Comparison of Quantitative Information Obtained from Real Time PCR and Expert System Analysis of Profiles Containing Male Female Mixtures

Robin W. Cotton, Ph.D.[1], Jeff Hickey, M.S.[1], David Sipes, M.S.[1], Mark W. Perlin, Ph.D., M.D., Ph.D. [2] Orchid Cellmark[1] and Cybergenetics[2]

As the process of DNA analysis of forensic samples continues to change and improve, the laboratory has an increasing number of tools which can be used to direct the processing of samples and enhance the quality of the results. There are many ways to evaluate the quality of DNA analysis results. One recent significant improvement which contributes to the quality of results is the use of real time quantitative PCR (qPCR) to produce a much more precise measure than was previously available of the amount of total human and male DNA obtained from a sample. This information contributes to the quality by providing reliable information regarding the amount of DNA needed for the amplification thus producing profiles with rfu within the analysis range of the instrument, reducing –A peaks caused by introduction of too much DNA to the amplification reaction and importantly not consuming excessive sample. Additionally, the measurement of both total and male DNA in a sample provides a ratio that predicts the male/female DNA ratio of the contributors. Since the observation of a secondary component in a mixture may be lost at ratios of > 1:20, the qPCR data provides advance information which predicts the success of observing a secondary male component in a mixture of male and female DNA. This information can direct the analysis to genomic or Y chromosomal loci which increases the opportunity of obtaining probative information from the sample.

It is anticipated that the introduction of expert systems will facilitate measurement of numerous profile parameters, thus routinely providing quantitative measures of profile quality. One of the many parameters which expert systems such as TrueAllele® System 3 from Cybergenetics can estimate from quantitative data is the ratio of components in a DNA mixture. This mixture estimate is calculated using information derived from quantitative signal information about the peaks that make up the profile.

In this study, known mixtures and no-suspect cases were analyzed using routine DNA analysis procedures. These procedures included the use of qPCR measurement of both total genomic and male DNA in the sample. DNA was amplified with Profiler Plus and COfiler with fragment separation using an ABI Genetic Analyzer. Profiles were analyzed using Genescan and Genotyper, and independently using TrueAllele System 3. The quantitative information generated by the expert system analysis was compared with the preamplification qPCR quantitation to illustrate the utility of both approaches.