OPTIMIZATION OF THE POWERPLEX® 18D SYSTEM FOR MICROFLUIDIC AMPLIFICATION OF STR LOCI

Jeffrey A. Hickey[1], Brian E. Root[1], Orion N. Scott[1], Carmen Reedy[1], An-Chi Tsuei[1],Robert Lovaglio[1], Lawrence Dirks[1],Michael Egan[2], Doug Storts[3], Joan M. Bienvenue[2], and James P. Landers[1]
[1]ZyGEM-MicroLab Diagnostics, Charlottesville, Virginia, USA
[2]Lockheed Martin Corporation, Bethesda, Maryland, USA
[3]Promega Corporation, Madison, Wisconsin, USA

STR analysis for human identification is a traditionally lengthy process, requiring 8-10 hours to complete under routine conditions. The largest contributor to this analysis time is a PCR amplification requiring 1.5 - 3 hrs. By translating sample processing to a microscale format, faster, more cost-effective analysis can be achieved. Integration and automation are also advantages realized through microfluidic analysis, as well as the ability to perform multiplex sample processing on a single device. The challenge for transitioning to microscale PCR is to continue to meet the quality standards for STR analysis set by the forensic community while concurrently reducing sample and reagent volume and amplification time.

Our group has previously presented the use of IR lasers for infrared (IR)-mediated heating for PCR amplification on a microfluidic device[1]. A robust STR kit has been developed specifically for this application in partnership with Promega. This kit contains the same primers available in the PowerPlex® 18D System, but has been optimized for use in our microfluidic devices. In the work presented here, we demonstrate the development of this kit on our beta microfluidic chips (PCR-ME only) and the transition of this kit to our new fully integrated cartridge and instrument. The PCR reagents have been optimized to work within the integrated cartridge, following sample preparation with a proteolytic enzyme, and no quantification step.

The IR laser is used in combination with a non-contact temperature sensing method for thermal cycling on a disposable, polymeric microdevice containing multiple PCR domains. In the course of transitioning the PCR chemistry to the new cartridge, studies were performed with DNA preparation from buccal swabs occurring off the cartridge, PCR performed in the fully integrated cartridge, and conventional separation and detection. Amplification of all STR loci was observed after microchip PCR through use of conventional separation and detection instrumentation. These studies indicate that the PCR reagents, disposable cartridge, and instrument are capable of producing DNA profiles of comparable quality to our previous microdevice. The development of this robust PCR kit will assist in the integration and automation of a microfluidics STR system, capable of fully-integrated sample-to-answer STR typing in a single, disposable cartridge.

References:

"Optimization of PCR for Microfluidic Amplification of STR loci", Advances in Polymerase Chain Reaction on Microfluidic Chips," K. Hagan, J. Norris, B. Root, O. Scott, R. Lovaglio, M. Egan, J. Bienvenue, and J. Landers. Presented at ISHI, 2011, Washington DC.

⌘