DEVELOPMENT OF A HIGHLY SENSITIVE QUANTIFICATION SYSTEM FOR ASSESSING DNA QUALITY IN FORENSIC SAMPLES

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Real-time PCR provides reliable results which are essential for determining the amount of amplifiable DNA in a biological sample. Currently several different approaches are used for fluorescence-based quantification assays, including SYBR Green, Plexor and TaqMan. 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 an evaluation of the quality assessment of degraded DNA samples using Ya5-lineage Alu genetic element[3]. 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[1]. 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.

This system utilizes two independent genomic targets to obtain quantification of an 80 bp short DNA fragment and a 290 bp long DNA fragment from a DNA sample in the multiplex 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[2]. *Alu* are Short Interspersed Elements (SINE), approximately 300 bp 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 TaqMan probes were designed using two independent intra *retrotransposon* insertions targets. The 80 bp "short" target sequence is from the Yb8-lineage *Alu* insertion whereas the 290 bp "long" target sequence is from a separate retrotransposon element. The primers and probes for the two targets are selected such that they have no interaction among themselves and are completely independent. 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.

Details of primers and probe designs for each target and the developmental validation studies for this system, will be presented including multiplexing PCR, sensitivity, precision, concordance and reproducibility, degradation, inhibition, and species specificity. 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 15-18 cycles for samples with no inhibition. Precision and sensitivity studies indicated that this system has a sensitivity threshold in the range of 3-4 pg, similar to those reported for other *Alu* based quantification systems. Studies comparing this system with other commercially available quantitation systems show concordance of quantitation values between systems.

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

References:

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