Simultaneous Amplification of Multiple DNA Targets with Optimized Annealing Temperatures

N. Pak[1], C. R. Phaneuf[1], D. C. Saunders[1], and C. R. Forest[1]
[1]Georgia Institute of Technology, Atlanta, GA

Introduction: The polymerase chain reaction (PCR) allows for highly specific and sensitive detection of pathogens by exponentially amplifying a specific region of DNA from as little as a single copy through thermal cycling a biochemical cocktail. Microfluidic PCR devices offer smaller volumes of costly reagents, integration with other analyses, and faster cycling times. However, most of these devices rely on indirect heating through silicon or glass microchips and can only run one temperature profile at a time [1]. We have created a microfluidic PCR device that uses spatially modulated infrared (IR) radiation and closed-loop temperature feedback control to directly heat multiple 1µL volumes on a polymer microchip with unique temperature profiles in order to optimize different thermal reaction conditions on the same chip, simultaneously.

Materials and Methods: The device consists of a single laser diode driver (Wavelength Electronics, PLD5K-CH) operated through a custom Labview program that powers two identical 1450 nm, 600 mW laser diodes. An optical shutter spatially modulates, via absorption, the radiation reaching the reaction chambers so that unique temperatures can be achieved. Other components include thermocouples, collimating lenses, heat sinks, and fans for each chamber. Furthermore, the chip is mounted on a pressure manifold delivering 40 psi nitrogen to inhibit bubble formation in the chambers. The Labview program measures the temperature at each chamber relative to a target and utilizes a proporational-derivative controller to modulate the shutter duty cycle through pulse width modulation and analog laser driving voltage to obtain accurate and repeatable thermal cycles for the 3 discrete temperature holds required for a typical PCR amplicon (e.g., denaturing at 94°C, annealing at 50-70°C, extension at 72°C).

Results and Discussion: A plot of a two temperature profiles run simultaneously on the same chip can be seen in Figure 1. The shutter is active during the annealing stage to decrease the temperature in that chamber to match the optimal annealing temperature of each primer. We will report progress on testing microfludic PCR of a human four-plex reaction [2] that is highly temperature specific in multiple chambers.

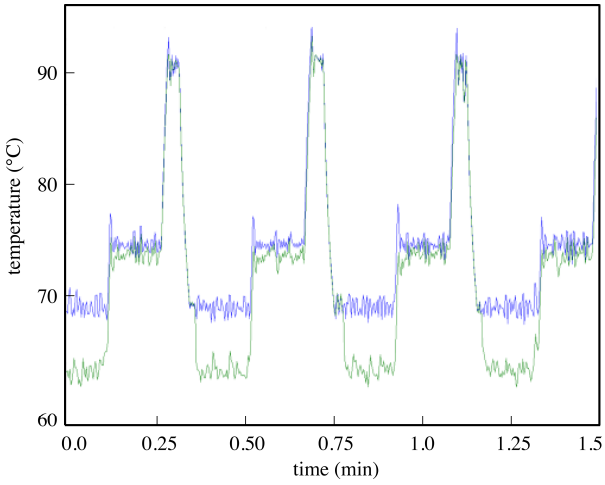

Figure 1. Temperature profiles of adjacent 1 µL reaction chambers, 1 mm apart, heated by the same laser diode source. Different annealing temperatures are achieved by modulating an optical shutter.

Conclusions: The infrared mediated thermocycler presented here provides a fast, non-contact, small volume option for performing PCR in a high-throughput manner. The ability to optimize the temperature profile of each individual chamber will greatly reduce the amount of time and effort required for viral screening and detection.

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References:

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