H110 Development of PowerQuant™ System: A New Robust Human and Male-Specific DNA Quantification System Which Monitors DNA Integrity

Anupama Gopalakrisnan, PhD, 2800 Woods Hollow Road, Madison, WI 53711; Margaret Ewing, MSFS, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711; Jonelle M. Thompson, MS, 2800 Woods Hollow, Madision, WI; Robert McLaren, PhD, Promega, 2800 Woods Hollow Road, Madison, WI 53711; Benjamin Krenke, MS, 2800 Woods Hollow Road, Madison, WI 53711; and Douglas R. Storts, PhD, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711*

After attending this presentation, attendees will better understand a new robust and sensitive DNA quantification system for determining total human and male DNA content in a casework sample. Also, attendees will learn about assess DNA integrity using the PowerQuant™ System and its utility in optimized casework sample processing for downstream Short Tandem Repeat (STR) applications.

This presentation will impact the forensic science community by serving as an information tool for learning about a robust and sensitive quantitative Polymerase Chain Reaction (qPCR) assay for human DNA detection and the use of quality indices for optimized sample processing in challenging casework samples.

Current qPCR-based human DNA-specific quantification systems allow for quantification of the amount of amplifiable human and male DNA present in a sample and whether autosomal or Y-chromosomal Short Tandem Repeats (Y-STRs) are likely to be more informative based on the auto/Y quantification ratio. While this information is useful, casework samples present additional challenges such as low quantity and quality (degraded DNA and/or presence of inhibitors). Any new qPCR quantification system should therefore be sensitive, robust to inhibitors, and able to provide information on the integrity of the human DNA sample in addition to the standard human and male DNA quantification results. Due to the robust performance in the presence of inhibitors of current commercial STR systems, it is also important that any new quantification system exhibits comparable tolerance to inhibitors. To address this need, the PowerQuant™ System has been developed. This is a five-color, four-target probe based qPCR assay that simultaneously quantifies the total amount of amplifiable human DNA and human male DNA in a single assay. The multicopy targets used allow for detection down to 0.1pg/ul DNA concentrations with minimal variation in auto/Y ratios across single-source male samples. This assay also includes a new larger degradation amplicon derived from a separate region of the sample autosomal quantification target that may be used to monitor the integrity of a DNA sample. As larger amplicons tend to be more sensitive to inhibition, the internal positive control has been designed to have a similar response to inhibitors as the degradation amplicon, thereby minimizing the potential for falsely flagging inhibited samples as being degraded.

Data will be presented demonstrating sensitivity, consistency of auto/Y ratios in male DNA samples, resistance to inhibitors, ability to detect DNA degradation, species specificity, and male specificity at various ratios of male-to-female DNA.

qPCR, Degraded DNA, Inhibitor