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Performance Improvement of PCB-based PCR chip with Calibrated Thermal Sensor

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Abstract. A thermistor sensor of PCB-based PCR chip is calibrated and the PCR performance is investigated for improvement. The chip temperature is controlled by the heater pattern of the substrate and the thermistor on the opposite face. Commercial chip thermistors with a 1% resistance tolerance are adapted. This paper presents the effect of the thermistor calibration on PCR performance. A comparison on the band brightness of gel images indicated that the calibration seemed to deliver a better performance.

Keywords: PCR, Micro-PCR chip, calibration, thermistor

1 Introduction

A polymerase chain reaction (PCR) test has advantages in that the test can be conducted with a small amount of DNA and is used in the diagnosis of various diseases [1]. Now that lab-on-a-chip systems are being used for DNA analysis, the development of a PCR chip that has a microfluidic channel is being carried out [1–3]. In a PCR chip, by using a very small amount of DNA, various data about the gene can be obtained quickly, and the gene can be analyzed. The PCR chip is used to diagnose various diseases through a test subject's reaction with its internal component [3]. To commercialize this capability, the PCR chip must be reliable, reproducible, and sensitive. Therefore, accurate temperature control and calibration of the temperature sensor may be necessary [4, 5].

Manufacturing costs are high for previously proposed PCR microfluidic channel chips. In this paper, to reduce the manufacturing costs of the PCR chip, a study was carried out for the temperature measurement and calibration of a PCR chip fabricated by using a thin polychlorinated biphenyl (PCB) substrate, polymer film, and double-sided tape, respectively, for the chamber floor, ceiling, and walls.

Temperature is one of the most important factors in the PCR amplification process. Controlling the temperature of PCR amplification is accomplished by using the PCB's heating pattern and a negative-temperature-coefficient (NTC) thermistor sensor. The

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PCR chip studied in this paper uses an NTC thermistor, which has a 1% resistance error, as a temperature sensor. In this paper, PCR performance was compared for cases when the NTC thermistor was calibrated and not calibrated. The effect of thermistor calibration on PCR performance was investigated by comparing the band brightness of gel images obtained through electrophoresis for amplified DNA in both cases

2 Material and Method

The proposed micro-PCR chip has a four-layer structure.By attaching box tape (#309 mini clear, 3M) made of PP (polypropylene) material on top, and 400-μm double-sided tape (5620A, Nitto) in the middle part of the chamber as chamber walls, a space inside the chamber was secured. The cover of the chamber was fabricated using PP. The bottom of the chip was a PCB which had a heater pattern and a thermistor was attached on.

The NTC thermistors employed in this study were chip thermistors with a resistance error of 1% (NCP15XH103F03RC, DigiKey). Considering the possibility of lot variations among the thermistors, they were supplied by two companies (Mouser and DigiKey) and the thermistors from each company formed a group of thermistors. The previously supplied sensors from DigiKey with the same part number compose another group resulting in three groups of thermistors.

Using the resistance values for four important temperatures (50ºC, 60ºC, 70ºC, and 95ºC) in the PCR from the temperature-resistance tables provided by the thermistor suppliers, the uncalibrated SH coefficients of A, B, and C were obtained. Using these values and the Steinhart-Hart formula ($\frac{1}{T} = A + B\ \ln(R) + C\ (\ln(R))^3$), the uncalibrated temperature values were calculated. The calibrated SH coefficients were determined by measuring the resistance values for each of four temperatures in a constant-temperature water tank. The calibrated temperature values were obtained by these coefficients.

In the PCR, the temperatures of three processes—denaturation, annealing, and extension—are important. In the current experiment protocol, the temperatures corresponding to the three stages are 95ºC, 60ºC (or sometimes 50ºC), and 72ºC. At 95ºC, eight chips that had thermistors showing the temperature values corresponding to the overall average value, and four chips showing maximum values and minimum values, were selected, for a total of 16 chips. The selected thermistors were divided into four groups, considering that there were four units of micro PCR systems used in the experiment. Each group consisted of one chip showing a maximum value, one chip showing a minimum value, and two chips showing the values corresponding to the average value. For each group, the PCR tests were carried out in four systems simultaneously.Throughout each round, the maximum-value and minimum-value chips were mounted once each in the four systems, randomizing the system variation.

With the four micro PCR systems two PCR experiments were carried out: one using the uncalibrated SH coefficients for 12 chips, and the other using the calibrated ones which were in the quick-response (QR) codes. In a PCR chip, the calibrated SH coefficients were converted to a QR code; and when the PCR chip was mounted on

the micro PCR system, the QR code was read and the calibrated SH coefficients were applied to the system. In the meanwhile, the uncalibrated coefficients, which could be pre-calculated from the resistance-temperature table provided by the manufacturer.

In this paper, the protocol was as follows: 3 min at 95ºC for pre-heating; 39 cycles of 10 s at 95ºC, 30 s at 60ºC, and 30 s at 72ºC; and 3 min at 95ºC and 30 s at 50ºC for the final extension. The following materials were used in the experiment: 11$\mu\ell$ of 1 ng/1$\mu\ell$ DNA (CT), 12.5$\mu\ell$ of the master mix, 10 pM/0.5$\mu\ell$ of primer F, 10 pM/0.5$\mu\ell$ of primer R, and 10 pM/0.5$\mu\ell$ of the probe.

3 Results

Investigating the gel image with the naked eye, it was difficult to distinguish between the band brightness of the ampliconsfrom calibrated chips and themfromuncalibrated chips.To quantitatively analyze the brightness of the bands, freeware called GelAnalyzer was used. Because the background brightness changed vertically, the change in background was compensated for by using the rolling-ball algorithm before detecting a peak.

As the ladder brightness of each gel image was different,each band volume was normalized with the volume of the last ladder band, which is the ladder band that is the most similar to the molecular weight of amplicons. The average of the normalizedband volumes of amplicons produced from the calibrated chips were slightly larger. This fact became clearer when comapring the box plots.

However, as results of the t-test and rank-sum test, the band volumes from the calibrated chips were not significantly larger (t-test p-value = 0.45, rank test p-value = 0.41). There is no basis for assuming that significant results will be obtained if more experiments are conducted. However, based on the box-plot result for the case of measuring temperatures with the thermistor, it is expected that better results will be obtained through calibration

4 Conclusion

In this study, the effect of thermistor calibration on the performance of a PCR was investigated by conducting experiments of the PCR process with chips that were calibrated and not calibrated with a NTC thermistor that has a 1% resistance error. This is required for measuring temperature in a micro PCR chip, which is a reaction chamber constituted on a thin PCB substrate.Using the Steinhart-Hart formula, eight chips in which a thermistor showed a temperature value corresponding to the overall average value at 95ºC, and four chips showing maximum and minimum values, were selected for a total of 16 chips. Using four units of the micro PCR as local systems, experiments were carried out.

The average brightness volume (1.15) of amplicons produced from the calibrated chips using GelAnalyzer was slightly larger than the average brightness volume (1.03) of amplicons produced from the uncalibrated chips. This was more apparent in a box plot. However, as a result of a t-test or ranksum test, the band volume from the calibrated chips was not significantly large (t-test p-value = 0.45, rank test p-value = 0.41).

Although significant results were not obtained, as a result of the box plot, it is expected that better results will be obtained through calibration when the temperature is measured with a thermistor.

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References

1. PatkoaD, MartonfalvicZ,Kovacsa B, VondervisztaF, KellermayercM, HorvathaR, Sensors and Actuators B 196, 352 (2014)
2. FockeM, KosseD, MullerC, ReineckeH, ZengerleR, and StettenabF, Lab on a Chip 10, 1365 (2010)
3. HAEBERLE, Stefan; ZENGERLE, R.:Microfluidic platforms for lab-on-a-chip applications. Lab on a Chip, 7.9: 1094-1110 (2007),.
4. Koo C, Malapi-Wight M, Kim HS, Cifci OS, Vaughn-Diaz VL : Development of a Real-Time Microchip PCR System for Portable Plant Disease Diagnosis. PLoS ONE 8(12): e82704. doi:10.1371/journal.pone.0082704 (2013)
5.Salm, E., Liu, Y. S., Marchwiany, D., Morisette, D., He, Y., Bhunia, A. K., & Bashir, R..: Electrical detection of dsDNA and polymerase chain reaction amplification. Biomedical microdevices, 13(6), 973-982 (2011)
6. Dittrich, Petra S., and Andreas Manz.: Lab-on-a-chip: microfluidics in drug discovery. Nature Reviews Drug Discovery 5.3,210 (2006)
7. Foudeh, Amir M.: Microfluidic designs and techniques using lab-on-a-chip devices for pathogen detection for point-of-care diagnostics. Lab on a Chip 12.18, 3249 (2012)
8. Kumar K. K., Jayaraman R., Narasimha, S. K., Radhakrishnan, R. M., Viswanaihan, S., Nair, C. B., ... &Venkataraman, V. U.S. Patent Application 12/682,555(2008).
9. Hoehl, M. M., Weißert, M., Dannenberg, A., Nesch, T., Paust, N., vonStetten, F., ...&Steigert, J.: Centrifugal LabTube platform for fully automated DNA purification and LAMP amplification based on an integrated, low-cost heating system. Biomedical microdevices, 16(3), 375(2014)
10. de Torres H. B,Rensch C, Fischer M, Schober A, Hoffmann M, & Müller J.:Thick film flow sensor for biological microsystems, Sensors and Actuators A: Physical, 160(1), 109 (2010)

