CONVECTIVELY DRIVEN POLYMERASE CHAIN REACTION THERMAL CYCLER

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ABSTRACT

Building on LLNL's history of making field deployable instruments we have designed and fabricated a new convectively driven thermal chamber for PCR that is inexpensive to manufacture and has low power consumption [1-3]. Both a 58 and 160 base pair segment of a multiple cloning site fragment of DNA have been successfully amplified in the convectively driven thermal cycler.

KEYWORDS

Convectively-driven PCR, disposable PCR

INTRODUCTION

With the increase in awareness and preparation for a biological terrorist attack, polymerase chain reaction (PCR) assays have become ever more important in identifying the causative agent of an attack. To be used by first responders a device needs to be easy to use, robust, low power and inexpensive. Forensic organizations or environmental specialists looking for the source of contamination in food or water, as well as clinicians looking for cheap PCR diagnostics could also benefit from such a device.

EXPERIMENTAL

Like flow through PCR devices [4-6] rather than temporally heating the entire chamber between the denaturation, annealing and extension temperatures, the convectively driven PCR thermal cycler has zones that are held at fixed temperatures for the entire duration of the experiment. However, unlike the flow through chambers we do not use an external force to drive the fluid through the different temperature zones. The disposable PCR thermal chamber uses buoyancy forces to drive the sample between the temperature zones needed for PCR amplification, Figure 1 [7,8].

RESULTS AND DISCUSSION

Fluid flow was visualized using digital particle image velocimetry. The average velocity was approximately 2mm/s. This velocity was in good agreement with modeling. Details of the modeling will be presented in this paper.

Both a 58 and 160 base pair segment of a multiple cloning site fragment of DNA have been successfully amplified in the convectively driven thermal cycler. The agarose gel detection of the amplicon is shown in Figure 2.

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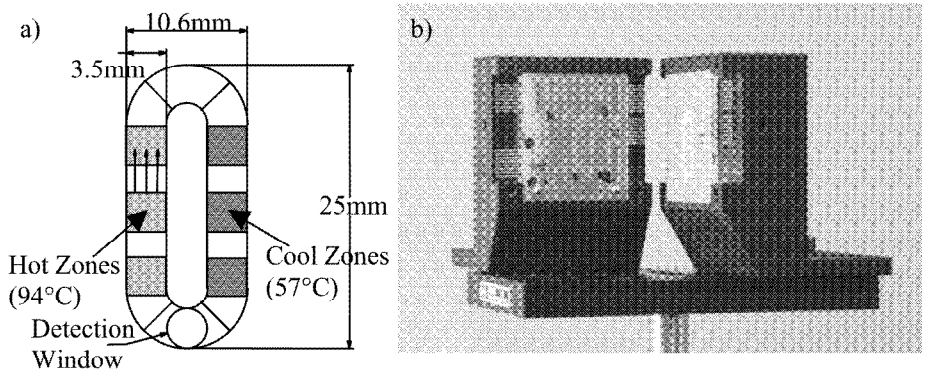

Figure 1: a) Novel thermal chamber uses natural convective forces to drive the fluid between the two temperatures needed for PCR amplification, b) Photograph of actual disposable convective PCR thermal cycler. Sample is placed in a polypropylene bag that is sandwiched between the two boards, forcing the fluid to conform to the channel.

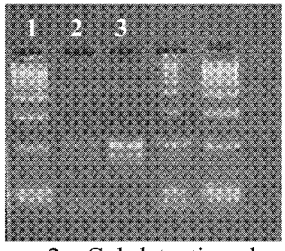

Figure 2: Gel detection showing successful amplification of 160bp DNA segment from multiple cloning site DNA. Lane 1: DNA sizing ladder (50,150,..bp), Lane 2: sample amplified in convective PCR thermal cycler, Lane 3: sample amplified in standard benchtop block thermal cycler.

Power analysis of the thermal chamber shows that a 30 minute amplification run takes 866±50J – allowing the thermal cycler to be operated on a battery. Analysis of the amplification time shows that amplification of just 20 minutes results in detectable amplification reducing the power consumption even further. An investigation of the efficiency of the PCR amplification in the convectively driven thermal cycler will also be presented.

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REFERENCES

1. Belgrader, P.; Benett, W.; Hadley, D.; Richards, J.; Stratton, P.; Mariella, R. Jr.; Milanovich, F. *Science* **2000**, 284, 449.
2. Belgrader, P.; Smith, J.K.; Wedn, V.W.; Northrup, M.A. *J. Forensic Science* **1998**, 43, 315-319.
3. Northrup, M.A.; Benett, B.; Hadley, D.; Landre, P.; Lehew, S.; Richards, J.; Stratton, P. *Anal. Chem.* **1998**, 70, 918-922.
4. Kopp, M.U.; de Mello, A.J.; Manz, A. *Science* **1998**, 280, 1046.
5. Obeid, P.J.; Christopoulos, T.K.; Crabtree, H.J.; Backhouse, C.J.; *Anal. Chem.* **2003**, 75, 288-295.
6. C.F. Chou, C.F.; Changrani, R.; Roberts, P.; Sadler, D.; Lin, S.; Mulholland, A.; Swami, N.; Terbrueggen, R.; Zenhausern, F.; *Micro Total Analysis Systems* **2001**, 151-152.
7. Bennet, W.J.; Richards, J.B.; Milanovich, F.P. U.S. Patent Application Number 09/802,549, "Convectively Driven PCR Thermal-Cycling". Filed March 09, 2001.
8. Wheeler, E.K.; Benett, W.; Ness, K.; Stratton, P.; Richards, J.; Weisgraber, T.; Papavasiliou, A. Laboratory Directed Research and Development FY2001 Annual Report, 2001, UCRL-LR-113717-01, 3-8.