Cost Reduction of PCR Thermal Cycler

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Abstract

This paper presents a PC-based thermal cycler with a local-host architecture, where a PC is employed as the host. The local system only executes the thermal cycling process and the PC is responsible for the management of PCR protocols with a convenient GUI. The presented local-host system greatly reduces the development time and the maintenance cost, providing an economical PCR thermal cycler.

Keywords: *PCR thermal cycler, local-host architecture, graphical user interface, PCR protocol management*

1. Introduction

Polymerase chain reaction (PCR) follows three steps: 1) denaturation to separate the two strands of DNA using heat, 2) annealing, where the primer combines to the end of the sequence that is targeted to be amplified while cooling down, 3) polymerization, or extension, synthesizing DNA through re-heating the sample [1]. PCR thermal cycler controls the temperature of a metal block with holes for the sample-containing tubes usually using Peltier thermoelectric devices [1]. PCR thermal cycler should possess not only the thermal cycling function but also system management functions such as the protocol data handling and the user interactions [1, 2]. Since even the simplest electronic devices now require a graphical user interface (GUI) and data accessibility through Internet, there are many advantages when assigning the system management function to a general computer [1-5]. The monitoring and controlling of the basic thermal cycling processes, however, needs to be implemented within a separate computing system in order to satisfy stability and the constraints of real-time and deadline. If the basic function and the GUI are integrated into one system, it would take an order of magnitude more time and human resources to implement and stabilize the interface requirements than with the basic control alone [2-5]. Such an integrated system has almost no trouble in realizing the basic functions such as handling the protocol or controlling the temperature. However, the UI functions including management of the protocol files or the user interaction can cause a problem. Furthermore, if it is necessary to provide a strong GUI as in recent thermal cyclers, more effort and time in the development along with higher maintenance cost will be required to provide GUI. Therefore it is more efficient to move the UI functions to a host by utilizing the host-local system. Moreover, there are additional benefits in using a PC as a host, such as convenient file management and being granted with the

development environment for user interactions. Considering the GUI design environment or user accessibility, both human and time resources will be conserved by using a Windows PC or its embedded versions as the host [2, 5].

In this work, an economical thermal cycler with the local-host architecture is implemented and tested. The functional partitioning into the host and local system is discussed. It is also presented that the additional cost-down can be achieved by active employment of price competitive IT components. Chapter 2 presents the hardware and function descriptions of the thermal cycler that has only a primitive user interface. The implementation issues of the user interactions are discussed in chapter 3. The PCR amplification performance of the presented thermal cycler is compared with the existing thermal cycler in chapter 4 and the conclusion is given in chapter 5.

2. Implementation of the Thermal Cycler

The presented thermal cycler has no other UI component excluding the LEDs to minimize the system cost related to the user interaction and to reduce the development time and the maintenance cost. LEDs only indicate the status of the power on-off, the connection to the host, and the errors.

As the heating block of the presented thermal cycler is designed to hold 25 tubes for targeting personal usage, the heat sink for the PC processors can be employed for cooling the Peltier device. A low-cost, compact heat sink is available from the IT industry. The lid is heated with the copper pattern of a printed circuit board (PCB) whose manufacturing process is also well-developed in the IT industry. Three NTC thermistors are applied: one at the top side of the Peltier device, one at the top side of the heat sink, and the other at the lid. The bottom of the heating block has a groove to fix one of the thermistors for the temperature measurement of the block. Below the Peltier device, an aluminum spacer is inserted for two purposes: fixing the thermistor on the heat sink and widening the distance between the Peltier device and the heat sink. The bottom side of the spacer is grooved like the heating block. The temperature of the heat sink has to be measured to cool the system and notify the failure of the system. The lid heater PCB has a hole for the last thermistor.

Since the thermal cycler has no other user interface excluding the LEDs, it is necessary to communicate with the host PC in order to input a command or to check the status details of the system. The communication failure can often occur by the window hangings or the accidental disconnection of the USB cable. In that case, if all the PCR protocol was handled by the host, the system would stop the cycle, thus damaging the PCR samples. To prevent this, the thermal cycler runs independently after receiving the PCR protocol from the host. After starting the cycle, the host only monitors the status or stops the cycle.

The system state is designed as simple as possible for the robustness. The system only has two states, ‘ready’ and ‘run’. The state of the PCR system changes from ‘ready’ to ‘run’ when it receives the ‘START’ command, which is sent by the host. The reverse change occurs in three cases: after the completion of PCR, when receiving the ‘STOP’ command from the host, or when any of the thermistors indicate the temperature is out of the predefined boundaries indicating the system failure.

Table 1 is an example of a PCR protocol. In the table, the first row labeled as ‘1’ is an extra denaturation step maintaining the chamber at 95°C for 30 seconds to easily separate the DNA strands before the normal cycling. The next three rows form one cycle of amplification, each representing denaturation, annealing, and polymerization.

'GOTO' label is for the flow control, indicating the cycle to return to label 2 for 34 times. The example therefore consists of 35 cycles in total. The last row is an extra polymerization step finishing the PCR. This step enables the strands that has not bond yet to bond with their complementary strand.

The system receives the protocol data like the above example just before starting the cycle. The protocol is fetched in sequence and parsed to control the Peltier device.

Table1. Protocol Examples

label	temperature (°C)	duration (sec)
1	95	30
2	95	30
3	55	30
4	72	30
GOTO	2	34
5	72	180

The temperature of the heating block is managed by a procedure that controls the current of the Peltier device by periodic measurement of the temperature. In the presented thermal cycler, the block temperature was measured and calculated to set the pulse width modulation (PWM) duration for the Peltier device driver. The temperature is controlled with proportional-integral-derivative controller (PID). The strength of the control is calculated by the linear combination of the difference of the chamber temperature and the aimed temperature, and the integral and the derivative of the difference. Therefore the PWM duration, u can be calculated as follows:

$$u = K_p e + K_i \int e + K_d \frac{de}{dt}, \quad e = T_t - T_c$$

Where T_t, T_c, K_p, K_i, and K_d are the target and current temperature, the proportion, the integral, and the derivative gains, respectively. The gains can be determined in various ways, but they are usually determined through experiments. Once the control strength u is determined, the output is controlled with the PWM duration of the Peltier driver. For a positive, the block is heated, and for a negative value the block is cooled.

3. The User Interface Implementation

PCR thermal cycling takes several hours and the users tend to test just one protocol for days. Therefore a simple UI is necessary in order to not occupy the PC screen where the other application windows are running. Therefore GUI has two windows: the main and the brief. The main UI is hidden and only the summarized information is displayed on the brief window. The whole information can be accessed on the main window by clicking on the tap view button of the brief window. The brief window only has the minimum information for the routine PCR experiments. It includes the protocol name that is currently running, a start button, the remaining time, and the LED buttons that show the same information as those of the thermal cycler. In the main window, the user can find the status details of the cycler such as the temperatures of the heating block and the lid. Also other properties such as the edit box

for the lid temperature setting, the protocol detail, and the unique number of the thermal cycler are displayed. The current active line of the protocol is flashed with the color background to identify which line of the protocol is running in the cycler. The unique number of the thermal cycler is needed for multi-cycler operation with one host. This functionality would be helpful for the users to find the optimal PCR condition as in the gradient PCR thermal cycler.

The protocols can be read from any text file with the predefined format. The windows file system is sufficient enough for various users to manage the various protocols. However, it is highly limited and expensive if it is implemented in the thermal cycler. This GUI developed with the Microsoft visual studio provides a more efficient and better environment for the UI design and debugging than that of the embedded system in the thermal cycler.

4. PCR Performance Comparison

The efficiency of the presented thermal cycler was verified through comparison with the conventional one that is more than 10 fold higher in cost. PCR was performed using HPV DNA (BIOMEDLAB, Co. Ltd., Korea) with the company provided protocol. 14 tubes of the positive and 7 tubes of the negative samples were prepared. The positive sample tubes were first amplified inserting 7 tubes to each thermal cycler. The 7 tubes of the negative sample were amplified later in the presented thermal cycler. The total of 21 amplicons was loaded into one gel for the electrophoresis. The gel image after electrophoresis is shown in figure 4. The first 7 bands except the ladder marker image are for the amplicons from the conventional cycler and the second seven bands are for those from the proposed thermal cycler. The last seven bands are for the amplicons of the negative samples. The visual inspection of the image shows that the presented thermal cycler has the same amplification performance as the conventional one. The negative sample did not show any bands as in the last 7 bands in the figure.

To provide a quantitative comparison, a trial version of commercial gel image analysis software was chosen. The band intensities were extracted with the software and their statistical characteristics were calculated. The mean of the band intensities and the coefficient variation (CV) were compared for each group of the bands from each thermal cycler. The relative mean difference was 86.20% and the CVs were 11% and 13% for the presented thermal cycler and the conventional one, respectively. The mean of the band intensities for the proposed thermal cycler was slightly lower than that for the conventional one, while CV was higher. The quantitative results also show that the presented thermal cycler performance is similar to the conventional one, considering the variations of the gel image processing.

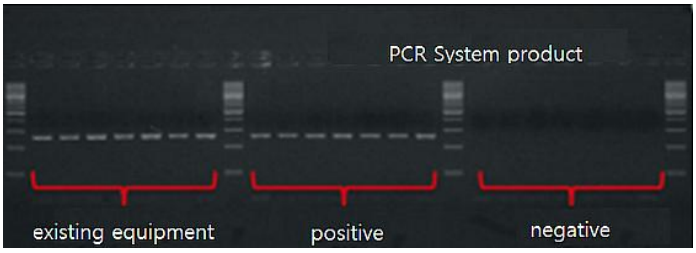

Figure 1. The gel Image for the Performance Comparison

5. Conclusion

This paper presents a host-local system architecture which shifts the user interface of the conventional PCR thermal cycler to a host PC, yielding a highly reduced cost. By employing the components from IT industry, further cost-down was achieved. The proposed scheme was implemented and the qualitative and quantitative comparison with the conventional thermal cycler was presented. The results showed that the proposed thermal cycler has a similar performance to the conventional one. Since the host has a strong GUI developing environment, the period required to develop the entire system can be shortened. Additionally the maintenance cost also decreased and resultantly the overall cost of the PCR system could be significantly reduced. Also, the suggested host-local architecture enables the user to easily edit and manage the PCR protocol on the PC windows, compared to the conventional thermal cycler where the user had to do with a poor user interface. By distinguishing each thermal cycler with a unique number, it was possible to control multiple devices on a single host.

Acknowledgments

This work (Grants No. 00047469) was supported by Business for Cooperative R&D between Industry, Academy, and Research Institute funded by Korea Small and Medium Business Administration in 2011.

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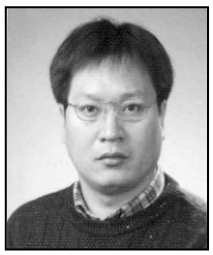

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