



A127 A Comparison of Two Real - Time PCR Systems for the Simultaneous Quantitation of Total Genomic DNA and Human Male DNA

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After attending this presentation, attendees will have a better understanding of the abilities of two different commercial real-time PCR dual quantitation kits, Promega Plexor HY, and Applied Biosystems Quantifiler Duo, to simultaneously quantitate total genomic DNA and human male DNA, overcome inhibition, and determine mixture ratios.

This presentation will impact the forensic community by comparing two products of equal purpose in an effort to assist laboratories in their determination of which one will most likely fit their current system.

DNA quantitation is an important aspect of forensic sample evaluation as it determines the amount of DNA necessary for successful STR amplification and analysis. Duplex real-time qPCR systems are an improvement to singleplex quantitative assays, which only quantitate either total human or total male DNA, as they simultaneously quantitate total genomic DNA and human male DNA in a single reaction. A large advantage to dual quantitation is that it allows for less consumption of sample, which is especially important in cases where only a limited amount is available. Additionally, duplex qPCR assays assess the presence of inhibitors and determine the relative female to male DNA ratios of mixed samples.

Several sample types were used to draw a comparison between Promega Plexor HY and Applied Biosystems Quantifiler Duo. The systems were used to quantitate samples of known DNA concentrations, ranging from 200-0.012 ng/mL. Reported human and male DNA concentrations were more concordant with Quantifiler Duo than with Plexor HY, especially for concentrations between 50-0.023 ng/mL. In a study using samples inhibited by denim, dirt, and leather, Promega Plexor HY showed a greater ability to overcome inhibition, as it reported DNA quantities greater than both Quantifiler Duo and Quantifiler Human. A mixture study of female to male DNA at varying ratios resulted in Quantifiler Duo reporting human and male DNA quantities more concordant with the expected values than Plexor HY. Additional results were obtained from known and non-probative samples meant to simulate actual forensic casework specimens. These assays both look to address the shortcomings of singleplex qPCR systems, but differ in their ability to accurately quantitate DNA, overcome inhibition, and report female to male ratios in mixtures.

Quantitation, Genomic DNA, Male DNA