Fundamental study on DNA denaturation and amplification by vibration

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1. Introduction

Currently, the polymerase chain reaction (PCR) method is an inevitable tool for DNA amplification. In the PCR method, a high temperature of 94 ° C is applied to DNA to dissociate (denature) double-stranded DNA into single-stranded DNA. It has been pointed out that the process of heat denaturation causes DNA to be damaged and enzyme to be inactivated. To overcome this problem, we have proposed and developed a novel method of denaturing and amplifying DNA by applying vibration using a wave transducer. In this study, we focused on vibration-driven DNA denaturation to improve the reproducibility of DNA amplification.

2. Experimental method

The conventional PCR is a powerful technology in medical and biological research to amplify a specific region of DNA up to several orders of the number of DNA through thermal cycles. The thermal cycle process is as follows (shown upper in **Fig.1**). First, the DNA solution is heated to 94°C so that double-stranded DNAs are denatured into single-stranded DNAs. Next, the temperature is lowered to 60°C, and subsequently, the single-stranded DNAs and the primers are bound (Annealing). Finally, the temperature is raised to 72°C to allow the dNTPs to bind to the primer chain (Elongation). However, the conventional PCR process has problems of DNAs being damaged and enzymes deactivated with exposed at such high temperatures.

To overcome these intrinsic problems in the conventional PCR, we have proposed and developed a novel method of vibration-driven denaturing and amplifying DNA [1]. In this technique, DNA solution itself in a plastic tube with a wave transducer is vibrated at audible frequencies as shown in **Fig.2**. Moreover, this method is mainly featured by being processed at 37°C, at which the enzymes are most activated, leading to significant reduction in process time.

The process of our vibration-driven PCR as follows (shown lower in **Fig.1**). Double-stranded DNAs are denatured into single-stranded DNAs by vibrating the entire plastic tube containing DNA solution. Then, annealing and elongation processes are performed at 37°C fixed.

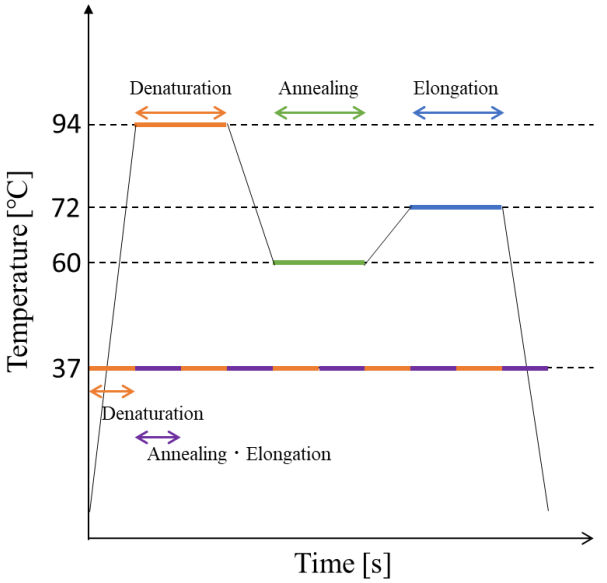

Fig. 1 Thermal cycle sequence. The conventional PCR (upper) and our vibration-driven PCR (lower).

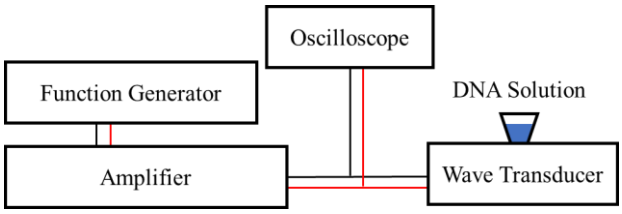

Fig. 2 Schematic of our vibration-driven PCR system.

In this study, we have focused on the analysis of DNA denaturation to achieve high amplification rate. A set of DNA solution in a plastic tube containing template DNA, primers, enzymes, and dNTPs is placed in a wave transducer as shown in **Fig.2**. After some cycles of vibration, the DNA solution was analyzed using capillary electrophoresis or gel electrophoresis to estimate the amount of double-stranded DNA before and after denaturation. The energy applied to DNA solution, U, is evaluated by [1]

$$U \propto f^3 A^2$$

A: vibration amplitude, f: frequency

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The vibration amplitude was measured using a laser displacement sensor.

3. Experiment results

Fig.3 and 4 show the amount of double-stranded DNA as a function of vibration amplitude at a frequency of 150 Hz. The values of the amount at A of 0.2mm and 1.85mm were set a reference, respectively.

These results show that double-stranded DNAs are denatured at over 1.85mm, demonstrating that vibration-driven DNA denaturation has a threshold of external energy.

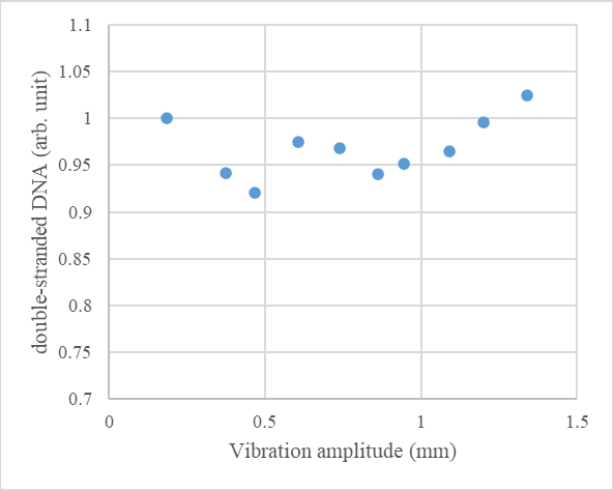

Fig.3 The amount of double-stranded DNA as a function of vibration amplitude A ($0<A<1.5$mm) at a frequency of 150 Hz.

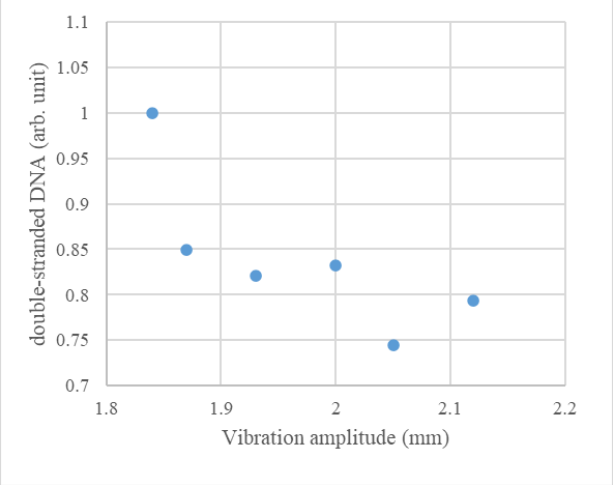

Fig.4 The amount of double-stranded DNA as a function of vibration amplitude A ($1.8<A<2.2$mm) at a frequency of 150 Hz.

4. Conclusion

We analyzed vibration-driven DNA denaturation at audible frequencies using a wave transducer. The results demonstrated that the denaturation process has a threshold of external energy and the denaturation rate increased with increasing vibration amplitude.

References

1. R. Kobayashi, S. Yoneda, and S. Yamaguchi, Proceeding of the 23rd. International Congress on Acoustics, 2019, Germany, p.4808-4812