Assessing DNA Quality, Quantity, and Inhibition using a highly sensitive multiplex quantification system for Forensic Samples

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Real-time PCR quantification of human DNA can provide an important estimate of the amplifiable DNA in a biological sample. Current methods in forensic DNA laboratories include SYBR Green, Plexor HY, andQuantifiler Duo TaqMan assays. The recent advances in mini STR analysis systems have made it possible to analyze highly compromised samples.

A quantification system that estimates the level of degradation in a forensic sample is a useful tool for DNA analysts. There are already some systems that provide a quality assessment of degraded DNA samples. One uses a Ya5-lineage Alu genetic element and a second uses a multiplex simultaneously assessing nuclear and Y chromosome targets ranging from 67bp to 190 bp. The advantage of an Alu 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. A large number of these fixed elements are present in every human genome enhancing sensitivity and minimizing the individual specific variation possible when using a multi-copy target quantification system.

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. Alus are Short Interspersed Elements (SINE), approximately 300 bp insertions, which are distributed in large copy number. The use of an internal primer to amplify a segment of an Alu element allows for higher primate specificity as well as high sensitivity when compared to a single copy target.

The new qPCR utilizes two independent genomic targets. Primers and TaqMan probes were designed using two independent intra retrotransposon insertions targets. The 80bp “short” target sequence is from an Alu insertion whereas the 207 bp “long” target sequence is from a separateretrotransposon element. The primers and probes are selected such that they have no interaction among themselves and are completely independent. The ratio of the quantity of long targets versus short targets provides a useful assessment of the quality of DNA. This quality index (QI) can have applications in predicting the profiling success of forensic samples. The use of a synthetic target as an Internal Positive Control (IPC) provides an additional assessment for the presence of PCR inhibitors in the test sample.

Our initial inter-laboratory testing indicates that the efficiency for both the long and short targets is consistently above 90%. The amount of synthetic IPC target was adjusted to provide reproducible Ct values between 18-22 cycles for samples with no inhibition. Precision and sensitivity studies indicated that this system has a sensitivity threshold down to 1 pg, similar to those reported for other Alu based quantification systems and comparable to other commercially available systems. Studies comparing this system with other commercially available quantitation systems show concordance of quantitation values between systems. Furthermore, the preliminary inter-laboratory results demonstrate the predictive value of the QI on degraded DNA, and the IPC results on humicacid (inhibitor) spiked samples.