ELSEVIER

Chemical Engineering Journal 141 (2008) 327–331

Chemical Engineering Journal

www.elsevier.com/locate/cej

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

Chao-Heng Chien *, Hui-Min Yu, Ming-Tse Lin

Department of Mechanical Engineering, Tatung University, Room 613, No. 7-1, Dehui St., ZhongShan District, Taipei city 104, Taiwan

Received 25 July 2007; received in revised form 10 January 2008; accepted 23 January 2008

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.

* Corresponding author at: Department of Mechanical Engineering, Tatung University, Room 603, No. 7-1, Dehui St., ZhongShan District, Taipei city 104, Taiwan. Tel.: +886 2 25973036; fax: +886 2 25973036.
E-mail address: chchien@mail.ttu.edu.tw (C.-H. Chien).

1385-8947/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.cej.2008.01.039

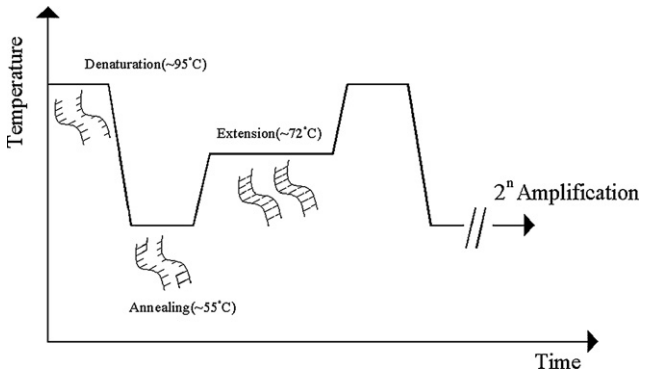

Fig. 1. Schematic diagram of the PCR process [6].

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.

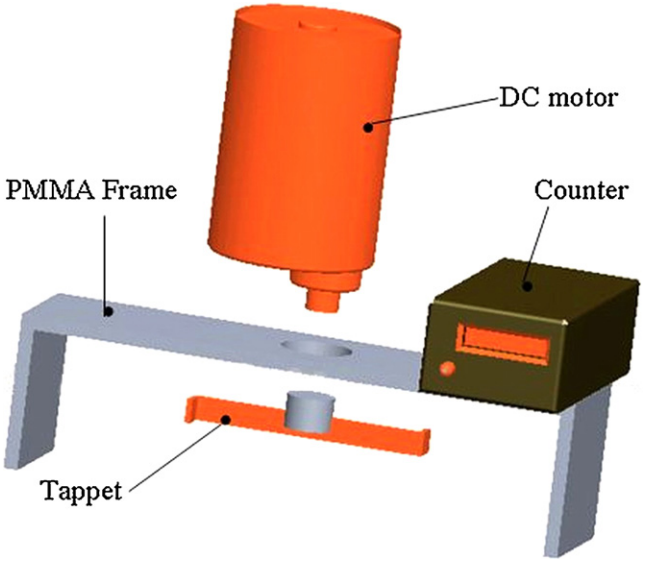

Fig. 2. Schematic of the rotation device of PCR apparatus.

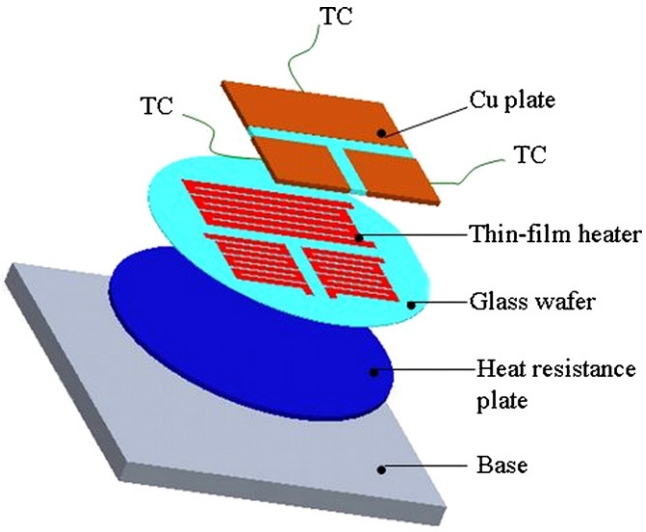

Fig. 3. Schematic of the heater device of PCR apparatus.

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

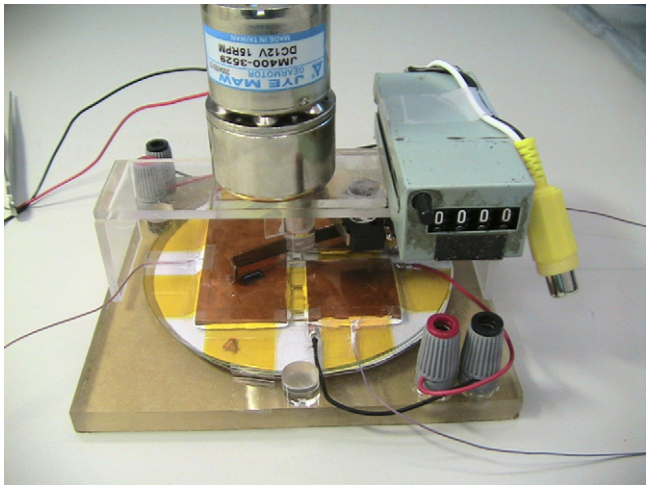

Fig. 4. The completed PCR apparatus.