THERMALLY-MULTIPLEXED MICROFLUIDIC PCR

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ABSTRACT

We present a new thermocycling method, termed thermal multiplexing, in which a heat source is uniformly distributed and selectively modulated for independent temperature control of an array of PCR reactions. This allows amplification of multiple targets simultaneously—each reaction segregated and performed at optimal conditions. We demonstrate the method using a microfluidic system consisting of an infrared laser thermocycler, a polymer microchip, and closed-loop pulse-width modulation control. We performed two reactions simultaneously with widely varying annealing temperatures (48°C, 68°C), demonstrating amplification of both targets as enabled by thermal multiplexing.

KEYWORDS: Polymerase Chain Reaction, Microfluidics, Multiplexing, Radiative Heating

INTRODUCTION

The polymerase chain reaction (PCR) is a standard technique for nucleic acid amplification [1], implemented for a multitude of applications in molecular biology, including sequencing, forensics, and disease diagnostics. Briefly, PCR allows the exponential replication of a specific region of DNA or RNA from as little as a single copy by thermocycling a biochemical cocktail. The specificity of the amplification is determined by short, custom-synthesized oligonucleotides known as primers that attach, or anneal, to the template DNA at regions flanking the sequence of interest, with every unique set of primers requiring a unique temperature for this annealing.

Despite advancements in the speed, throughput, and minimum sample volume of PCR instruments, operation is typically limited to uniform thermal conditions. Yet, different genetic targets have corresponding optimal annealing temperatures that depend on the length and GC content of their primers. Consequently, conventional instruments only allow a single type of reaction per run. Multiplex PCR is a technique for overcoming this limitation in which sets of primers corresponding to multiple targets are designed to anneal at a common temperature. Drawbacks to multiplex PCR include amplification bias due to efficiency variations, PCR drift, and the high likelihood of adverse primer-primer interactions [1,2]. In addition, the technique requires skilled primer design and cannot always accommodate all desired target sequences [3]. The limitation of this biochemical approach can be addressed through instrumentation.

Here we introduce thermal multiplexing via spatial modulation of a multi-channel laser source for flexible sample-to-sample temperature control. This expands upon previous work [4-6] in which we presented a rapid, low-cost PCR platform that combines infrared radiative thermocycling with a polymer microchip, and is distinct from similar work [7,8] in which laser heating is implemented with droplet-based microfluidic platforms.

THEORY

To perform thermally multiplexed PCR, a heat source is uniformly distributed, independently modulated, and directed to an array of reaction chambers as depicted in Fig. 1a. The heat source delivers a constant power, q, divided equally amongst n chambers as q/n. Each attenuator (e.g., shutter, valve, filter, variable resistor) modulates the power q/n according to a unique programmed function (e.g., pulse width modulated square wave), $f_i(t)$ for $1<i<n$ and $0 \leq f_i(t) \leq 1$. Each chamber is thus heated to temperature profile $T_i(t)$, shown in Fig. 1b, by the power profile $q_i(t)=(q/n)f_i(t)$.

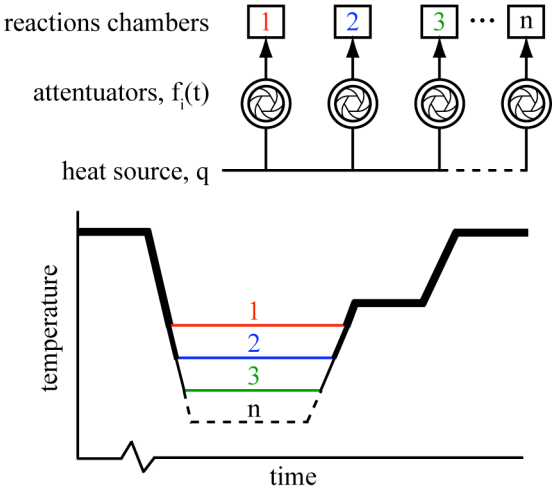

Figure 1: Thermal multiplexing can be generalized to a uniformly distributed and independently attenuated heat source delivered to an array of reaction chambers (top). Theoretical temperature profiles for n reactions illustrate the capability of maintaining a set of distinct annealing temperatures for optimal amplification of multiple DNA targets (bottom).

EXPERIMENTAL

Thermal multiplexing was implemented using 1450 nm infrared laser radiation as the heat source for parallel reactions, shown in Figure 2. A PMMA microchip features two 1 µl reaction chambers with ports designed for easy sample injection using a micropipette. Samples are loaded as a plug between volumes of mineral oil for sample confinement and passivation purposes [5,6]. Miniature solenoid shutters, driven independently by 10 Hz pulse-width modulated signals, serve as attenuators. A pressure manifold delivers 40 psi to the microchip ports to prevent the expansion of air bubbles during thermocycling. Temperature feedback is provided by 125 µm diameter thermocouples embedded in the microchip. An algorithm programmed in LabVIEW controls thermocycling. A proportional-derivative (PD) controller modifies the duty cycles of the solenoid control signals to independently attenuate the infrared radiation for accurate temperature holds. Two unique PCR reactions with high and low annealing temperatures were performed. The high temperature reaction targets a 500 bp amplicon from λ-phage DNA with a 68°C annealing temperature, while the low temperature reaction targets a 600 bp amplicon from Epstein-Barr virus (EBV) DNA with a 48°C annealing temperature.

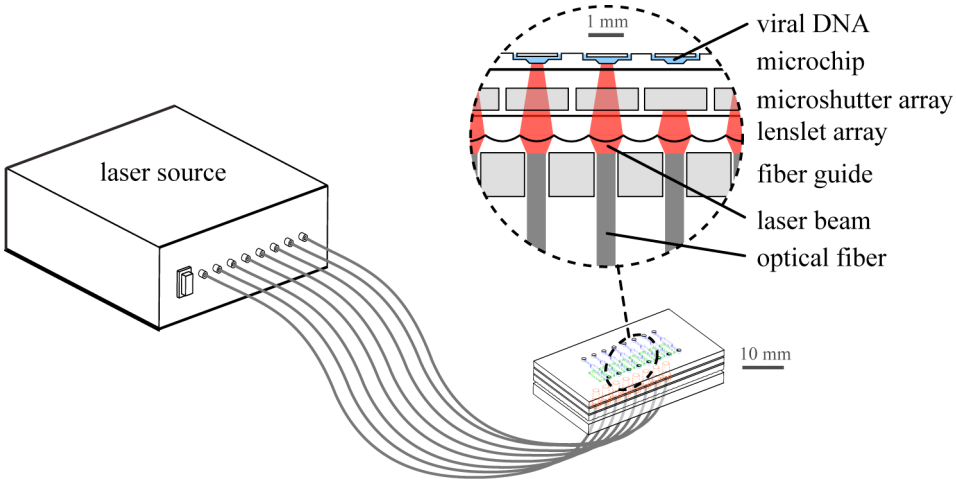

Figure 2: An infrared laser delivers radiation to an array of 1 µl reaction chambers. An optical system (inset), including a solenoid shutter array, provides spatial modulation to enable thermal multiplexing.

RESULTS

The two distinct thermocycling profiles required to simultaneously perform the λ-phage and EBV amplifications is shown in Figure 3. Total runtime was roughly 110 min to complete 40 cycles with average heating rates of 2.54°C/s and cooling rates of 2.53°C/s (n=5). Cycle-to-cycle temperate accuracy was within 0.64°C (average absolute difference over three consecutive cycles), with an average standard deviation of 0.46°C within a cycle.

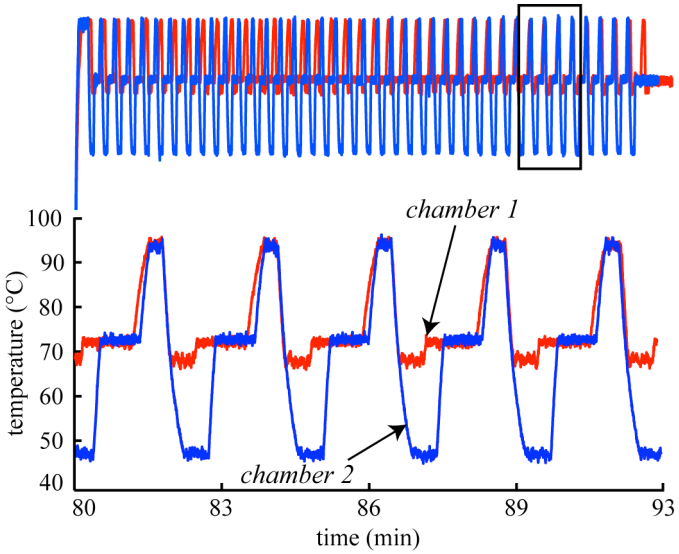

Figure 3: Independent temperature profiles for λ-phage (red) and EBV (blue) amplifications were generated simultaneously with distinct annealing temperatures of 48°C and 68°C. This system utilized closed-loop control via thermocouples feedback and shutter-based optical modulation of infrared laser radiation in 1 µl reaction chambers on a polymer microchip.

Electropherograms for the microchip-based λ-phage and EBV amplifications can be seen in Figure 4, illustrating that thermal multiplexing was required to simultaneously amplify both targets.

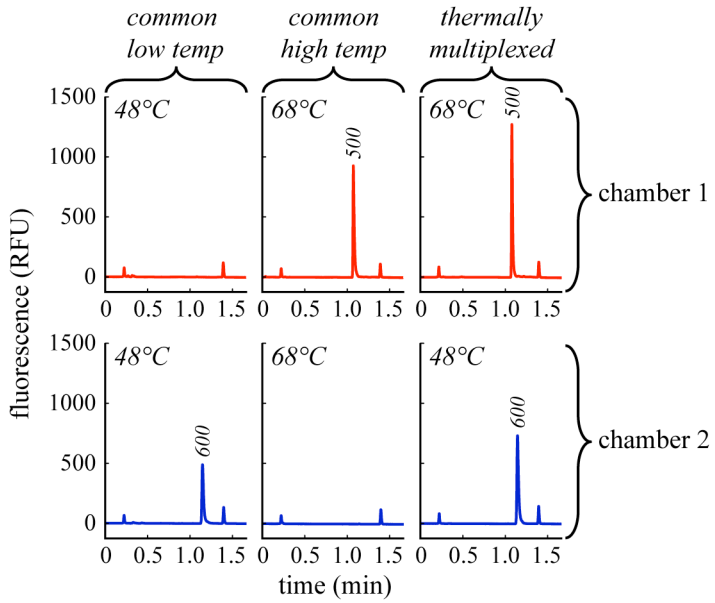

Figure 4: Electropherograms show PCR products for λ-phage (top row) and EBV (bottom row) amplifications run in parallel using three different thermocycling runs (represented by each column). From left to right, annealing temperature was uniform across the two reaction chambers at low and high values and then thermally multiplexed to maintain the ideal temperatures for each reaction. Small peaks correspond to sizing and quantification markers (15 bp, 1500 bp).

CONCLUSION

Without the need for specialized reaction design as required for multiplex PCR, thermal multiplexing allows the consolidation of any set of existing reactions into a single instrument run. We are currently scaling the platform to eight reactions and will report progress using it for patients from the Children's Healthcare of Atlanta (CHOA) Pediatric Hospital in which nasopharyngeal swabs will be screened for a variety of pathogens.

ACKNOWLEDGEMENTS

We acknowledge funding from the Georgia Tech Translational Research Institute for Biomedical Engineering & Science (TRIBES) Seed Grant Awards Program and support from Georgia Tech through the Institute for Bioengineering and Biosciences Junior Faculty Award. We would like to acknowledge the members of the Division of Viral Disease at the Centers for Disease Control and Prevention as well as Mark McJunkin and Marty Jacobson for their assistance.

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