B159 Developing Rapid PCR Multiplex Assays With miniSTR Loci

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The goal of this presentation is to evaluate and discuss the conditions required for developing rapid multiplex PCR assays.

This presentation will impact the forensic science community by demonstrating how the development of rapid multiplex PCR assays will reduce the overall time required for DNA typing. The information provided in this presentation will assist those developing integrated DNA typing devices and provide further understanding of PCR to the forensic community.

Learning Objective and Outcome: Currently DNA typing is conducted in approximately 8 to 10 hours. The process includes DNA extraction, quantitation, PCR amplification, and fragment length detection. With the advent of miniaturization technologies such as microfluidic and micro-capillary devices there is a desire to reduce the overall time it takes to type DNA samples. Such miniature devices could be used for initial screening in the field. An essential component of a rapid DNA test is reducing the time required for amplification of STR loci.

Existing commercial multiplex STR typing kits were not designed for rapid PCR thermal cycling conditions. Currently it takes approximately three hours to amplify a multiplex of up to 15 STR loci. miniSTR loci are promising candidates for a rapid PCR multiplex due to their small amplicon size (<150 base pairs) and flexibility in terms of primer design. Non-CODIS miniSTR loci[1] were used to test conditions for developing a rapid PCR amplification.

Materials and Methods: A useful rapid screening assay should consist of highly variable miniSTR loci. Three miniSTR loci were tested in the multiplex amplification. The forward primer for each locus was labeled with a unique fluorescent dye (FAM, VIC, NED). Amplification was carried out using commercially available polymerases intended for rapid PCR. The temperature ramp rate of a standard thermal cycler was increased from 1°C/sec to 4°C/sec to accomplish the rapid cycling. In addition, temperature dwell times for denaturation, annealing, and elongation were reduced to ten seconds or less. Standard PCR parameters such as Mg^{++}, total reaction volume, annealing temperatures were optimized to produce a balanced multiplex PCR.

Summary of Results: A set of three miniSTR loci (D2S441, D10S1248, D22S1045) were simultaneously amplified in a single PCR. Fast processing polymerases and rapid cycling protocols allowed for the evaluation of a 'rapid' STR multiplex PCR amplification. The total time required to run 28 cycles on a standard thermal cycler employing a heating rate of 4°C/sec was under 40 minutes. Capillary electrophoresis experiments indicated even peak balance and good signal intensity with slight adenlyation artifacts. Genotyping results are concordant with miniSTR amplification utilizing a standard 2 hour (non-rapid) thermal cycling procedure.

Conclusions: A three locus multiplex PCR can be carried out in less than 40 minutes using miniSTR loci. The loci are well balanced and the assay is robust enough to routinely amplify 0.5 ng of template DNA. Understanding conditions and primer design parameters that allow for rapid multiplex amplification can also be applied to researchers focusing on performing rapid multiplex PCR on non-standard thermal cycling devices. Since the miniSTR fragments are less than 150 base pairs the time required for separation on a miniaturized device will also be reduced.

References:

1 Hill, C.R., Coble, M.D., Butler, J.M. (2007) Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. *J. Forensic Sci., in press*.

MiniSTR, PCR, Rapid PCR

 ** Presenting Author*