B139 Development of Human and Human Male DNA Quantitation Systems Using a Novel, Fluorescent, Two-Primer Real-Time PCR Method

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The goal of this presentation is to present information on a new approach to DNA quantitation.

This presentation will impact the forensic community and/or humanity by demonstrating a new commercial alternative for real-time PCR.

Heavily multiplexed Short Tandem Repeat (STR) analysis has become the dominant technology in DNA-based human identification. Although highly informative, these assays require a defined range of template quantity to produce optimal results. Additionally, resources can be conserved with accurate assessment of DNA quality and assessment of minimum quantity.

Currently, many practitioners observe either high levels of false negative results (due to lack of sensitivity) or subjective conclusions (due to visual comparison of band intensities) based on common hybridizationbased methodologies. Amplification-based methods for quantitation provide a high level of sensitivity while real-time methods can deliver a dynamic range that often exceeds end point assays. A numerical output also increases the objectivity of the data interpretation.

A real-time PCR method has been developed for the quantitation of total human and human male DNA in purified samples using the specificity of interaction between two modified nucleotides to achieve quantitative PCR analysis. One of the PCR primers includes a modified nucleotide (iso-dC) adjacent to a fluorescent label on the 5′ end. The second PCR primer is unlabeled. The reaction mix includes deoxynucleotides and iso-dGTP, which has been modified to include dabcyl quencher. The only nucleotide incorporated at the position complimentary to iso-dCTP is dabcyl iso-dGTP. The incorporation of the dabcyl iso-dGTP adjacent to the fluorescent dye results in a reduction in signal that allows quantitation during amplification. Associated analysis software has been developed to visualize amplification data from various instrument platforms, plot standard curves and calculate DNA concentrations of unknowns. Relative to other real-time approaches, this methodology provides specificity through the use of fluorescently-labeled primers compared to DNA binding dyes and simplicity compared to probebased quantitative PCR approaches. Data will be presented demonstrating the performance of assays using human autosomal (total human) and human Y-chromosome (male human) targets for quantitation.

Forensic Science, DNA Quantitation, Real-Time PCR

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