A173 Development of a Highly Sensitive Quantification System for Assessing DNA Quality in Forensic Samples

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After attending this presentation, attendees will understand the scientific basis for primer and probe design of a human DNA test method that can simultaneously estimate the quantity and quality of DNA in a potentially degraded forensic sample.

The presentation will impact the forensic science community by providing forensic DNA analysts with information regarding a new DNA quality and quantity assessment method, a highly sensitive system and valuable tool when making decisions regarding the appropriate test kit to analyze potentially degraded forensic DNA samples.

Real-time PCR provides reliable results which are essential for determining the amount of amplifiable DNA in a biological sample. The recent advances in mini STR analysis systems have now made it possible to analyze highly compromised samples. A system which can assess the extent of degradation in a forensic sample will be a useful tool for DNA analysts. Recent scientific literature reports the evaluation of the quality assessment of degraded DNA samples using Ya5-lineage Alu genetic element.[1] This presentation utilizes two independent genomic targets to obtain quantification of an 80bp short DNA fragment and a 250bp long DNA fragment in a degraded DNA sample. A multi-copy intra *Alu* based approach, to quantify human specific DNA in an evidence sample, has been successfully used to obtain DNA quantification with high sensitivity.[2] *Alu* are Short Interspersed Elements (SINE), approximately 300bp insertions which are distributed throughout the human genome in large copy number. The use of an internal primer to amplify a segment of an *Alu* element allows for human specificity as well as high sensitivity when compared to a single copy target. In this study, primers and probes were designed using two independent intra *Alu* insertions targets. The 80bp target sequence is from aYb8-lineage specific *Alu* insertion, whereas the 250bp target sequence is from Ya5-lineage *Alu* insertion. Use of a multi-copy target, two different size Alu markers along with a synthetic target, as an Internal Positive Control (IPC), provides an additional assessment for the presence of PCR inhibitors in the test sample.

The average age of Yb – lineage subfamily is estimated as 2.39 million years. It is estimated that the human genome contains over 1800 *Alu* Yb family elements and, out of those, approximately 50% are from the Yb8 subfamily. Another advantage of the *Alu* Yb 8 system is the presence of a large number of fixed insertions. It has been reported that only 20% of the Yb-lineage *Alu* elements are polymorphic for insertion presence or absence in the human genome.[3] Because a large number of these fixed elements are present in every human genome, this minimizes the individual specific variation possible when using a multi-copy target quantification system.

The precision and sensitivity studies indicated that this system has a sensitivity threshold in the range of 3-4pg, similar to those reported for other *Alu* based quantification systems. The slope of the standard curve ranged between -3.53 and -3.3. The amount of synthetic IPC target was adjusted to provide reproducible Ct values between 12-14 cycles for samples with no inhibition. A correlation study of estimated quantification for both 80bp and 250bp fragments with the STR analysis results obtained from DNAse I degraded DNA samples will be presented.

In conclusion, a DNA-based qualitative/quantitative/inhibition assessment system, that accurately predicts the status of a biological sample, will be a valuable tool for deciding which DNA test kit to utilize when processing forensically compromised samples for DNA testing.

References:

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3. Carter AB, Salem AH, Hedges DJ, Keegan CN, Kimball B, Walker JA, Watkins WS, Jorde LB, Batzer MA. Genome-wide analysis of the human *Alu* Yb-lineage. Human Genomics 2004;1(3):1-13.

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