B12 Rapid Direct Polymerase Chain Reaction (PCR) of a Y-Chromosomal Short Tandem Repeat (Y-STR) Multiplex as a Screening Tool for the Presence of Male DNA

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After attending this presentation, attendees will better understand how the use of rapid direct PCR along with microfluidic separation and specially designed Y-STR multiplexes can screen crime scene samples for the presence of male DNA and thus aid in the analysis of time-sensitive cases.

This presentation will impact the forensic science community by providing results that show how the amount of time and reagents used can be vastly decreased with a combined rapid and direct PCR screening method on which little research has been performed. This presentation will add to the body of research being carried out in forensic DNA analysis that focuses on reducing the amount of time and steps involved in sample analysis. This would enable more samples to be run in a determined amount of time, thus increasing throughput. In addition, the removal of the extraction step would decrease the potential for sample loss and in-laboratory contamination, both of which are important problems when dealing with samples from a crime scene.

It is often extremely important to rapidly screen crime scene samples and unknown individuals who may have been involved in a crime, namely in situations where many samples may need to be run for sorting through excessive amounts of evidence or before detention of a suspect is possible. Examples include seized evidence potentially linked to a suspect or the determination of which blood stains present at a crime scene may be probative.

Mass disasters in particular create a need for rapid, inexpensive screening of DNA samples with a minimum of sample pretreatment. Recently developed DNA typing methods provide the best biometric information for identity, kinship, and geographical origin, but they are not sufficiently fast to permit the detection of a suspect's DNA in real time. Rapid direct PCR procedures can greatly accelerate the processing time because no extraction is necessary.[1-6] This decrease in processing time and reagent volumes leads to a quick turnaround and inexpensive processing of larger numbers of samples. Such procedures have previously been designed for STRs.[7, 2-3] The goal of this project is to develop a rapid and direct method for profiling Y-STRs as a fast and effective screening tool to determine the presence of male DNA in collected samples.

To do this, specially engineered enzymes, high speed thermal cyclers (capable of running 28 cycles in less than 14 minutes), and microfluidic chip-based electrophoresis will be implemented to process a specifically designed Y-STR multiplex.[1, 8-14] The goal is to reduce the analysis time to less than 25 minutes.[7] The designed multiplex includes four Rapidly Mutating (RM) Y-STRs (DYS526a/b, DYS576, DYS626, and DYS570) between 137bp and 402bp in size, with mutation rates of 10^{-2} per meiosis or greater. By using off-the-shelf instruments and commercially available enzymes, it is possible to create a procedure that acts as a quick, highly informative sample-screening process that also retains sufficient DNA for later manual processing using standard STR or Y-STR kits.

In the first phase of this study, a 4-locus Y-STR multiplex was designed and utilized on a conventional 310 Capillary Electrophoresis (CE) and a beta version of a denaturing Microfluidic Electrophoresis (ME) system. This was tested on control DNA standards 2800M Control DNA and HY DNA as well as with donated saliva samples from five adult males. The multiplex was then analyzed using a rapid PCR protocol, using a variety of rapid polymerases in an effort to optimize the speed and balance of the amplification. This procedure was further optimized with the use of Z-Taq and a direct PCR buffer (Any Direct F buffer) to obtain a rapid direct PCR method, which when coupled with microfluidic separation cuts down sample analysis time to less than 40 minutes, with the possibility of decreasing this further.

The results of this study demonstrate the application of rapid direct PCR for the analysis of Y-STRs for evidence screening. Because the process utilizes a small set of rapidly mutating Y-STR loci, it can also provide useful preliminary data on the presence of male DNA for use in suspect identification.

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