B58 Developmental Validation of a Real Time PCR Assay for the Quantitation of Total Human and Male DNA

Benjamin E. Krenke, MS, Cynthia J. Sprecher, BS, Nadine Nassif, MS, Melissa R. Schwandt, PhD, Curtis Knox, BS, and Douglas R. Storts, PhD, Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711*

After attending this presentation, attendees will understand the utility, performance and limitations of the Plexor™ HY System in forensic sample quantitation.

This presentation will impact the forensic science community by presenting a full developmental validation data set from a new qPCR system for the quantitation of forensic samples.

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. In addition to accurate sample quantitation, simultaneous assessment of sample quality and highly sensitive detection are necessary to fully answer the question of how best to proceed with sample analysis.

Quantitative PCR has displaced hybridization-based methods for human-specific quantitation. This change has reduced the rate of false negative results (due to lack of sensitivity) and increased the objectivity of data interpretation (numerical output rather than visual comparison of band intensities). However, some current qPCR methods do not allow simultaneous quantitation of total human and human male DNA as well as sensitivity that consistently exceeds that of the subsequent STR assays.

The Plexor™ System, a quantitative PCR method, has been developed using the specific interaction of two modified nucleotides. 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 buffer includes the complementary iso-dGTP, which has been modified to include dabcyl quencher. Incorporation of the dabcyl iso-dGTP adjacent to the fluorescent dye reduces signal that allows quantitative data to be obtained. Multiple targets can be simultaneously detected through use of a different flurophore for each target. The non- destructive nature of this approach permits melt/dissociation analysis of amplified products. This post-PCR analysis can compare similarity of the amplified sequence between the standards and unknowns, providing a useful quality confirmation.

The Plexor™ HY System is a multiplex assay that has been developed for the simultaneous quantitation of total human DNA and human male DNA. An internal PCR control (IPC) has been included to monitor inhibition in the quantitation process. This assay uses three dyes to detect amplification and a fourth dye to provide a passive reference signal. The autosomal target is a multicopy 99bp target on chromosome 17. The Y-chromosomal target is a multicopy 133bp target on the short arm of the Y-chromosome. The IPC target is a synthetic sequence added to all wells. The amplified IPC is 150bp, the longest amplicon in the assay.

Associated Plexor™ Analysis Software has been developed to visualize amplification data from multiple instrument platforms, plot standard curves, and calculate DNA concentrations of unknowns. An STR normalization module has been built in to the software that, with simple user inputs, allows the software to: (a) compute sample input volumes required for amplification in autosomal and Y-STR reactions, (b) calculate necessary dilutions for concentrated samples, and (c) flag low quality and inhibited data. Protocols for use with the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems and the Stratagene Mx3000P® and Mx3005P® QPCR Systems have been developed. A discussion on use with other instrumentation will also be presented. In addition to analysis software, development of automated methods for qPCR set-up, DNA normalization and STR amplification set-up will be described.

Data will be presented demonstrating the performance of this assay and the interface of the analysis software. Developmental validation studies include: (a) within run and between run reproducibility, (b) Y-assay male specificity, (c) human specificity (non-human DNA analysis), (d) post-quantitation normalization and STR amplification, (e) inhibitor impact and purification method studies, (f) concordance with existing quantitation systems including non-probative samples, (g) quantitation of degraded DNA, (h) male/female mixture studies, and (i) interlaboratory comparisons.

DNA Quantitation, Real Time PCR, Forensic Science

 ** Presenting Author*