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The rapid temperature transfer apparatus for *E. coli* K12 DNA segment amplification

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Abstract

Thermal cycler machine was extensively used machine for temperature transfer of polymerase chain reaction (PCR) to amplify DNA sample. One of the major problems is consumption for cooling and heating. In order to improve the efficiency of time, this research presented a novel method to reduce the time for temperature transition during the DNA amplification reaction process. Based on the concept for designing the apparatus, the DNA sample was placed in the silicon chamber, which was pushed by a tappet through three temperature regions around a center. The DNA segments could be amplified rapidly after 30 thermal cycles. The polymerase chain reaction apparatus consisted of two parts, the heater device and the rotation device. The photolithography and bulk micromachining technologies were utilized to construct the thin-film heater and DNA reaction chambers. The temperature transition rate of DNA chamber was simulated by CFD-ACE+ software. Finally, 1 μl 100 base pairs (bp) DNA segment of *E. coli* k12 was amplified successfully within 36 min in the PCR apparatus.

Keywords: DNA amplification; PCR; Bulk micromachining; Thin-film heater; *E. coli* K12

1. Introduction

Since the micro-electro-mechanical system (MEMS) technologies had been developed, it was widely applied for many fields and applications such as optical MEMS, bio-MEMS, power MEMS, etc. In bio-MEMS field, many important issues have been developed, for example, bio-detection, polymerase chain reaction (PCR) chip and dielectrophoresis (DEP) chip for recent years. However, it had many advantages, for example, lower consumptive sample, portable, miniature, short reactive time and automation. These years, the MEMS technology was also applied to fabricate the chip for amplifying DNA, such as continuous-flow PCR [1–4] flow-through thermocyclers [5,6], or closed chamber PCR-chip [7,8].

In the past decade, PCR has been considered as a very important role in the field of molecular biology. It was a very powerful technique that could amplify a little amount of nucleic acid to generate plentiful material for diagnosis. Typically, PCR included three steps which were denaturation of double-stranded DNA, annealing of oligonucleotide primer pairs, and extension of new DNA strands catalyzed by DNA polymerase at temperatures of approximately 95 °C, 55 °C and 72 °C, respectively [9]. Following the cycles of different temperature zones, theoretically, a DNA segment was amplified 2^n times after n cycles of PCR [10]. Fig. 1 showed the DNA replication process of PCR. In fact, the enzyme efficiency was decayed after each cycle so that the efficiency of DNA amplification was $A = (1 + e)^n$ where e approximated 0.8–0.9 [6].

In general, PCR thermal cycles were performed in a thermal cycler that took 2–3 h for thirty cycles. However, most of the reaction time was spent on cooling and heating during the reaction process. In this research, an apparatus which had a rapid temperature transfer and could adjust the ratio of reaction time according to the DNA sample had developed. The thin-film heater was employed as the heater device and the rotation device were mounted on the heater device to perform temperature transfer of DNA chamber between the three temperature zones. Finally, the *E. coli* K12 DNA segments as the amplified sample used the PCR apparatus and the ratio of reaction time was 1:1:2.

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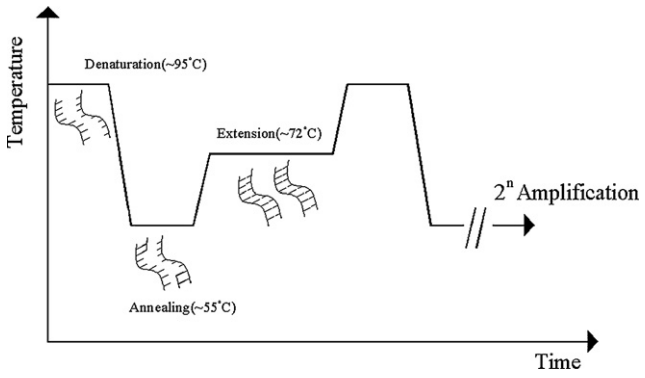

Fig. 1. Schematic diagram of the PCR process [6].

2. Design and fabrication

2.1. Design of PCR apparatus

The PCR apparatus in this research consisted of two parts that were the rotation device and the heater device as shown in Figs. 2 and 3. In the rotation device, both of a dc motor and a counter were placed on the polymethylmethacrylate (PMMA) frame. The rotation rate of tappet was controlled via the dc motor. The DNA chamber on the heater device was driven by the tappet, and the cycle number was counted. In the heater device, it consisted of four components including the polymer base, a heat resistance plate, a thin-film heater and copper plates. The heat resistance plate was the isolation layer between the copper heater and the polymer base to induce most of the heat flow of the heater transmitted into the copper plates. The copper plate with 2 mm thickness to adhere the thin-film heater was to make uniform temperature distribution. The thermal couples (TC) were then used to insert into the copper plate for the measurement of temperature. The copper was chosen as the material for the thin-film heater and then deposited and patterned on the glass wafer. The completed PCR apparatus was shown in Fig. 4.

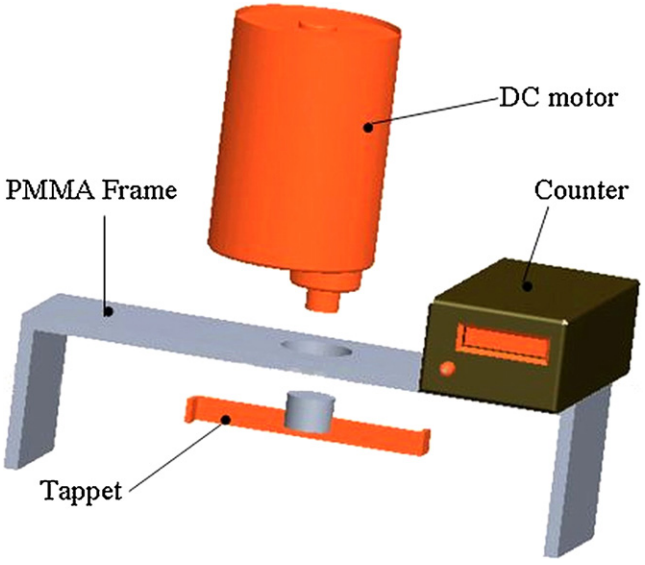

Fig. 2. Schematic of the rotation device of PCR apparatus.

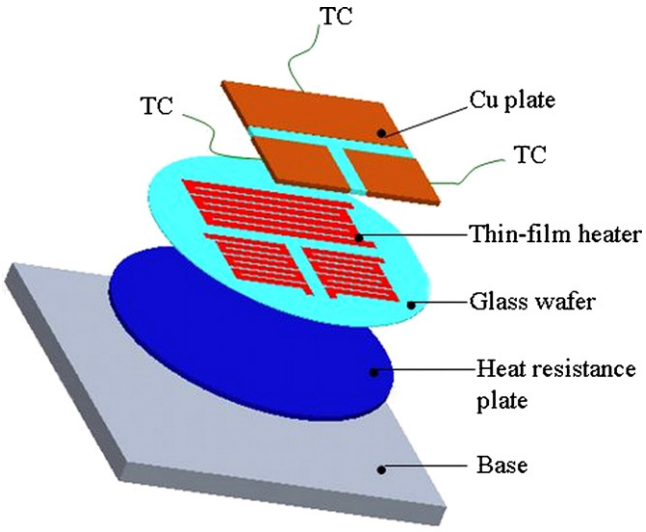

Fig. 3. Schematic of the heater device of PCR apparatus.

The trace of DNA chamber on the heater device was described in Fig. 5. The anticlockwise rotation of DNA chamber passed through the regions of 95 °C, 55 °C and 72 °C per each cycle. The ratio of trace was L1:L2:L3 = 1:1:2 when the center of the tappet was located on the "A" point. According to the different DNA sample, the ratio of the trace could be adjusted with the location from the tappet center.

2.2. The thin-film heater and temperature control

In the temperature control system, the proportional integral derivative (PID) controller was used to regulate the temperature. The thermal couples were employed as the temperature sensor to insert into the copper plates shown in Fig. 3. The copper of 2000 Å thickness was deposited on the glass wafer to be as the thin-film heater by the E-beam evaporator. Fig. 6 showed the fabrication process of the thin-film heater. The Ti/Cu/Ti was deposited with the thickness of 500 Å, 2000 Å and 500 Å, sequentially. The purpose of titanium material deposited was to

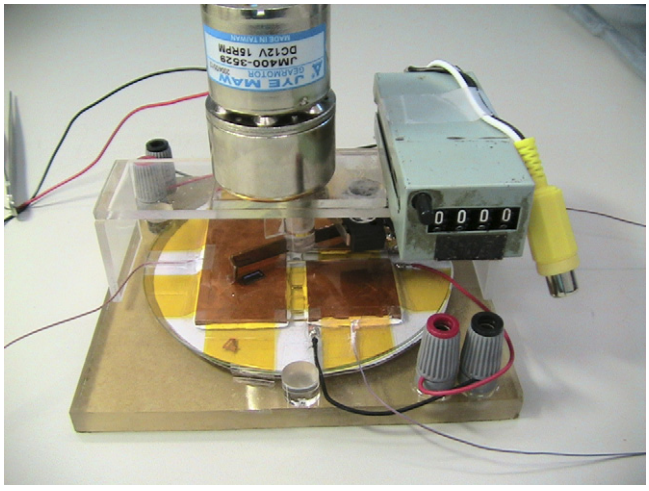

Fig. 4. The completed PCR apparatus.

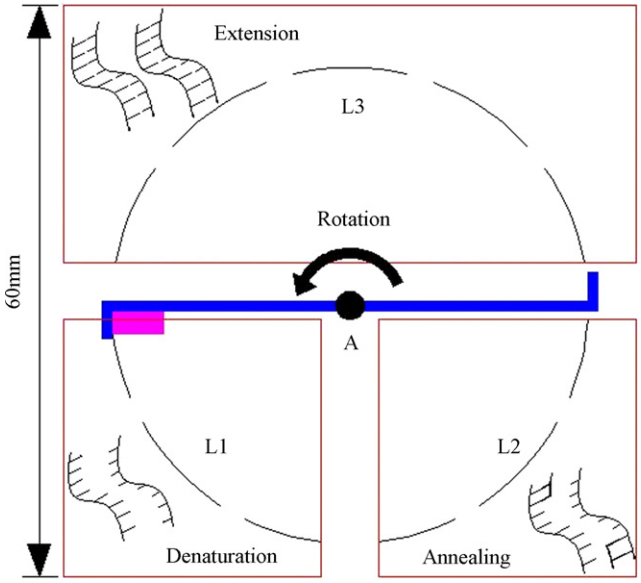

Fig. 5. The trace of DNA chamber on the heater device; L1:L2:L3 = 1:1:2.

be the adhesive layer and to avoid the oxidation of the copper thin-film heater. At last, the wires were soldered on the pad to complete the thin-film heater.

2.3. Fabrication of DNA chambers

The correct duplicated DNA was depending on the uniform temperature distribution on the PCR sample. The accelerated heating/cooling rate decreased the reaction time in the DNA amplification. Therefore, the silicon material was chosen to be fabricated as the DNA chamber because it had a high thermal conductivity, better temperature distribution and less time consuming. Fig. 7 showed the fabrication processes of DNA chamber. The ⟨1 0 0⟩ silicon wafer of 525 μm thickness with the double side silicon nitride (Si_3N_4) was the substance of chamber. The photolithography technique was utilized to pattern the etching hole before removing the Si_3N_4 by reaction ion etching (RIE) as shown in Fig. 7(b). The 3D chamber was etched of 300 μm depth by wet anisotropic KOH solution (KOH:DI water = 1:5) to make the side wall of 54.74° with ⟨1 1 1⟩ [11], then the upper Si_3N_4 was all removed and shown in Fig. 7(c)–(d). In the last step, the thermal oxide was formed with 0.2 μm thickness on the chamber surface in order to avoid the inhibition of the enzyme reaction on the silicon material [12], shown in Fig. 7(e). The DNA chamber, with the volume of each chamber 4.5 μl, is as shown in Fig. 8. The relationship between the etching depth and time was shown in Fig. 9.

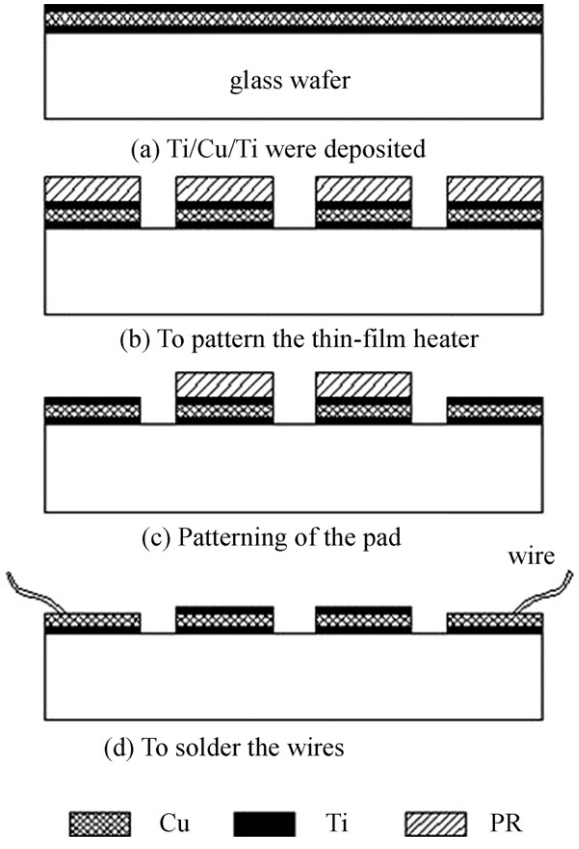

Fig. 6. The fabrication processes of the thin-film heater.

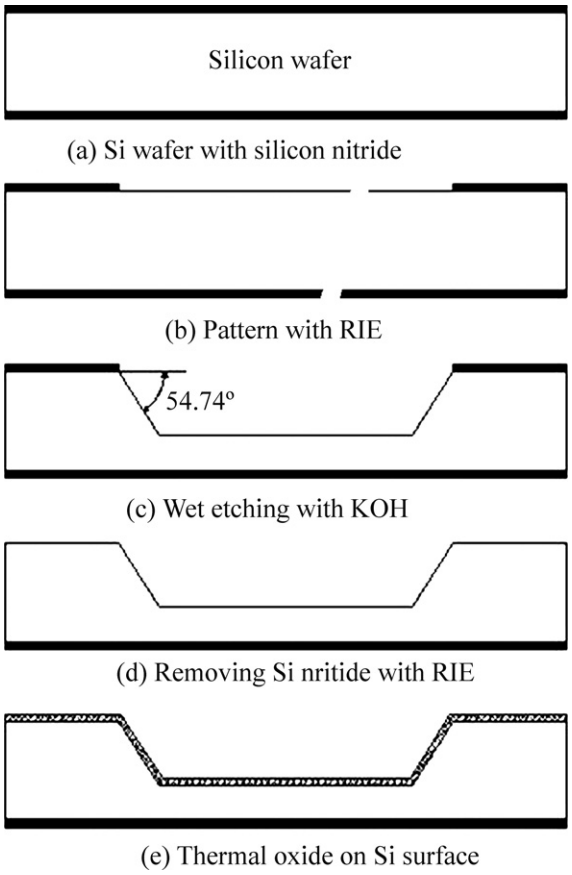

Fig. 7. Fabrication processes of DNA chamber.

3. Temperature simulation of DNA chamber

The commercial computational fluid dynamics (CFD) simulation software of CFD-ACE+ (CFD Research Corporation) was applied to simulate the temperature distribution of DNA chamber. The CFD-ACE+ software provides the simulation and analysis of fluid flow and associated multiphysics, such as

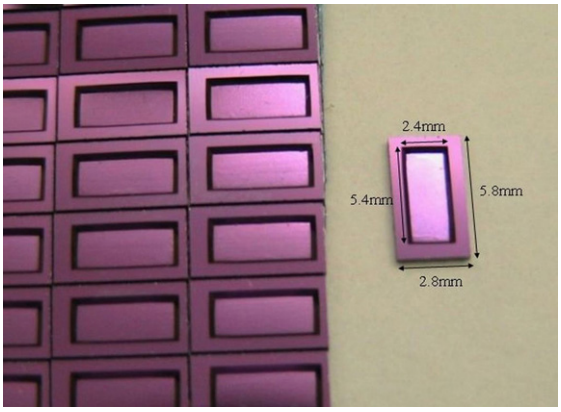

Fig. 8. Photograph of the completed DNA chamber.

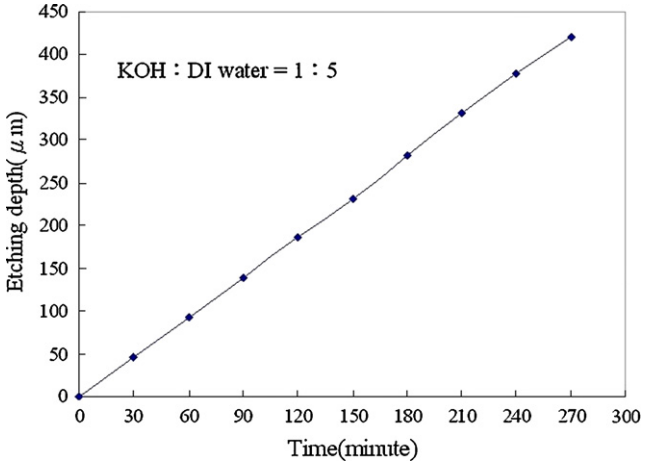

Fig. 9. The relationship between the etching depth and etching time.

electric, heat transfer, structure. It is applied in industry, semiconductivity, fuel cell, bio-MEMS, etc. The CFD-ACE+ solver is finite volume- and pressure-based. The grid could be divided into structure grid and unstructured grid. Fig. 10 showed the 3D symmetrical model of the DNA chamber. The model was constructed by structure grid in CFD-GEOM with 41,952 elements.

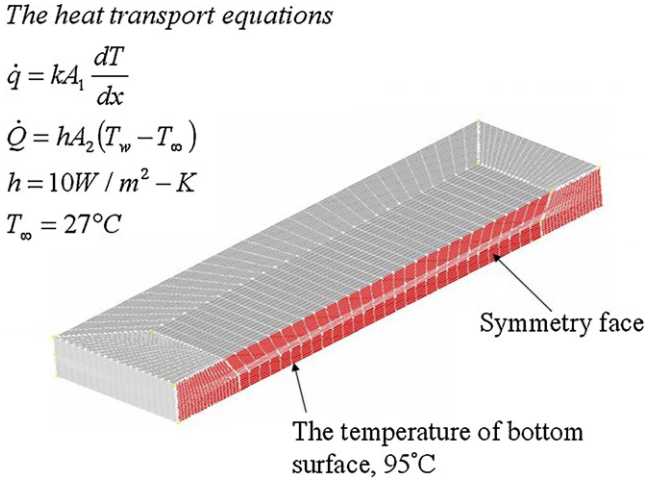

Fig. 10. The 3D grid symmetrical model of DNA chamber.

Table 1
The material properties

	ρ (Kg/m^3)	k (W/m K)	C_p (J/Kg K)
Silicon	2330	148	712
Mineral oil	900	0.15	2000
Water (sample)	997	0.613	4179

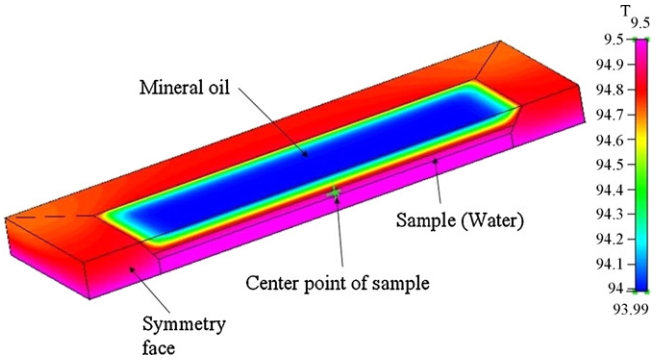

Fig. 11. The simulation of temperature distribution of DNA chamber in steady state.

The surface boundary condition was supposed the heat convection coefficient h of 10 W/m^2 K for free heat convection at the ambient temperature $T_\infty = 27\,°C$. The temperature of surface of chamber was 95 °C. Table 1 showed the material properties of density, ρ, thermal conductivity, k, and specific heat, C_p.

Fig. 11 indicated the temperature profile of DNA chamber of the 3D symmetrical thermal model. The silicon was the material of the container, and the water was supposed as the target material under the mineral oil been used to avoid the evaporation of the water. The total temperature difference range in the whole container was within 1.01 °C, and the temperature difference of 0.2 °C was at the center point of the target sample. The simulation result indicated uniform temperature distribution within DNA chamber. Fig. 12 showed that the heating/cooling rate of the sample in the silicon chamber exchanged between the three temperature regions. The simulation predicted a high heating and cooling efficiency. The average cooling rate was 20 °C/s between 95 °C and 55 °C, the average heating rate was 9 °C/s

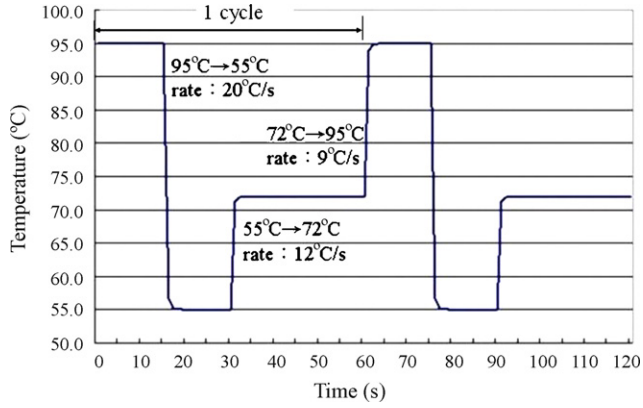

Fig. 12. Simulation result of the thermal cycles.

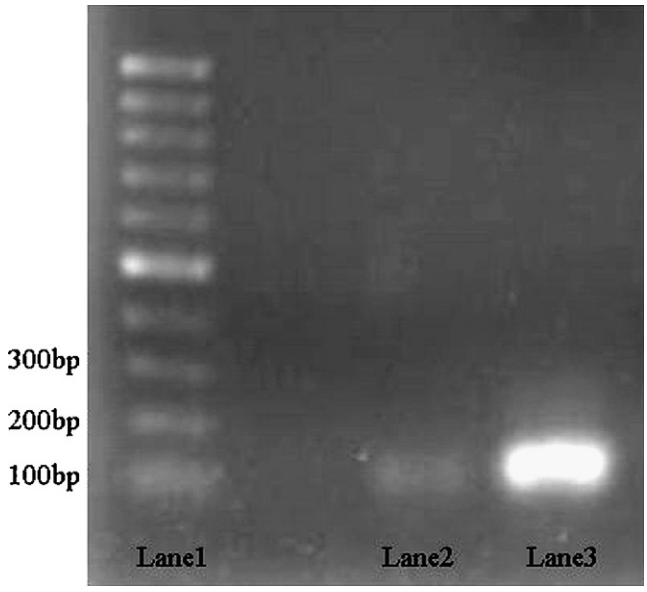

Fig. 13. Result of PCR amplification of 100 bp segments from *E. coli* K12. Lane 1: DNA size marker at every 100 bp; lane 2: the PCR apparatus (∼0.8 μl); lane 3: the commercial thermal cycler (5 μl).

from 55 °C to 72 °C and 12 °C from 72 °C to 95 °C at the center of the sample.

4. Experiment and results

The PCR reaction mixture contained 1 ng DNA template, 0.2 mM dNTP, 0.48 μM of each primer, and 1 U TaqEnzyme (Super-Therm). The sequences of primer were set up to amplify a 100 base pairs (bp) target segment of 16s rRNA gene where 5′-GGA TTA GAT ACC CTG GTA GT-3′ (forward) and 5′-CTT GCG RYC GTA CTC CCCA-5′ (reverse). The *E. coli* K12 genomic DNA used as the template amplified. The three dc powers were supplied to the three thin-film heaters for 95 °C, 55 °C and 72 °C, individually, with the 4.51 W, 1.35 W and 3.74 W of power consumption. The DNA chamber was cleaned and dried in the oven with 120 °C for 5 min. 1 μl of the test sample was dropped into the DNA chamber. Afterward, the mineral oil was covered on the chamber to prevent evaporation of the sample because the boiling point of mineral oil was higher than the DNA sample. The DNA sample was denatured at 95 °C during 2 min of the first step in PCR. The tappet was then started to rotate the chamber to go through the three temperature regions which were the 15 s of denaturation at 95 °C, the 15 s of annealing at 55 °C and the 30 s of extension at 72 °C for each cycles. After 30 cycles were executed, the chamber should be kept on 72 °C for 4 min in the final step. The total reaction time was 36 min and the collected samples were analyzed by slab gel electrophoresis. Fig. 13 showed the results of gel electrophoresis of PCR productions. As results of lane 2, the 100 bp of PCR productions was observed and there were no specific products to be found. Thus, the PCR apparatus could amplify DNA segment and to save a lot of time between the temperature changes. However, the amount product of PCR apparatus was less than product of the traditional PCR thermal cycler so the fluorescence intensity being not so good.

5. Conclusions

A simple apparatus for rapid DNA amplification was developed in this research. The major components of the PCR apparatus included dc motor, tappet, heater device, PMMA frame and the temperature control system. The silicon material was chosen as the DNA chamber substance due to the high coefficient of thermal conductivity promoted the temperature distribution uniformly. The commercial simulation software of CFD-ACE+ was utilized to simulate the temperature distribution and transition time of the DNA chamber. The simulation results predicted that the test sample had the temperature difference within 0.2 °C and could achieve fast temperature transition. Finally, a 100-bp DNA segment of *E. coli* K12 was demonstrated successfully for amplification. The total reaction time spent 36 min with 1 μl for 30 cycles. Only the DNA chamber was contaminated in the PCR apparatus and it could be used again after cleaning. In the further experiment, a closed system to integrate with the PCR apparatus has been to constructed for controlling the ambient temperature to obtain the identical convection in each reaction process.

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