Y21 The Implementation of a Method for Rapid Direct Polymerase Chain Reaction (PCR) on Diluted Saliva

Mallory Baud, BS, Miami, FL 33174; Georgiana C. Gibson-Daw, PhD, Lincoln, NE 68521; Bruce R. McCord, PhD, Florida International University, Miami, FL 33199*

Learning Overview: After attending this presentation, attendees will better understand a method for rapid and direct PCR on diluted direct saliva.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing results that show how run time and reagent costs can be reduced using this combined rapid and direct PCR method. This project will improve current forensic DNA analysis by reducing time and steps involved in sample analysis. The removing of the extraction step in sample analysis decreases extraction-based sample loss as well as potential lab contamination due to sample transfer steps. Attendees will see results of an experimental design-based method to optimize direct and rapid PCR amplification of diluted saliva. These results provide a procedure to reduce the time of the PCR leading to the rapid production of a 7-locus DNA profile for sample screening. This method can be very useful for suspect screening, (e.g., booking stations, customs, and mass disasters).

While current DNA analysis procedures are highly automated, multiplexed, and efficient, they are relatively slow on a per-sample basis. Thus, there is still a need for rapid screening of single-suspect samples. One solution to reduce analysis time is to use rapid direct PCR, which combines cell extraction and PCR amplification in the same tube. Rapid direct PCR procedures can greatly reduce the processing time by screening suspects for presumptive results. This decrease in processing time and reagent use leads to a quick turnaround and inexpensive processing of large numbers of samples.

In order to do this, specially engineered enzymes, such as OmniTaq (a triple mutant of Taq polymerase that makes it resistant to inhibitory effects of body fluids and soils), and high-speed thermal cyclers, capable of temperature changes as fast as 15 degrees per second, will be used to process a specifically designed 7-locus multiplex. The goal is to reduce the analysis time to less than ten minutes by elimination of the extraction step. The MP7 primers consist of the following Short Tandem Repeat (STR) markers: D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, and amelogenin loci. These loci cover from 100 to 450 base pair. Work was also performed on a miniSTR multiplex consisting of THO1, FGA, CSF1PO, D21S11, D5S818, D7S820, TPOX, D18S1, and amelogenin—STR loci that cover 51 to 281 bases. These shorter loci are suspected of needing less time to amplify. Those primers, being much smaller, would reduce the annealing time leading to a shorter reaction. Next, 2µL of DNA is added to 8µL of PCR reaction, which is amplified during 32 cycles at 98°C for 3 seconds and 62°C for 14 seconds. Once amplified, the sample is run on ABI® Prism® 310 Genetic Analyzer or a microfluidic device. The PCR reaction mixture was optimized using experimental mixture designs. The experimental design considered salt concentration, buffer addition, dNTP addition, primer addition, and PCR enhancers to develop experiments to maximize amplification of straight saliva. Those modifications created a faster PCR reaction which was obtained in 12 minutes 36 seconds. The output was optimized base on the overall peak heights of a 7-locus multiplex.

The result of this study demonstrates the application of rapid direct PCR for the analysis of STRs to obtain a presumptive DNA profile in under 13 minutes. Without any extraction, the PCR reagents and enhancers have been shown to work with the inhibitors present in saliva.

Rapid PCR, Direct PCR, Saliva