A82 Evaluation of Direct PCR for Forensic DNA Profiling and the Development of a Direct PCR Multiplex

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After attending this presentation, attendees will understand the basic principles of direct PCR and how it can be used in the analysis of forensic DNA profiling. Other items for discussion include the use of direct PCR with various types of samples retrieved from commonly encountered porous and non-porous substrates, the effect these substrates have on the recovery of DNA, and, finally, the development of a novel multiplex that has been validated to be used with direct PCR.

This presentation will impact the forensic science community by providing insight into the benefits of using direct PCR compared to conventional DNA profiling protocols to analyze various biological samples for DNA profiling. This research also focuses on the development and validation of the direct PCR multiplex for the simultaneous amplification of autosomal and Y- STRs, and two internal PCR controls for the identification of sample quality.

The goal of this research was to evaluate the use of direct PCR with different types of biological samples and to develop a direct PCR multiplex. Direct PCR is a technique in which DNA samples are subjected to PCR without having to first undergo extraction and quantification. With different extraction techniques, there is an associated loss of DNA which is caused by extraction inefficiencies. The multiple tube changes during extraction also introduce the opportunity for contamination and handling errors. With direct PCR, better DNA profiles could be obtained faster and cheaper as there is no loss of DNA associated with extraction steps and it does not use expensive commercial extraction and quantification kits. In this study, genomic DNA preparations and buccal cell counts of various concentrations were deposited on commonly encountered substrates, recovered, and amplified using direct PCR before subjecting them to capillary electrophoresis. The electropherograms obtained were compared to those obtained using the standard DNA profiling protocol which involves extraction prior to amplification and fragment analysis. Direct PCR was found to be more successful than the conventional DNA profiling protocol and was further tested with fingerprints, touch DNA on fabric, and blood and semen stained fabrics. All these tests were successful with direct PCR, indicating that this technique has the potential to be incorporated into routine forensic DNA testing. Supplementary tests were carried out to compare the efficiency of the swabbing technique utilized throughout this study and the effect that different substrates had on DNA recovery. Four non-porous substrates—glass, stainless steel, plastic, and ceramic—and four types of dyed fabrics—white cotton, light blue denim, nylon, and brown cotton—were used and the resulting DNA profiles were evaluated. The results obtained from this experiment indicated that the substrate on which DNA is deposited affects the amount of DNA retrieved, and, subsequently, the generation of DNA profiles. Several commercial multiplexes were used to amplify these samples using direct PCR and it was found that some of these multiplexes were more suitable than others for amplifying using direct PCR and several pitfalls were identified. With this in mind, a novel multiplex consisting of five autosomal and two Y-STRs, which also provides the inhibitor status of the sample, was developed and validated with direct PCR samples. This multiplex also addresses the issues of sensitivity and robustness that were encountered with the commercial multiplexes tested. This direct PCR kit successfully amplified various mock crime scene samples without prior extraction, while being able to amplify as low as 25pg of pristine DNA. Allelic ladder, panels and bins were created to be used with this multiplex to aid in sample designation when subjected to capillary electrophoresis.

Direct PCR, DNA Profiling, Multiplex Development

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