Real-Time Observation of Vibration-Driven DNA Denaturation

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1. Introduction

Currently, the PCR method is mainly used for DNA amplification, and requires high temperatures during the process of dissociating (denaturing) double-stranded DNA into single-stranded DNA. However, this process may cause DNAs to be damaged and enzymes to be inactivated. To overcome this problem, the authors have proposed and developed a vibration-driven PCR method in which DNA is dissociated (denatured) and amplified by vibration at a constant temperature of 37℃[1].

The conventional thermal cycle process is shown in **Fig.1(a)**. First, the DNA solution is heated to 94℃ so that double-stranded DNAs are denatured into single-stranded DNAs. Next, the temperature is lowered to 54 ℃, 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 (Extension).

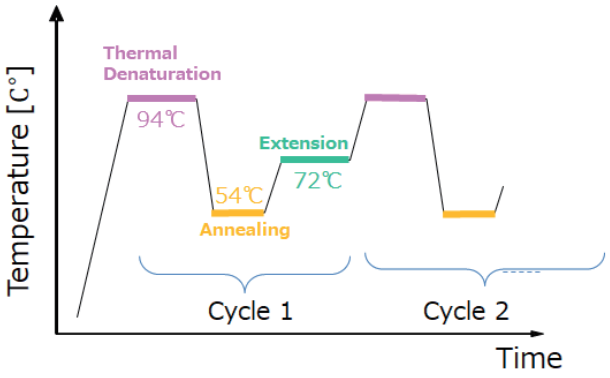

Fig.1(a) Conventional thermal PCR cycle

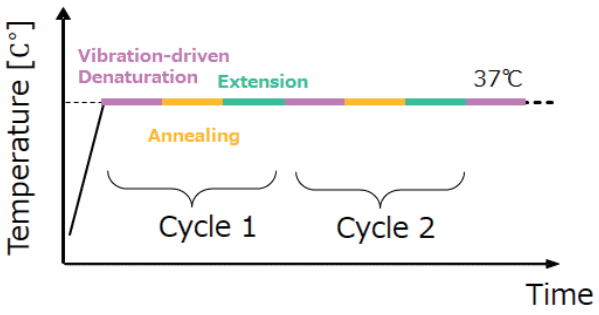

Fig.1(b) Vibration-driven PCR cycle

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In our previous study, we paid attention to vibration-driven DNA denaturation to improve the reproducibility of DNA amplification[2]. In this study, to analyze the denaturation process in detail, we aimed to construct a system where vibration-driven DNA denaturation process can be observed in real time.

2. Experimental method

Vibration-driven denaturation was so performed that DNA solution in a plastic tube was vibrated at audible frequencies at a constant temperature of 37℃. As shown in **Fig.2**, real-time observation system was constituted with a combination of a spectrofluorophotometer and a vibration denaturing system. In the system, the intensity of the fluorescence from the dye intercalated into the double-stranded DNA was measured while vibrating the tube.

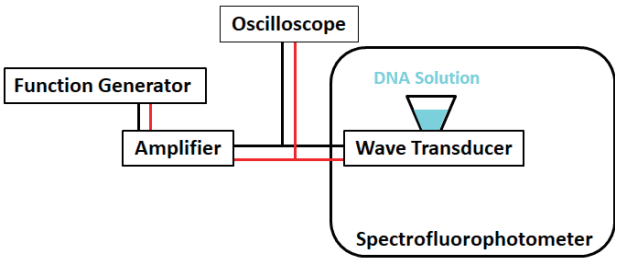

Fig. 2. Real-time observation system

3. Experimental results

One cycle was composed of a vibration time of 50s and no vibration time of 15s. The amplitude was 0.4mm to 0.8mm. Three cycles were performed. For each cycle, the denaturation rate was calculated with reference to the previous cycle.

Under the same conditions, measurements were made at frequencies of 90Hz and 110Hz with different amplitudes, and the denaturation rate was calculated.

Figure 3 shows the duration time dependence of DNA vibration-driven denaturation rate as parameters of amplitude ranging from 0.4 mm to 0.8 mm. It was found that in the first cycle, the

denaturation rate increased as the amplitude increased.

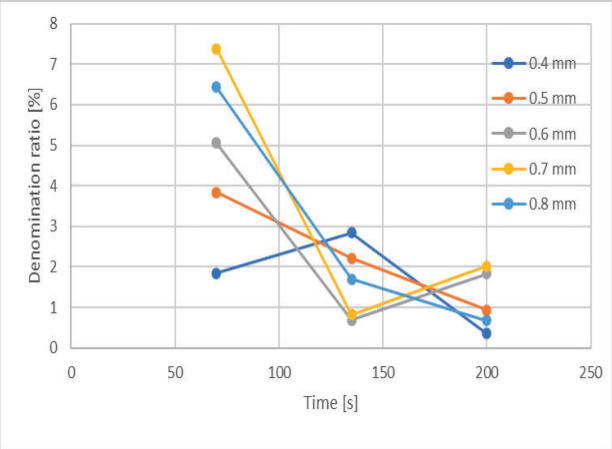

Fig.3 Duration time dependence of DNA denaturation rate

Figure 4 shows the denaturation rate as a function of amplitude when the frequency was changed from 90 Hz, 100 Hz, to 110 Hz. The denaturation rate was found to be higher at higher frequencies for the same amplitude.

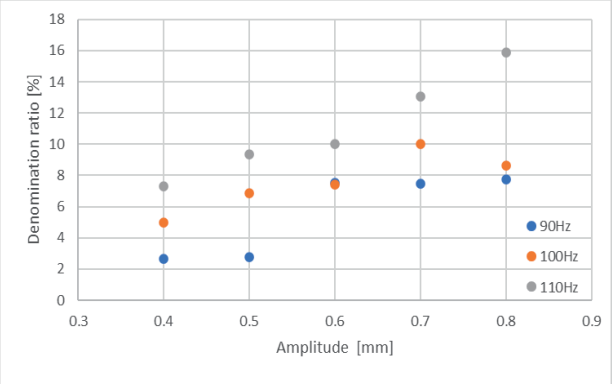

Fig.4 Degeneration rate at different frequencies

4. Conclusion

We aimed to construct a real-time observation system for DNA denaturation driven by vibration at audible frequencies and at a constant temperature of 37℃. The results through the system showed that the denaturation rate increased as the amplitude increased and as the frequency became higher.

5. Acknowledgment

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References

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