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Reagent and Sensor Temperature Comparison for PCB-based PCR Chip

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Abstract. The protocol executed during DNA amplification by PCR (polymerase chain reaction), which includes three processes, namely, the so-called denaturation, annealing, and extension of the DNA, is closely related to temperature. While it is important for the thermistor, which adjusts the temperature in a microfluidic channel, to reach its target temperature accurately, successful PCR actually depends on the temperature of the sample in the microfluidic channel when it precisely reaches the target temperature. Therefore, in this study, we examined whether the temperature of the thermistor in a micro PCR chip and the temperature of a DNA sample are the same and analyzed the time the target temperature is attained. In the experiment, we used an existing micro PCR chip to produce chambers 200 μm and 400 μm high and performed experiments for all chambers in order to examine the differences in temperature and speed. To examine the temperatures of the NTC-thermistor installed outside the chip on the microfluidic channel, and of the DNA sample in the channel, omega thermocouples were used to examine the time taken to reach the target temperature and whether the temperature reached the target value. From the result, we confirmed that both temperatures reached the target and that the times taken to reach the target value showed no difference in the range of 60–72°C, while in the other ranges, the speed was higher for 200 μm than for 400 μm.

Keywords: microfluidic channel internal temperature, polymerase chain reaction, NTC-thermistor

1 Introduction

In polymerase chain reaction (PCR), two strands of DNA are separated by the application of heat in denaturation; at a low temperature, the primer is annealed to the sequential terminal for amplification, and at slightly higher temperature, the DNA is synthesized—this process is called polymerization or extension. A test involving PCR is referred to as a PCR test. A PCR test is advantageous because only a small amount of DNA is needed to run a test, and it can be applied for diagnosing many diseases [1, 3- 7]. However, current commercial PCR devices have disadvantages such as long

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testing time, poor transportability because of their large sizes, and high cost [1, 2]. With the active development of lab-on-a-chip systems, which are analytical systems that overcome the disadvantages of the existing genetic diagnostic techniques and allow on-site genetic diagnoses, for overcoming such disadvantages, micro PCR chips that contain microfluidic channels are also being developed [1, 2]. A lab-on-a-chip system employs technology such as the MEMS (Micro Electro Mechanical System) and performs all preprocessing and analytical steps, including the dilution, mixing, reaction, separation, and determination for a sample on a single chip [1, 2].

A micro PCR chip, one of the lab-on-a-chip systems, can quickly draw various pieces of genetic information with a very small amount of DNA, enables genetic analyses, and is used for diagnosing various diseases through reactions to the internal components of a test subject [2].

One of the most important factors involved in PCR amplification is temperature. Temperature control in micro PCR, which will be examined in this study, is achieved by means of the heating pattern of a micro PCR chip and by the temperature sensor of an NTC-thermistor (Negative Temperature Coefficient-thermic resistor). If the temperature is effectively controlled, a quicker PCR test is possible even with the same protocol. Further, the miniaturization of PCR devices makes them better than the existing PCR devices by increasing transportability and portability, thus, increasing efficiency.

Because a thermistor is installed on the bottom of a micro PCR chip, however, it is necessary to confirm whether the temperature of a testing sample inside the chamber is actually the same as that of the temperature sensor. In this study, for experiments, we installed two thermocouples inside the chamber of the micro PCR chip and performed experiments to examine if the sensors show exactly the same temperature and to compare the times of delay of the two temperatures.

2 Materials and Methods

A heating pattern to heat up a sample in the chamber is layered above the PCB that forms the bottom layer of the chip. An NTC thermistor is attached below the bottom layer to measure temperature. Because the epoxy coating of the PCB is a PCR inhibitor, a PP (polypropylene) box tape is layered above the PCB that is overlaid with a double-sided tape. The double-sided tape is cut out in the middle in the shape of the chamber. A 180-μm PP film with inlet and outlet holes form the top layer of the chip.

The local system measures and sends the temperature of the thermistor to the host system. Then, based on the target temperature, the host system calculates the PWM to heat up the heater, and the calculated PWM is sent back to the local system so that the PWM of the heater or fan can be set. A Windows PC was selected as the host, and the local system has PIC18F4550.

The thermistor in the micro PCR chip has a resistance error of 1% and operates in the range -40–125°C (NCP15XH103F03RC, Digikey). By referring to the resistance values for four temperatures (50, 60, 70, and95°C), which were provided with the basic data sheet for the pertinent part number, we determined the Steinhart-Hart cali-

bration coefficients and temperature values by using the Steinhart-Hart equation (1/T=A+B ln (R)+C(ln(R))3).

To examine the time of transferring the internal temperature of the channel and the bottom temperature of the channel to the top, we created channels by installing two thermocouples, one placed above the box tape and the other below the cover film; the heights of the channels were 200 μm and 400 μm, respectively. To examine temperature errors of the thermocouples, they were put in a constant water bath, and their temperatures were measured at 50, 60, 72, and 95°C. A TRH Central program, basically provided by Omega Engineering, to show the temperatures of a thermocouple was used to examine temperatures, and we confirmed that the temperatures of the thermocouples were the same as those of the constant water bath. The protocol that was used in the experiment for measuring the internal temperatures was composed of four cycles of the PCR protocol.

3 Experiments and Results

No steady state error in temperature was observed for both 200μm and 400μm. However, as expected, the temperatures of the chamber changed faster than those of the chip thermistor.

The time taken for temperature transfer for the greater chamber height appeared to be longer; As suggested by the data in these case, excluding the event in which a fan was used to lower the temperature, when the temperature increased, the speed of temperature change of the thermistor was higher by more than a factor of two, and the speed for the 200-μm-high chamber was much higher than that for the 400-μm-high chamber.

When the fan was used for cooling, the thermistor and thermocouples had a similar speeds in terms of temperature change in the case of the chip with a 200-μm-high chamber; this is attributed to the fact that the top and the bottom of the chip were cooled off at the same speed by the fan that blew air in the sides of the chip.

On the other hand, for 200-μm-high chamber, the speed of temperature change was not necessarily proportional to the size of the chamber; the speed in the medium chamber was the lowest. We attribute this phenomenon to the larger heating area of the large chamber because the dimensions of the heating pattern of the PCB substrate remained constant.

Because the overall temperature change in the chamber was insensitive to overshooting, even when the thermistor's temperature change was overshot, the thermocouples were not subject to overshooting. Because we received a quick reaction when the thermocouples were put in the pre-heated constant water bath, the reaction speed of the thermocouples was irrelevant. Therefore, during temperature control, it seems fine even if we control the temperature quickly to the degree where a bigger overshooting occurs as a reaction from the thermistor.

4 Conclusion

This study observed temperature changes of the thermistor in a PCR chip in which a reaction chamber was installed with commercial tapes over a PCB substrate and of the samples inside the chamber. Measurements of temperature changes were made for two different chamber heights and three chamber sizes. The results showed that regardless of the height or size of a chamber, there was almost no steady state error. As expected, greater the chamber height, the lower is the speed of temperature transfer of a sample; however, because the size of the heating area remained constant, the speed was not proportional to the chamber size. Because the direction of air of the cooling fan was in parallel with the chip, the lower and upper parts of the chip cooled down at the same time; therefore, the temperature changes of the chip's thermistor and the sample within the chip were similar.

Because the temperature change in the chamber was insensitive to overshooting, even if the thermistor's temperature change overshot, the thermocouples were not subject to overshooting.

Because we received a quick reaction when the thermocouples were put in a preheated constant water bath, the reaction speed of the thermocouples was found to be irrelevant. Therefore, during temperature control, it is fine even if we control the temperature quickly to the degree where a bigger overshooting occurs as a reaction from the thermistor.

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