K-type thermocouple with an accuracy of ±0.5 was also connected to the chip and presented the data through a BTM-4208SD both online and saved on a SD card. The thermocouple was placed close to the sensor and the data from the thermocouple and the sensor demonstrated a difference lower than 0.5 degree. The temperature data charts read from the sensor also were both plotted online and offline.

3. Controller and temperature control
As previously mentioned, in a PCR reaction, three temperature sections of denaturation, annealing, and elongation, each for 30 seconds and at temperatures of 90-95, 50-65, and 70-74 degrees of Celsius, must occur alternatively for 34 cycles. Furthermore, system was set to work for 3 minutes once in the beginning and at the denaturation temperature, and once for another 3 minutes at elongation temperature in the end. Codes of the micro-controller were written so that they could fix the temperature of the system around the set-points. This controller adjusted the temperature by controlling the on and off time of the relays, one connected to the lamp and the other to the fans. Temperature read by the sensor was given to the system as the feedback. To control the system and considering that the on and off time of each source had to change with respect to the error from the set-point, a PID and a PD controller were designed for the system. Each integral, derivative, and proportional coefficient was determined according to the system response and by trial and error.

There were two main ideas to control the system. First, a PID controller was designed that controlled denaturation and elongation stages only by turning the lamp on and off and the annealing stage by turning only the fans on and off. It was expected to achieve a good accuracy at the cost of speed. The PID controller in comparison with the PD code was more time consuming, but more accurate due to the integral proportion. Each cycle using the PID code took about 250 seconds in which, regarding that in each cycle there is only a 90-second-period needed for the stages, about 160 seconds were extra. In other words, the ramp of changing the temperature between each two stages was too slow. The temperature data are plotted in Figure 2.
In the second experiment, integral proportion was eliminated from the controller and additionally, each stage was controlled by turning both the heating and cooling sources on and off, where the total time for each cycle reduced to about 160 seconds (Figure 3).

Here, increasing the ramp of the temperature variation between two stages was the first priority. However, calculation of the standard deviation using for both tests (Table 1 and Table 2), demonstrated slight increase in the error, at the second test.
Table 1. Temperature and standard deviation data of the system controlled by PID controller.

|                         | Denaturation | Annealing | Elongation |
|-------------------------|--------------|-----------|------------|
| Set-point               | 92.00 °C     | 58.00 °C  | 72.00 °C   |
| Average temperature     | 91.86 °C     | 57.59 °C  | 71.37 °C   |
| Standard deviation      | 0.56         | 1.55      | 0.72       |

Table 2. Temperature and standard deviation data of the system controlled by PD controller.

|                         | Denaturation | Annealing | Elongation |
|-------------------------|--------------|-----------|------------|
| Set-point               | 92.20 °C     | 56.00 °C  | 72.00 °C   |
| Average temperature     | 92.20 °C     | 56.25 °C  | 72.33 °C   |
| Standard deviation      | 1.05         | 0.72      | 0.90       |

4. Tests and results
A PCR sample consists of different components, such as primers, gene samples, nucleotides, Taq polymerase enzymes, buffers, etc. For this test, a 324 kb DNA was aimed to be multiplied. The sample was prepared and injected into a 0.2 µl vial (Figure 4).

![Figure 4](image1.png)

**Figure 4.** Sample containing vial placed on an ice pack.

After each test, the sample was evaluated using a gel electrophoresis device to measure the quality and quantity of the proliferation. Figure 5 shows the result of the reproduction, when using the PID controller (column 3) and using our commercial PCR device (column 1). As can be seen, there is no bond in the range of the target DNA and the halo represents the dimer among the primers which has been formed due to the unfavorable temperature conditions.

![Figure 5](image2.png)

**Figure 5.** Gel electrophoresis results of the multiplied sample when using PID controller.

Figure 6 represents the result of the gel electrophoresis analysis on the tested sample, when system was using the PD code as its controller. Unlike the previous case, a bright belt in the length range of the target DNA was visible, which means that along with the primer dimers, DNA has been multiplied as well. Although the low brightness of this belt and the primer-primer dimers demonstrate the low quantity
and quality of the proliferation, respectively, this device has been accomplished to do the reproduction task.

![Image of gel electrophoresis results](image_url)

**Figure 6.** Gel electrophoresis results of the multiplied sample when using PD controller.

Comparing the two tests and their results, it can be concluded that the ramp of the temperature variation is much more important than its accuracy. Reducing the extra time of each cycle drastically improved the functionality of the device.

5. Conclusion

A low-cost, light, and portable PCR device was designed, fabricated, and tested which utilized radiation and convection heat transfer simultaneously, to control each stage. Two controllers were designed for the system, and the functionality of the system using each two was assessed by multiplying a 324-bp DNA and analyzing the reproduction using a gel electrophoresis device. Hereunder comes a brief conclusion:

1. The ramp of the temperature variation is more important than the accuracy of adjusting the temperature. In other words, reducing the extra time of each cycle will result in better proliferation. By reducing the cycle time from 250 to 160 seconds, we could observe the proliferation.

2. As the system uses radiation besides convection for heating the sample, the response time of the sample will be comparatively high; meaning that the difference between the vial wall and sample temperature will be low. On the other hand, as the system uses only forced convection heat transfer and the vial wall is made of plastic which is not a good heat conductor, temperature inside and outside of the vial will be considerable. To eliminate this problem, annealing and elongation time can be raised.

3. Radiation heat transfer can increase the temperature rising ramp; thereby, improving the system functionality.

4. The annealing stage could be considered as the point of design; for removing the energy from the sample is harder than giving internal energy to it.

6. References

[1] Venter, J. Craig, Mark D. Adams, Eugene W. Myers, Peter W. Li, Richard J. Mural, Granger G. Sutton, Hamilton O. Smith et al. "The sequence of the human genome." *science* 291, no. 5507 (2001): 1304-1351.

[2] Mullis, Kary, Fred Faloona, Stephen Scharf, R. K. Saiki, G. T. Horn, and H. Erlich. "Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction." In *Cold Spring Harbor symposia on quantitative biology*, vol. 51, pp. 263-273. Cold Spring Harbor Laboratory Press, 1986.

[3] Kopp, Martin U., Andrew J. De Mello, and Andreas Manz. "Chemical amplification: continuous-flow PCR on a chip." *Science* 280, no. 5366 (1998): 1046-1048.
[4] Obeid, Pierre J., Theodore K. Christopoulos, H. John Crabtree, and Christopher J. Backhouse. "Microfabricated device for DNA and RNA amplification by continuous-flow polymerase chain reaction and reverse transcription-polymerase chain reaction with cycle number selection." *Analytical chemistry* 75, no. 2 (2003): 288-295.

[5] Norian, Haig, Ryan M. Field, Ioannis Kymissis, and Kenneth L. Shepard. "An integrated CMOS quantitative-polymerase-chain-reaction lab-on-chip for point-of-care diagnostics." *Lab on a Chip* 14, no. 20 (2014): 4076-4084.

[6] Sun, Yingnan, Xiaoguang Zhou, and Yude Yu. "A novel picoliter droplet array for parallel real-time polymerase chain reaction based on double-inkjet printing." *Lab on a Chip* 14, no. 18 (2014): 3603-3610.

[7] Nanayakkara, Imaly A., Weidong Cao, and Ian M. White. "Simplifying Nucleic Acid Amplification from Whole Blood with Direct Polymerase Chain Reaction on Chitosan Microparticles." *Analytical Chemistry* 89, no. 6 (2017): 3773-3779.

[8] Cho, Yoon-Kyoung, Jintae Kim, Youngsun Lee, Young-A. Kim, Kak Namkoong, Heekyun Lim, Kwang W. Oh et al. "Clinical evaluation of micro-scale chip-based PCR system for rapid detection of hepatitis B virus." *Biosensors and Bioelectronics* 21, no. 11 (2006): 2161-2169.

[9] Consolandi, Clarissa, Marco Severgnini, Andrea Frosini, Giancarlo Caramenti, Marco De Fazio, Francesco Ferrara, Anna Zocco, Alessandra Fischetti, Michele Palmieri, and Gianluca De Bellis. "Polymerase chain reaction of 2-kb cyanobacterial gene and human anti-α 1-chymotrypsin gene from genomic DNA on the In-Check single-use microfabricated silicon chip." *Analytical biochemistry* 353, no. 2 (2006): 191-197.

[10] Kim, Soo Ho, Jermim Noh, Min Ku Jeon, Ki Woong Kim, Luke P. Lee, and Seong Ilil Woo. "Micro-Raman thermometry for measuring the temperature distribution inside the microchannel of a polymerase chain reaction chip." *Journal of Micromechanics and Microengineering* 16, no. 3 (2006): 526.

[11] Bustin, Stephen A. "How to speed up the polymerase chain reaction." *Biomolecular Detection and Quantification* 12 (2017): 10-14.