A166 Investigations Into Fast STR Assays for Rapid Screening of DNA Samples

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The goal of this presentation is to provide information on the investigations into decreasing PCR cycling time of multiplex STR assays to enable faster screening of DNA samples.

This presentation will impact the forensic science community by providing information on the effects of utilizing a more processive DNA polymerase on the performance of STR multiplexes.

Forensic DNA typing currently requires approximately one day to process the sample. The workflow involves DNA extraction, quantification of DNA, PCR amplification of STR loci, separation of fragments by capillary electrophoresis followed by data analysis. Significant effort has been invested into decreasing the separation time from 45 minutes to approximately 15 minutes using microfluidic devices. However, the STR amplification step takes approximately 2.5 to 3 hours to complete representing a significant portion of the workflow.

Many companies have advertised "fast" PCR master-mixes for shortening PCR times to less than one hour. However, these products have predominantly been used on single marker targets which are then used for other downstream applications and therefore do not need to meet the quality standards required by a forensic laboratory. Interest in developing fast master-mixes or utilizing fast PCR protocols for multiplex STR assays has attracted the attention of forensic scientists as a method for improving the time to results. Novel DNA polymerase enzymes for PCR amplification have been developed by our research group. The efficacy of these alternative and more processive DNA polymerase enzymes were investigated to decrease PCR cycling time. It was necessary to balance the benefit of shortening the time to result against the need to address factors which can impact the interpretation of a DNA profile. Examples of these factors are: generation of stutter products, non-template addition, intra-locus balance, accuracy, and species specificity.

In this presentation, the results will be presented of the evaluation of different DNA polymerase enzymes, optimization of a new DNA polymerase for a fast multiplex PCR assay, initial feasibility studies, and experiments to overcome PCR inhibition and species cross reactivity. These results show that this assay can decrease PCR cycling time to less than one hour.

Fast PCR, STR Multiplex, DNA Polymerase

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