A42 Rapid Pentameric STR Screening Using Short Microchip Capillary Electrophoresis

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After attending this presentation, attendees will understand the development of a fast and portable DNA screening method that uses microchip electrophoresis for the detection of a set of three newly designed pentameric STRs. Attendees will also gain an understanding for how this system works, the limitations of the system and how these limitations were surmounted to achieve the desired resolution on the microfluidic chip.

This presentation will impact the forensic science community by addressing the problems and limitations encountered with the current commercial microfluidic systems such as poor resolution, and the ability to only detect double stranded DNA.

Forensic DNA analysis involves the amplification and separation of length polymorphisms in the human genome for the purposes of identification. The power of this technique for assisting law enforcement in solving crimes has resulted in a rising backlog of untested samples needing to be screened and analyzed. As a result of this problem and a similar need to develop procedures to screen evidence in remote locations, there is need for the development of rapid and portable genotyping systems. While short tandem repeat (STR) DNA analysis by capillary array electrophoresis is capable of high resolution and has a large power of discrimination in forensic identification, these instruments are not portable and require a relatively long sample run time. It is because of this problem that the project to develop a portable DNA screening method using a commercially available microchip system that utilizes short fluidic channels was started.

Generally speaking microfluidic systems require fairly long channels and complex detection systems for proper resolution and accurate identification using multiplex STR loci for forensic DNA samples. However, there remains a need for portable systems with a small footprint for use in evidence screening. The Agilent 2100 Bioanalyzer uses small two cm microfluidic chips, which are approximately the size of a postage stamp. Due to the short path length of these chips and the fact that they were designed to analyze double stranded DNA (dsDNA), most four base repeats will not properly

separate. As a proposed solution, a set of primers for known pentameric STRs that permits the use of smaller, easier to separate polymorphic amplicons was designed. Pentameric nucleotide repeat units have been shown to reduce the amount of stutter in the amplified sample, a useful characteristic when dealing with mixtures. In addition, pentameric STRs are highly polymorphic and have relatively few microvariants, which make them ideal for forensic analysis. Secondly, a novel mixture of polyvinyl pyrrolidone (PVP) and hydroxyethyl cellulose (HEC) that permits facile DNA separations in the shorter fluidic channels was developed. Lastly a modified chip platform that permits single stranded DNA analysis was utilized. The result is a dramatically improved separation. The data demonstrates that baseline separation is possible for pentameric STR markers using very short fluidic channels. In addition because this new technique is based on a beta modification of an existing commercial system, chip loading is relatively quick and easy. As a result this system should provide a useful tool for quick and portable screening in forensic DNA analysis.

The net result of this research will be to address the problems and limitations encountered with the current commercial microfluidic systems such as poor resolution, and the ability to only detect double stranded DNA.

Pentameric STR, Forensic DNA, Microfluidics

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