

## A RAPID SEMI-AUTOMATED METHOD FOR HUMAN-SPECIFIC DNA QUANTIFICATION

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The National Guidelines for DNA Profiling define quantification of human DNA as a component of all casework. Commonly employed methods for human-specific quantification are cumbersome to perform and not easily automated. Furthermore, these methods are only semi-quantitative at best, being affected by the size of the original DNA fragments present in the sample and more fundamentally by intrinsic variation in the number of copies of the target D17Z1 sequence present in different samples. In this presentation, we describe our development, validation, and implementation of a simple, inexpensive, microtiter plate-based, semi-automated method for human-specific DNA quantification.

Our procedure employs TH01 primers to amplify genomic DNA samples. The primate-specific nature of the selected primers insures that only primate DNA is amplified. The amplified products are transferred to a microtiter plate containing the dye, PicoGreen. This dye binds the amplified DNA products. A fluorometer is employed to activate the dye and the fluorescent output of each well microtiter plate is measured. This tabular output is transferred to an Excel spreadsheet. Following minor operator manipulation, the spreadsheet uses the output generated from a set of samples of known quantity and amplified at the same time as the unknowns to calculate a standard curve and determine the concentration of each unknown DNA sample automatically.

Manipulation of amplification conditions and dye concentration improved quality of the method by raising the ratio of signal observed with amplified samples over the background present in negative controls. Comparison with spectrophotometric methods for DNA quantification revealed the new method provides accurate quantification. The method was also validated for primate-specificity and reproducibility. Comparison with other commonly used forensic quantification methods will be described.

All protocols and the spreadsheet for automated calculation will be provided without cost or limitation for use upon request to the authors.