Journal of Systemics, Cybernetics and Informatics (2022) 20(3), 76-80
https://doi.org/10.54808/JSCI.20.03.76

An Interdisciplinary Design Project for Undergraduate Engineering Training - Portable Real-Time Polymerase Chain Reaction System

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ABSTRACT [1]

Our world has been permanently changed by the pandemic outbreak of COVID-19 starts around the end of 2019. In the first few months of 2020, the whole world was in urgent need of an effective, easy, and quick method for the identification of the infection of the new virus. Polymerase Chain Reaction (PCR) machine, which can test DNA samples by rapidly making millions of copies of a specific DNA sample through the PCR process, including the COVID-19 virus, can perfectly fit this demand. In this study, a design project on PCR is introduced for undergraduate education in electrical and mechanical engineering. The objective of this project is to develop a low-cost, ease-of-use, wallet-size, portable real-time PCR (RT-PCR) machine for accurate testing of various bacteria or viruses. The key function of the PT-PCR system is to precisely control and maintain the temperature of the bio-sample solution within a range between 55℃ and 95℃. This project provides students opportunities in studying and practicing a wide range of engineering technics and skills, including mechanical design, electronics design, microcomputer programming, data acquisition and processing, etc. Students can gain comprehensive understanding of the design of multiphysics system after they overcome various challenges emerging in the project. From the view of engineering education, the process of this project development has demonstrated the importance and benefits of adopting complex interdisciplinary engineering problems for student teams to solve, especially those involve contemporary issues.

Keywords: Polymerase Chain Reaction, Virus detection, Real time, Undergraduate education, Interdisciplinary engineering problems.

INTRODUCTION

Our world has been permanently changed by the pandemic outbreak of COVID-19 started around the end of 2019 from a wet market in Wuhan, China. In the first few months of 2020, the whole world was panicked with the soaring number of patients and in urgent need of an effective, easy, and quick method for the identification of the infection of the new virus. Polymerase Chain Reaction (PCR), as Nobel prize awarded (1993) revolutionary technology, can perfectly fit this demand. PCR process enables researchers to clone, purify, and identify DNA sequences. A PCR machine can test DNA samples by rapidly making millions of copies of a specific DNA sample through the PCR process, including the COVID-19 virus. PCR devices have been widely equipped in most biological laboratories which require well-trained professional personnel to operate.

PCR process is based on the original way cells replicate their DNA. During DNA replication, the two strands of each DNA molecule separate, and DNA polymerase, an enzyme, assembles nucleotides to form two new partner strands for each of the original strands. In the PCR process, three critical steps enable the copying of the DNA strands. The first step, denaturing, is when the double-stranded template DNA is heated to separate it into two single strands. In the second step, annealing, the primers anneal, or attach, to the DNA template. In the third step, Taq polymerase adds nucleotides to the annealed primer. Real-time PCR (RT-PCR) is a much faster way of conducting a PCR that has furthered the disease testing capability [1-3]. In RT-PCR the amplification of DNA is detected in real-time as PCR is in progress using fluorescent reporters [4]. RT-PCR enables high sensitivity, selectivity, and efficiency for detection which may significantly improve the testing capability and accessibility to contribute to the control of viral spread. Furthermore, with proper engineering design, the RT-PCR can be made automatic and portable to be easily operated in remote areas with minimum training required.

[1] The authors acknowledge peer-editors of the final version of the paper: Dr. Jiayue Shen and Dr. Diganta Dutta.

Based on this observation, we organized an interdisciplinary undergraduate engineering student team that worked the design and development of a low-cost portable Real-Time PCR system based on thermal conduction for point-of-care diagnostics. The team is fully engaged in the project during the year 2020 and has practiced a wide range of skills in electronics, mechanics and computer programing.

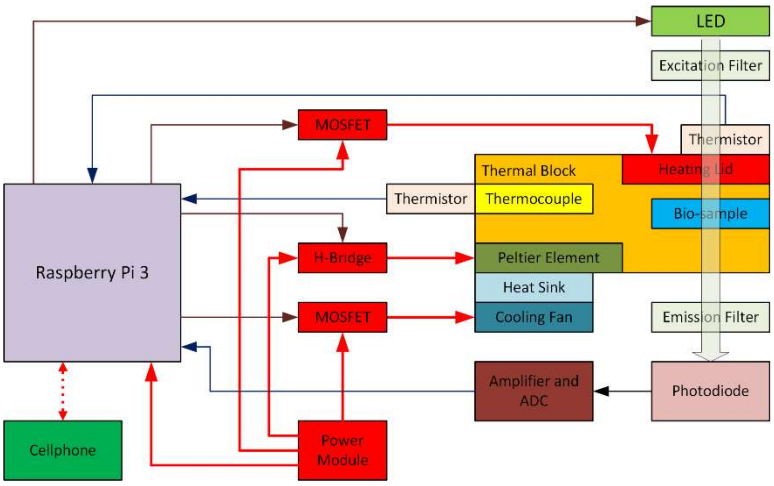


Figure 1. Portable RT-PCR System Diagram

1.1 System Overview

The diagram of the system is shown in Figure 1. The key function of the RT-PCR system is to control and maintain the biosample temperature within a wide range between 55℃ and 95℃. The central unit of the PCR system is a microcomputer Raspberry Pi 3 which monitors thermistors and controls other electric components for the repetitive PCR cycles, including a Peltier element, a heating lid, and a cooling fan. For the DNA diagnosis, the Raspberry Pi 3 controls an LED that generates light in a special wavelength to the biosample and measures fluorescent from the biosample through a photodiode for viral detection. The Raspberry Pi 3 communicates via Bluetooth to a cellphone application developed for system operation and data visualization.

1.2 Thermal Cycling Unit

The basic function of the thermal cycling unit is to provide the proper thermal environment for the continuous PCR cycles for the DNA to replicate repetitively to reach the detection threshold. The thermal block comprises a heating lid, a thermal block, a finned heat sink with a cooling fan, a Peltier (thermoelectric) element in between, and two thermistors.

The thermoelectric (TE) element is deployed for fast heat transfer to meet the dynamic temperature needs. The TE element is made of semiconductor material that works based on the Peltier phenomenon. When current is passed through, heat will transfer from one side to the other. By switching the direction of the current the fast heating and cooling can be achieved effectively. In the system, a 72W, 40mm×40mm TE module is selected for the function.

As shown in Figure 2, the thermal block is made of high thermal conductivity aluminum alloy. It can hold up to six biosample tubes to test every time. The dimension of the thermal block is chosen to be 1"×1.5"×1". Based on the calculation, the cooling time required for the Peltier element to lower the temperature from 90℃ to 45℃ is around 20 seconds which meets the system design requirements. The heat transient evaluation is also verified in SolidWorks.

The heating lid enables biosamples to be heated efficiently, minimizing the formation of nonspecific annealing and primer dimers, and prevents water condensation on the lid and evaporation of biosamples. The heat sink dissipates the extracted heat into the ambient environment. Two thermistors, one is in the heating lid and the other is attached to the thermal block through a thermocouple, provide the temperature measurements for the thermal cycling control.

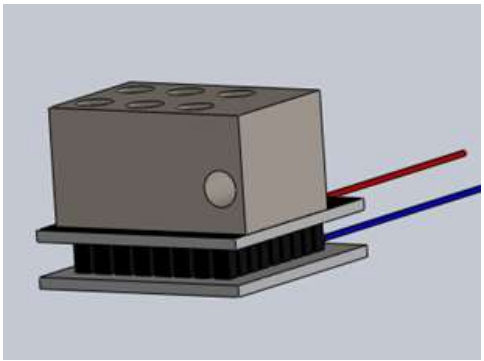


Figure 2. Thermal Block Model

1.3 Detection Unit

The fluorescent detection unit is shown in Figure 3. The fluorescent detection unit is composed of LED, excitation filter, capillary tube, emission filter, and photodiode. The LED, configured with the Raspberry Pi, emits light at a wavelength not exceeding 497 nm. An excitation filter is placed to filter out light otherwise when passing through. The light path is to be directed to the capillary tubes which contain the biosample. The light will be absorbed by the SYBR Green dye mixed into the primer solution of each sample. As the PCR processes of annealing, denaturing, and extending are implemented the dye SYBR Green [5] combines with the target DNA copies. SYBR Green absorbs blue light at 497 nm wavelength and emits fluorescent light at 520 nm wavelength as it combines with DNA copied strands. A photodiode is positioned to detect the light emitting from the sample at 520 nm wavelength that passed through the emission filter. The filters are present to control the signal to noise ratio so the system can receive accurate results. The signal received from the photodiode will be measured by the Raspberry Pi 3 to determine whether each sample is positive or negative. In building the system, we selected both filters in the dimensions of 5×5×1mm from Chroma [6].

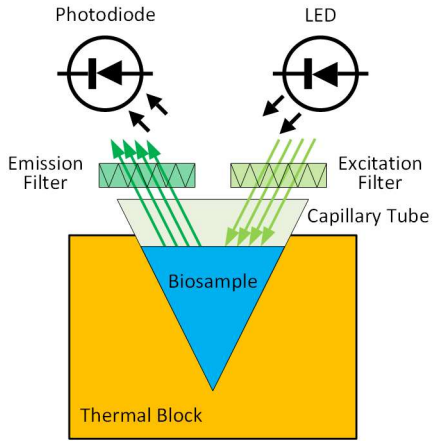


Figure 3. Fluorescent Detection Unit

1.4 PCR Control System

The RT-PCR system is centrally controlled by a microcontroller Raspberry Pi 3. It receives temperature measurements from thermistors and operates the heating lid, the TE module, and the cooling fan to regulate the temperatures required in repetitive thermal cycles. There are three phases in each thermal cycle, corresponding to the three PCR steps. First heating to temperature T1, cooling to temperature T2 and then heating to temperature T3. At the beginning of each cycle, the thermal block temperature will be heated to the desired temperature T1 and held at a defined interval. Then the Raspberry Pi 3 stops the heating lid and switches the TE module from heating to cooling with the cooling fan on until the temperature drops down to T2. Then the Raspberry Pi 3 turns off the cooling fan, switches the TE unit, turns on the heating lid again to temperature T3 and holds until the cycle completes. In our system, the three temperatures T1, T2, and T3 are set to 95℃, 55℃, and 75℃ for our temperatures.

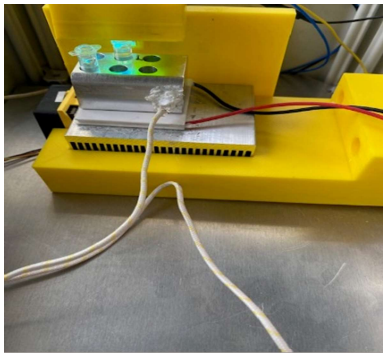
A 15V/150W power module is deployed to power all electrical components in the system. The Raspberry Pi 3 operates the heating lid and cooling fan through MOSFETs. An H-Bridge motor driver is identified and integrated into the system for the Raspberry Pi 3 to control the polarity of the Peltier element.

1.5 Cellphone Application Design

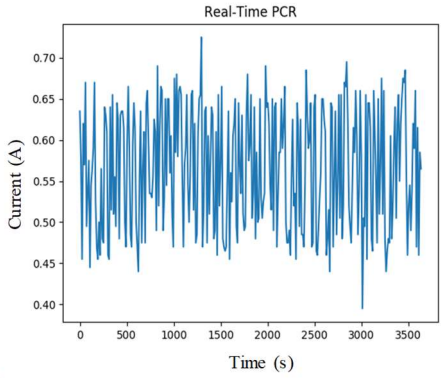
A cellphone app is developed for the RT-PCR device. There are two basic functions in the app. The first is the operation of the device and the second is to receive, access, review, and visualize the testing results. The MIT App Inventor was utilized for the app interface design. It uses programming blocks in the design which is very friendly to students new to cellphone app development. The device needs to be connected to the device wirelessly through Bluetooth. The design of the main interface of the cellphone app includes the Bluetooth functionality, operation of the PCR device, and the result data access. After the completion of the testing, the app will receive the fluorescence data for every one of the six biosamples from the Raspberry Pi 3 and can plot each data onto an image of a graph. The app also provided multiple ways to secure the data, either to save on a USB attached to the Raspberry Pi 3 or send via email.

SYSTEM TESTING AND RESULTS

First, the basic system functionality is tested after the assembling of all components. Then a real PCR testing is conducted in a biological laboratory in the Pharmaceutical department of our university under the surveillance of specialists. During the testing, the RT-PCR device is tested with two samples, positive and negative. The negative sample is pure water. The positive sample included 100ml of the SYBR green mix, 10nM of primers, 40ng of the DNA template, and 40ml of water to have the complete mixture for the PCR amplification process.



(a)



(b)

Figure 4. (a) PCR testing with the positive sample, and (b) testing result

For successful testing, the positive control sample and the negative control sample are required to display a fluorescence that is significantly separated in value. Since the fluorescent value is unknown for the separation, it will be determined after the analysis. If there is not enough separation in value, the threshold between the two sample fluorescent values cannot be determined. For inconclusive samples, there will be a certain margin that the threshold is center. Any sample whose fluorescence lies in that margin will be determined as inconclusive. Only with various testing of different positive samples and negative samples a true threshold can be determined.

Figure 4(a) shows the setup when the PCR process of the positive sample is being conducted. While the process was undergoing the observers could observe the fluorescence from the phone application that generates a time graph. After 3 hours of PCR cycling, the results of the test were generated and saved to a USB file which is a line graph of the fluorescent detection results in Figure 4(b). And Figure 5 shows the PCR testing result of the negative sample.

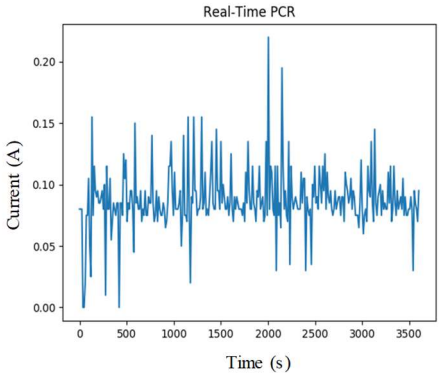


Figure 5. PCR testing result with the negative sample

In conclusion, as can be seen from the results, although a clear difference can be observed between the two samples, the results are less conclusive than expected for the samples. Although, during the process, the fluorescent detection did positively increase but not to a high value due to the sensitivity of the SYBR Green dye. The results from the negative control sample show no fluorescent detection which is a good indication that the PCR process for both the positive and negative samples was conducted properly. The functions of the machine work properly but as a PCR machine, it is less effective. The fluorescent detection of the machine needs to be exponentially higher than the start of the experiment. There is not a high enough separation between the positive sample value and the negative sample value to determine a threshold. As the results show the values fluctuate every second and will skew the correct labeling of “negative” or “positive”.

There is a wide range of possible reasons that the fluorescent level did not reach the desired level. One cause is that the sample size for reagent and sample inside the capillary tube was greater than the normal amount that the laboratory where it was tested normally uses. Also, there are very specific sets of temperatures held for specific amounts of time for each type of sample and reagent used. This must be accurately adjusted when variating the sample size. These factors need to be addressed with more test samples for better PCR testing of the device.

CONCLUSION AND FUTRUE PLAN

In this project, an interdisciplinary team with two engineering undergraduate students worked a whole year in developing a low-cost, ease-of-use, wallet-size, portable RT-PCR machine for accurate testing of various bacteria or viruses including COVID-19. Besides, the machine can be used for many other applications from community medical support to environmental monitoring and so forth.

This project provided students opportunities in independent learning and solving real-world engineering problems. The two students in the team have collaborated

effectively in studying and practicing a wide range of technics and skills, including CAD design, thermal dynamics, electronics design, PCB design, power electronics, cellphone app development, and microcomputer programming. With the comprehensive training they received through dealing with various challenges they experienced, their engineering confidence and competence are significantly improved, which may positively impact their career development in the long term. From the view of engineering education, the process of this project development has demonstrated the importance and benefits of adopting complex interdisciplinary engineering problems for student teams to solve, especially those involve contemporary issues.

On the other hand, the world is getting cured of COVID-19 day after day, but the portable RT-PCR device developed can find many other applications and needs to be essentially improved for better performance. We are living on a planet full of viruses and bacteria. A low-cost automatic DNA tester can be applied in many areas including disease control, environmental protection, agriculture, national security, etc. The project is still going on and we will continue the study on all related topics and issues and update the design for better engineering and education outcomes.

ACKNOWLEDGEMENT

The authors acknowledge peer-editors of the final version of the paper: Dr. Jiayue Shen and Dr. Diganta Dutta.

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