

DNA amplification by sound and ultrasound frequency vibration

Ryu Kobayashi¹; Seiji Yoneda¹; Shigeo Yamaguchi¹

¹Kanagawa University, Japan

ABSTRACT

In the Polymerase Chain Reaction (PCR) method, which is a strong DNA amplification technology, a step with a high temperature of 94 °C is inevitably included to denature double-stranded DNA to single-stranded DNA. However, the thermal denaturation has problems causing DNA damage and enzyme deactivation. To overcome them, we have proposed and developed a new technology for DNA denaturation and amplification, namely, vibration-driven DNA amplification method (the frequency ranges from 100 Hz to 25 kHz). In our method, DNA is denatured and amplified by vibrating a small plastic tube with at sound to ultrasound frequencies using a wave transducer. In the tube, DNA, enzyme, and other materials are mixed and dissolved in water. Using the DNA amplification system we developed, we examined the optimum condition for DNA denaturation and amplification by changing amplitude, frequency and time of vibration. Maximum amplification rates were obtained at frequencies from 100 Hz to 800 Hz. Moreover, through the agarose gel electrophoresis, we found that no DNA was damaged by vibration.

Keywords: DNA, PCR, Vibration

1. INTRODUCTION

In the DNA amplification technology, the PCR method conventionally used includes a process of dissociating DNA from double-stranded into single-stranded by high temperature of 94 °C. However, this heat denaturation has problems such as DNA damage and enzyme deactivation. Therefore, we have proposed and developed DNA denaturation and amplification method using vibration in the audio frequency band without using high temperature conditions.

In our method, DNA is denatured by vibrating the DNA solution at audible frequencies using a wave transducer. To analyze the results of DNA amplification by vibration, a microchip-type electrophoresis was used. Furthermore, the energy applied to DNA by vibration was estimated, and the relationship between the estimated values and the amplification of DNA was discussed.

2. METHOD

2.1 The PCR method

The conventional thermal PCR is a method of amplifying a target DNA about 2^N times by performing N cycles of a three-step thermal cycle reaction using a primer and an enzyme that bind to both ends of the DNA at the target site.

The thermal cycle is explained as follows. First, double-stranded DNA is denatured into single-stranded DNA by applying heat of 94 (denaturation). Second, the temperatures of the single-stranded DNA and the primer are lowered to 55 °C to mutually complement bind (annealing). Finally, by raising the temperature to 72 °C, the enzyme works to extend the primer. These three steps are one cycle.

Figures 1 and 2 show the DNA model during PCR and, the thermal cycle of the PCR, respectively.

¹ r201870084yq@jindai.jp

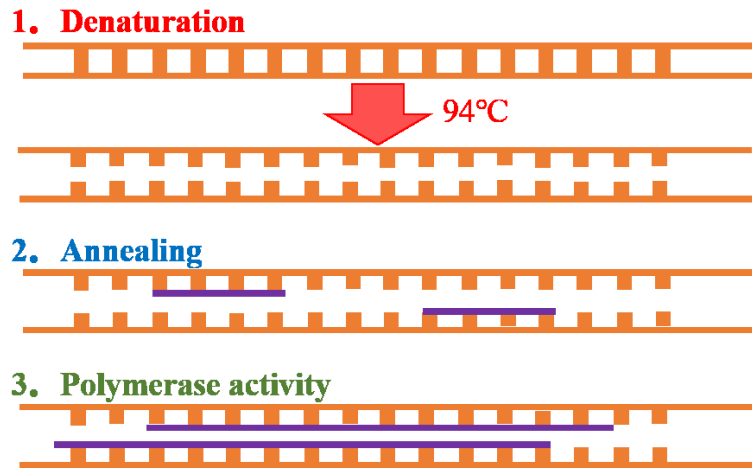


Figure 1 – DNA model during PCR

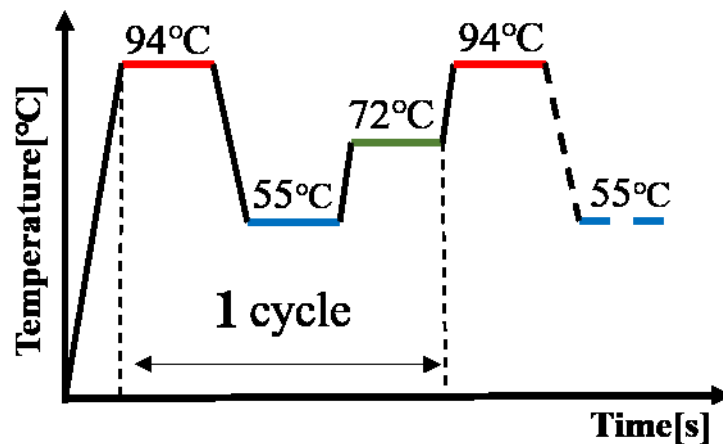


Figure 2 – Thermal cycle of PCR

2.2 Vibration-driven PCR

The conventional PCR method has a problem that it damages DNA and enzymes because it uses high temperature. Therefore, we have proposed and developed a new technology of denaturation and amplification of DNA by vibration in stead of using heat. In the vibration-driven PCR method, accurate amplification, significant reduction of time, and high efficiency are expected.

The method was performed by placing a wave transducer in a thermostatic chamber set to the activation temperature (37 °C) of the enzyme to be used.

Figure 3 shows device configuration of the vibration PCR method.

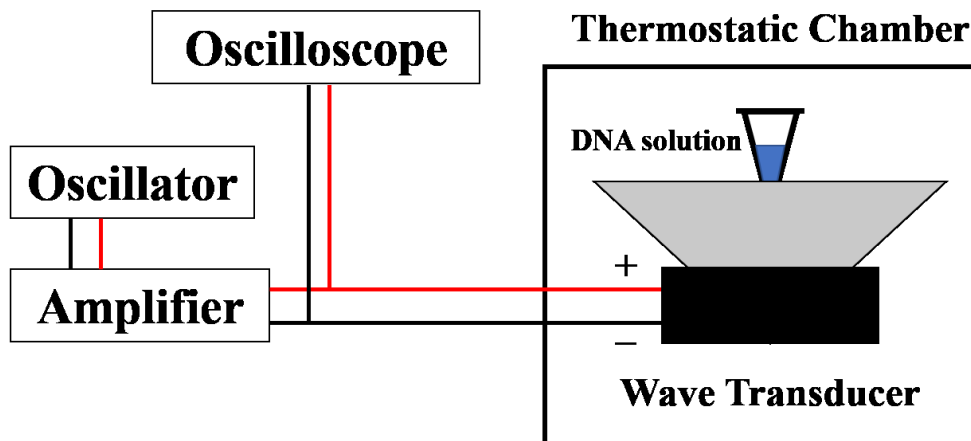


Figure 3 – Configuration of the vibration-driven PCR method

The vibration PCR method is performed through two-step process. First, the DNA solution is denatured by vibration. Next, the annealing in the vibration-free state. These two steps are one cycle. Figure 4 shows the thermal cycle of the vibration PCR method.

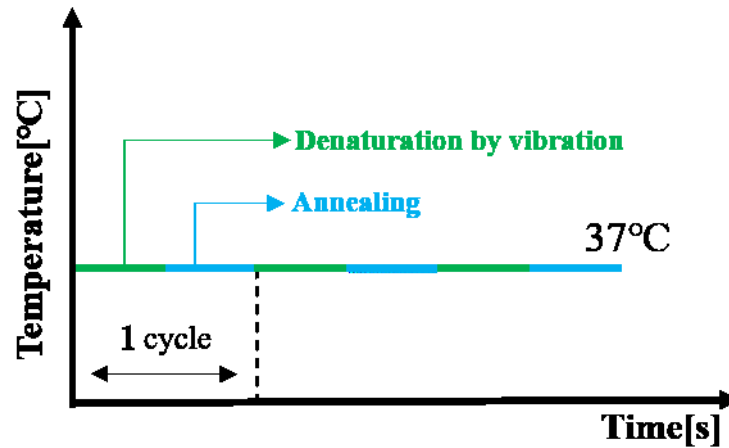


Figure 4 – Thermal cycle of the vibration PCR method

In this study, we simply calculated assuming a single sphere of DNA. The vibrational energy applied to DNA per vibrational cycle is expressed as the following equation.

$$\varepsilon_{v,1cycle} = 2m\omega^2A^2 = 8\pi^2mA^2f^2 \quad (1)$$

Here, m is total mass of DNA in the tube, A is vibrational amplitude of the tube, f is frequency of the tube. Considering the vibration time, it is expressed as the following equation.

$$\varepsilon_v = \varepsilon_{v,1cycle}f\Delta t = 8\pi^2mf^3A^2\Delta t \quad (2)$$

In addition, since the DNA size used in this experiment is 500 bp, the polymer worm strand model of the following equation considering anti-flexibility was applied rather than the complete random coil model. For a DNA of $q = 44$ nm (full length $L = 170$ nm), the radius of inertia is estimated 35nm.

$$\sqrt{\langle S^2 \rangle} = \frac{1}{3}Lq - q^2 + \frac{2q^3}{L} \left[1 - \frac{q}{L} (1 - e^{-L/q}) \right] \quad (3)$$

The number of DNA in the tube is on the order of 10^{10} , and the maximum vibrational energy applied per DNA sphere (including water) from the radius of inertia is estimated to be 9.1×10^{-18} J from equation (1).

3. EXPERIMENTAL METHOD

First, using a wave transducer, the entire microtube containing a solution of DNA (size 500 bp; bp = base pair) is vibrated. The vibration conditions at that time are a frequency of 100 to 800 Hz (amplitude of 0.44 to 0.015 mm), a voltage of 5 V, a vibration time of 10 seconds, and no vibration time of 30 seconds, 5 cycles and 10 cycles.

Next, the size and amplification rate of DNA after vibration are measured by capillary electrophoresis.

Furthermore, the energy applied to the DNA in the tube from the outside is calculated from the analysis results of capillary electrophoresis and the equations (1) to (3).

4. EXPERIMENTAL RESULT

Figure 5 shows the amplification rate at 5 cycles estimated by capillary electrophoresis, and Figure 6 shows the amplification rate at 10 cycles. Figure 7 shows the vibrational energy characteristic calculated from equation (2).

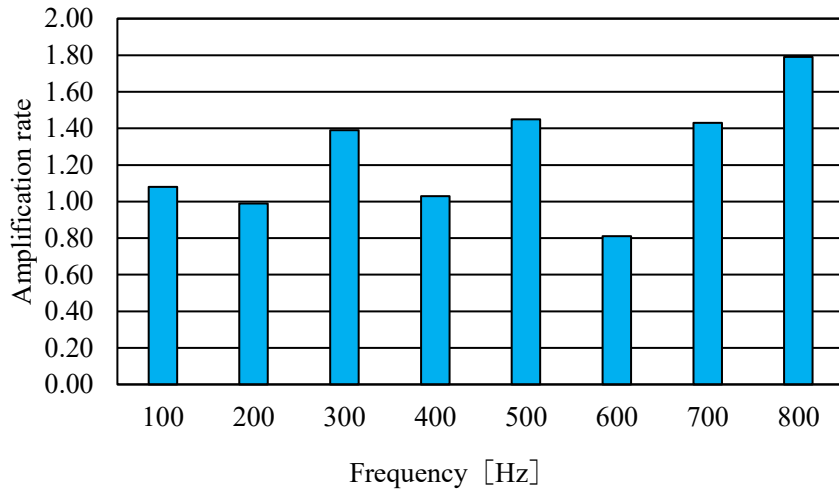


Figure 5 – Amplification rate at 5 cycles

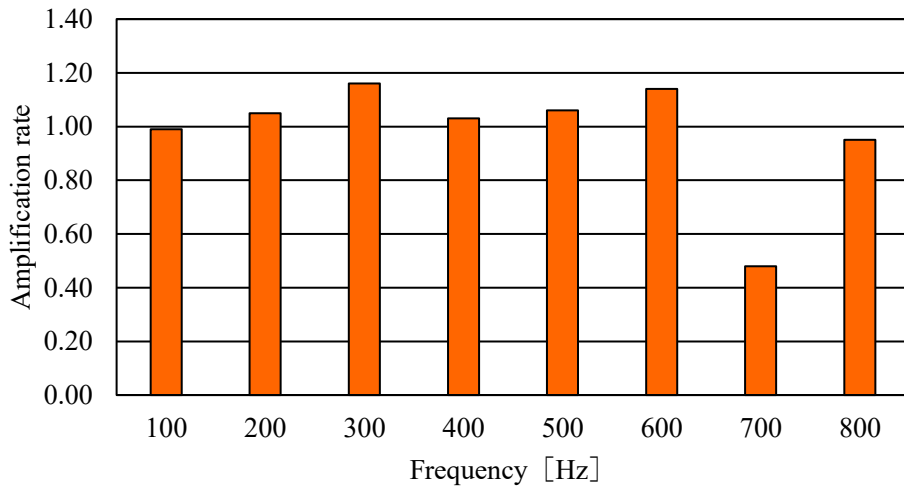


Figure 6 – Amplification rate at 10 cycles

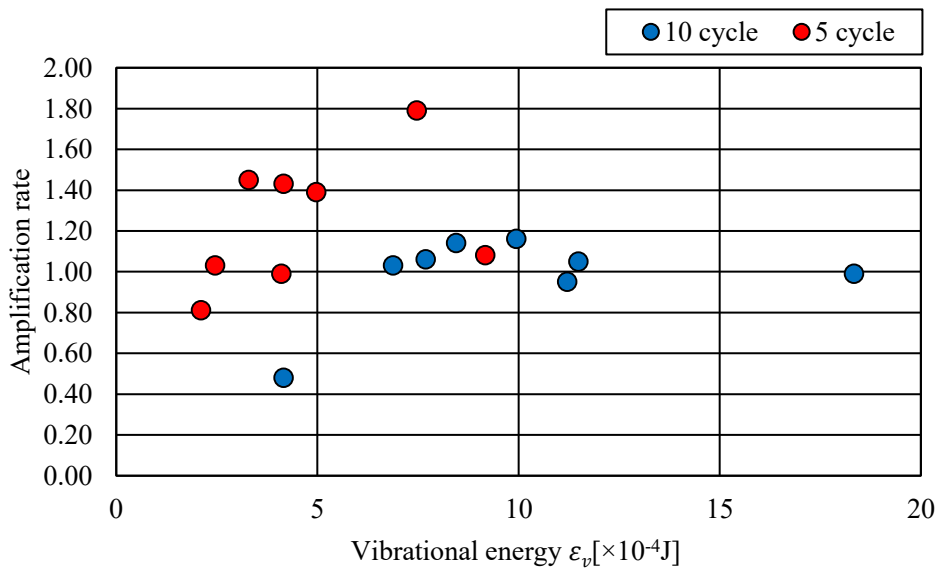


Figure 7 – Vibrational energy characteristics calculated from equation (2)

5. CONCLUSIONS

Amplification, rate was 1.8 times at the maximum. The vibration condition at that time was 800 Hz for 5 cycles. The amplification rate was, as a whole, higher in 5 cycles than in 10 cycles. These results indicate that when the vibrational energy applied to the DNA is too high, the enzyme is deactivated.

ACKNOWLEDGEMENTS

This research is funded by JSPS research grant JP18H03524.

REFERENCES

1. O. Kratky and G. Porod, Recl. Trav. Chim. Pays-Bas, 68, 1106 (1949).
2. E. S. Sobel and J. A. Harpst, Biopolymers, 31, 1559 (1991)