Topic No. 7.02 (first priority), 8.04(second priority) Abstract Reference No: 0912
Oral or Poster

40 CYCLE PCR USING HUMAN GENOMIC DNA IN LESS THAN 1 MINUTE ON A MICROFLUIDIC CHIP

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We introduce a 1-2 second/cycle Polymerase Chain Reaction (PCR) on a microfluidic chip with only 50 ng (14, 500 copies) of a 102 bp target (from human genomic DNA) as starting template concentration in the reaction mix. The target was amplified in 40 cycles (between 40 – 80 seconds) to produce a sharp, distinct peak (see Figure 1 bottom) on a high resolution melting instrument. In our knowledge this is the fastest PCR ever accomplished on a microfluidic chip with a low concentration of target template, the target is part of the human genome, and can be amplified to a significant concentration so that it can be easily detectable with commercial systems.

Polymerase Chain Reaction (PCR) is one of the most important reactions in molecular diagnostics. Consequently the microfluidics research community, since its inception in the early 1990s has always tried to make contributions to the capability of PCR with regard to instrumentation. These efforts have been mostly targeted towards increasing the speed of PCR, and also reduction in footprint of a PCR instrument to make it more adaptable to point-of-care testing [1]. Though successful efforts have been made in both of these aspects none of the demonstrated approaches have been able to sustain the efficiency and yield when cycle times were reduced from 20 s to 2 s. Furthermore, most of the target templates were significantly in higher concentrations or were from plasmids, viruses, and bacteria; which are smaller and more easily amplified quickly. The uniqueness of our approach is that we match the rapid heat transfer rates available in microfluidics with rapid reaction kinetics of a unique reaction mix; a PCR reaction mix developed by Farrar & Wittwer, referred to as Extreme PCR [2].

The design/working principle of our microfluidic chip is also innovative, since it is a blend of thermal gradient continuous flow PCR [3,4] and shuttle PCR (that moves a reaction mix between two/three isothermal zones on a chip) [4]. The chip has 20 cycles built into it. A plug of reaction mix (10-15 μl) is transported back and forth once by off-the-shelf pressure regulator to complete 40 cycles. The chip is made by cutting the microchannel in polyimide tape (double-side adhesive) by a knife plotter and then bonded between two microscope glass slides. The lower glass slide is spray painted dull black so that the plug of PCR mix is easily visible to the naked eye. A 102 bp NQ01 gene of the human genome is used to test the system for extreme PCR. Once all cycles are completed the product was melted in a high resolution melting analysis system for product identification. Figure 1 shows the melt curves of the negative control (before and after being flowed through the chip) and the PCR product. Figure 2 depicts a 2D sketch of the chip with top-glass slide surface temperatures, and COMSOL simulation results of the microfluidic chip to achieve desired temperatures for successful amplification. Figure 3 is a picture of the actual chip.

Word Count: 497

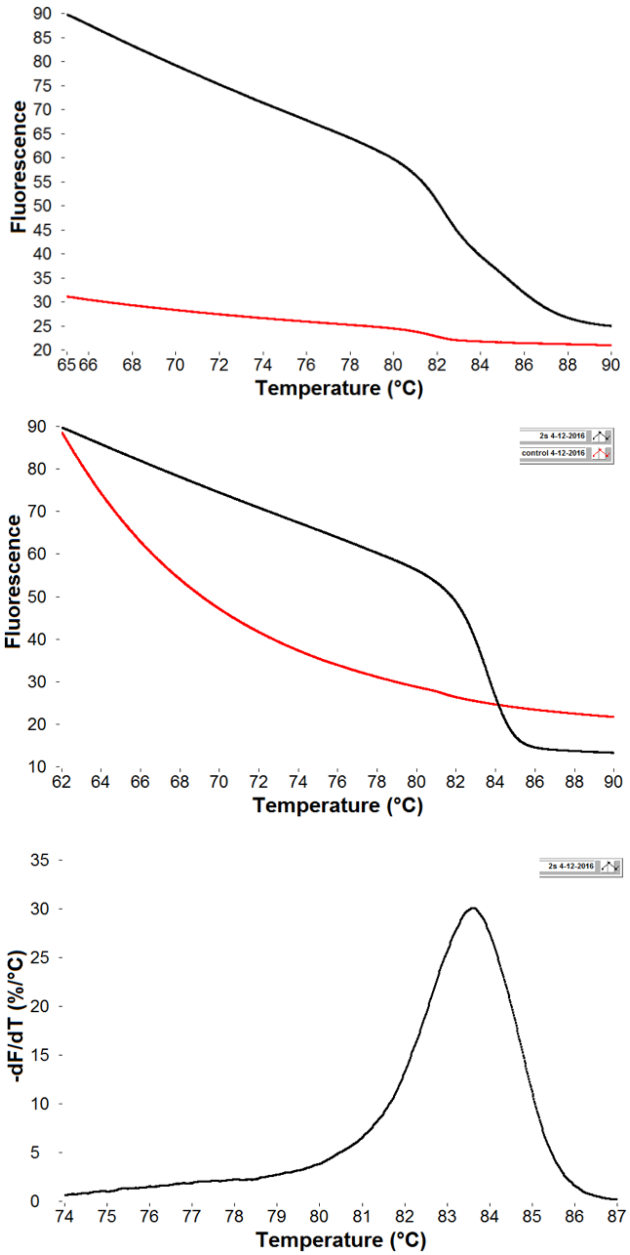

Figure 1: Melt curves for PCR product (102 bp NQ01) and negative control.

Top: PCR product + probe (black), and negative control + probe (red) that was run through the chip. The probe is used to further confirm the identification of the PCR product.

Middle: PCR product (black,) and negative control (red) that was not run through the chip.

Bottom: PCR product (black) with a distinct peak based on derivative of fluorescence intensity with respect to temperature against temperature.

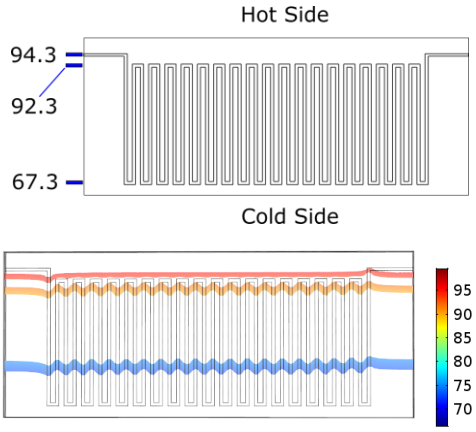

Figure 2. All temperatures are in ˚C.

Top: 2D depiction of the chip design with hot/cold side and experimental values of steady-state temperatures at top-glass surface at different levels of the chip.

Bottom: COMSOL simulation results showing the presence of thermal regions for all three steps of PCR at fluid flow rates of ~ 2 seconds/cycle. The vertical length of each of these regions is crucial to ensure that all three steps of PCR get enough time to execute.

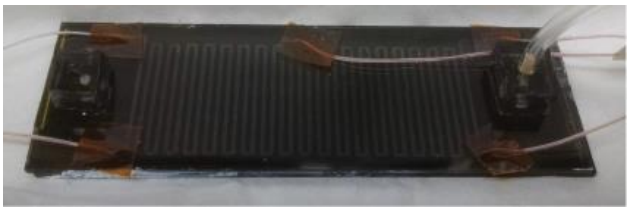

Figure 3. The actual chip for extreme PCR with attached thermocouples and access ports for inlet/outlet

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