PCR AMPLIFICATION OF STR LOCI USING AN INFRARED LASER SOURCE

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ABSTRACT

In order to effectively reduce the analysis time required for conventional processing of forensic biological samples, the most time-consuming analysis step, PCR amplification, must be expedited. The transition from conventional to microfluidic PCR amplification allows for substantial (5-fold) time reduction. This work demonstrates the first use of an IR laser source for IR-mediated heating combined with non-contact temperature sensing for PCR amplification of STR loci for forensic DNA analysis on a microdevice. A conventional 3h 30 min amplification time is reduced to as little as 36 min in the microfluidic regime without sacrificing quality standards set by the forensic community.

KEYWORDS: Polymerase Chain Reaction, Deoxyribonucleic Acid, Forensic STR Analysis

INTRODUCTION

Short tandem repeat (STR) typing has become the accepted gold standard for human identification over the past two decades. Although highly successful and reliable, the 8-10 hours required to complete the analysis under routine conditions with conventional instrumentation is largely due to a polymerase chain reaction (PCR) amplification requiring >3 hours. Translating sample processing and analytical methods to the microscale format permits integration, automation, and miniaturization that will provide the end user with a system that affords expedited and cost-effective analysis, as well as the ability to perform multiplex sample processing using a single device. However, the challenge for microscale PCR is to meet the standards set by the forensic community for quality and interpretation of the STR results.

THEORY

Many examples of PCR amplification using microfluidic devices have previously been demonstrated [1]. Fast thermal cycling using noncontact heating via infrared (IR) excitation of vibrational modes of water molecules has been pioneered by the Landers group. While IR radiation heats the solution in the PCR chamber, the surrounding substrate will heat due to energy transfer and the partial absorption of the IR by the substrate. The decreased ramp times for heating and cooling along with reduction of volume to the micro- or nanoliter scale allow for a dramatic decrease in amplification time. Here we describe, for the first time, the successful transition from the use of a broadband tungsten lamp for IR-mediated heating [2] to an IR laser for amplification in a polymeric microdevice. This transition enables multiplexed PCR on a single microfluidic device because the IR laser source is smaller in size and able to be directed to specific locations on the chip.

EXPERIMENTAL

Purified DNA from a buccal swab (obtained from both low and high buccal cell shedders) and conventional kit-based PCR reagents are guided into the chamber of a disposable, polymeric microfluidic chip (digital image in Figure 1a and IR heating image in Figure 1b). A variety of polymeric substrates were milled to fabricate the PCR microdevices, and the

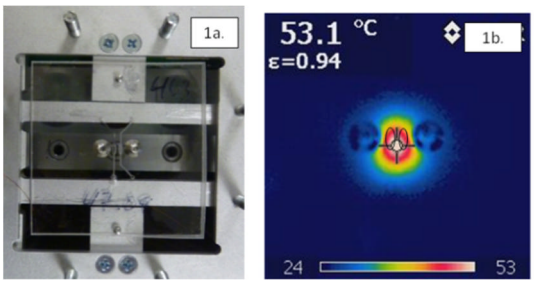

Figure 1: (A) Polymeric PCR microdevice attached to IR laser system. (B) IR radiation of PCR chambers (outlined in black) using IR laser; image was produced using IR camera.

results here are from one particular substrate. With this polymeric material, the milling process dictated the volume of the PCR chamber, which ranged from 1-1.6 µL for all devices used in this work. The IR laser is then used in combination with a noncontact temperature sensor for thermal cycling. The PCR process, requiring up to 3.5 hours with conventional thermal cycling, is completed in as little as 36 minutes (Figure 2) using the IR laser system. Shortened hold times and decreased heating/cooling ramp rates achieved using IR-mediated heating help realize the expedited amplification time.

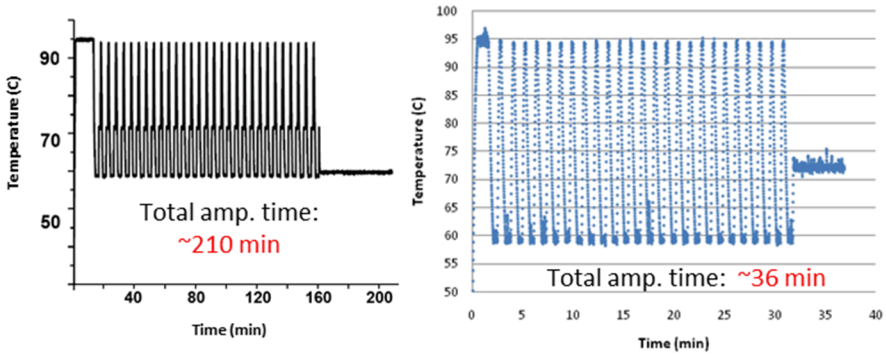

Figure 2: (A) Conventional thermal cycling profile and (B) thermal cycling profile of a microchip PCR amplification using an IR laser.

RESULTS AND DISCUSSION

Efficient IR-PCR amplification of all STR loci was demonstrated using conventional separation and detection analysis (Figure 3). Amplification using the IR laser source results in STR amplifications with intracolor and intercolor (Figure 4) balance required by the forensic community for samples with the varying amounts of input template DNA mass inherent to forensic samples. The average amplicon peak height is also well above the threshold required for forensic data, which is typically set around 100 RFU, dependent upon individual laboratory validation (Figure 5). These results show that amplifications for forensic STR analysis can be performed using the IR laser system and a polymeric PCR microdevice.

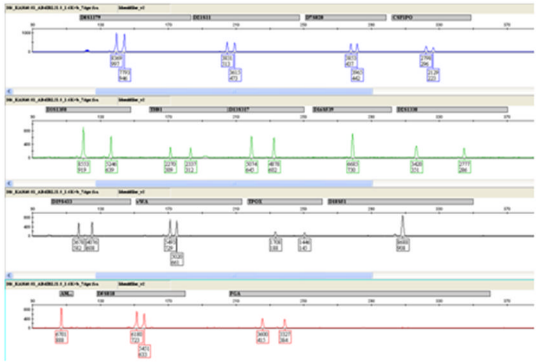

Figure 3: STR profile generated from DNA amplified using the IR laser system and a polymeric microfluidic device. Fragments were amplified using conventional kit-based PCR reagents and separated on an ABI 310 Genetic Analyzer. Amplification was performed in 36 minutes.

Figure 4: Intercolor balance of STR profiles for shedders and non-shedders using the IR laser system and a polymeric microfluidic device. Fragments were amplified using conventional kit-based PCR reagents and separated on an ABI 310 Genetic Analyzer.

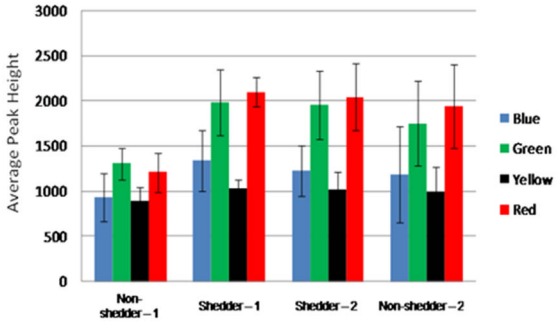

Figure 5: Average STR peak height for shedders and non-shedders using the IR laser system and a polymeric microfluidic device. Fragments were amplified using conventional kit-based PCR reagents and separated on an ABI 310 Genetic Analyzer.

CONCLUSION

PCR amplification of STR loci has been successfully demonstrated utilizing an IR laser and non-contact temperature sensing method, resulting in STR profiles in line with forensic quality standards. A 5-fold reduction from conventional PCR amplification time was achieved with amplifications performed on polymeric microdevices in as little as 36 min. It is reasonable to expect that this technique for PCR amplification on a microdevice can be integrated with on-chip downstream sample processing steps. The time savings associated with IR-PCR, in combination with the possibility to create a fully automated, multiplexed analysis system, provides the potential to significantly alter the means by which evidence is processed in crime laboratories today.

ACKNOWLEDGEMENTS

The authors would like to acknowledge superb guidance of Paul Kinnon, CEO, ZyGEM-MicroLab, John Mears, Program Director, Lockheed Martin Corporation, and Doug J. South, Lockheed Martin Corporation.

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