Inter-Laboratory Testing of a Highly Sensitive Quantification System for Assessing DNA Quality in Forensic Samples

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Abstract

Real-time PCR quantification of human DNA can provide an important estimate of the amplifiable DNA in a biological sample. Currently, methods being utilized in forensic DNA laboratories include SYBR Green (1), Plexor HY (2) and Quantifiler Duo TaqMan (3) assays. The recent advances in mini STR analysis systems have now made it possible to analyze highly compromised samples.

A quantification system that estimates the level of degradation in a forensic sample will be a useful tool for DNA analysts. There are previous reports of systems that provide a quality assessment of degraded DNA samples. One uses a Ya5-lineage Alu genetic element (6) and a second uses a multiplex simultaneously assessing nuclear and Y chromosome targets ranging from 67bp to 190 bp (5). 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 (4). 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 (7). Alus 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 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 80 bp "short" target sequence is from an Alu insertion whereas the 207 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. **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 humic acid (inhibitor) spiked samples**.

Methods

This system (InnoQuant™) utilizes two independent genomic targets in a multiplex to simultaneously obtain quantification of an 80 bp fragment and a 207 bp fragment (See Fig. 1). The 80 bp "short" target sequence is from the Yb8-lineage Alu insertion whereas the 207 bp "long" target sequence is from a separate retrotransposon element, SVA3. Use of a synthetic 172 bp target as an Internal Positive Control (IPC) provides an additional assessment for the presence of PCR inhibitors in the sample.

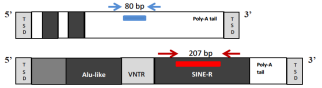

Figure 1. Illustration of *Alu* and SVA (full-length retrotransposons are not drawn to scale). As represented, the REs have a target site duplication (TSD) consisting of identical DNA sequences at the beginning and end.

Real-time PCR reactions were processed on the Applied Biosystems 7500 Real-Time PCR System using Agilent Technologies' Brilliant Multiplex QPCR Master Mix with the following parameters: 10min at 95° C, followed by 32 cycles of: 15 sec at 95° C and 2 min at 61° C. The ratio between the DNA quantity of the short target divided by DNA quantity of the long target gives an indication of the degree of DNA degradation for the quantified sample, and is termed the "Quality Index", or "QI".

STR amplification was performed using the Identifiler ID Plus Kit (Life Technologies, Thermo Fisher, Foster City CA) or the Powerplex 16HS Kit (Promega, Madison, WI) according to the manufacturer's protocol. Separation and detection of PCR amplicons was performed using ABI 310 capillary Electrophoresis (CE), 3130 Genetic Analyzer and GeneMapperID software from Life Technologies, Foster City, CA) according to the manufacturer's protocol.

Results

Figure 2. PCR efficiency for both the long and short targets is consistently above 90%

Figure 2a. Reproducible Duplicate Short Target Standard Amplification down to 2.25pg

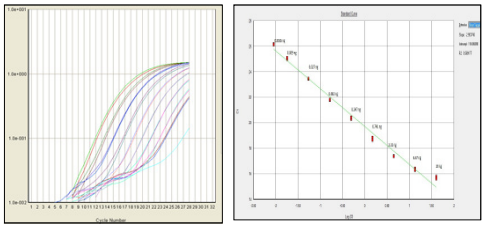

Figure 2a. Short Target raw data and standard curves on 20ng down To 2.25pg. Correlation coefficients ranged from 0.99-0.998 and slopes ranged from -3 to -3.4 for the short target. PCR efficiency ranged from 90-96.7%.

	Inno. Short	Inno. Long	SJSU Short	SJSU Long
PCR Efficiency	96.7	98.2	90	99%
Slope	-3.4	-3.37	-3	-3.75
R2	0.998	0.996	0.99	0.992

Figure 2b. Reproducible Duplicate Long Target Standard Amplification down to 2.25pg

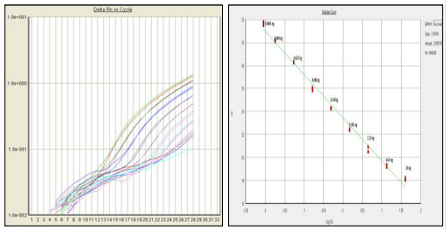

Figure 2b. Long Target raw data and standard curves on 20ng down To 2.25pg. Correlation coefficients ranged from 0.992-0.996 and slopes ranged from -3.4 to -3.75 for the long target. PCR efficiency ranged from 98.2-99%

Reproducible PCR efficiencies > 90%

Figure 3. Sensitivity down to 1 pg

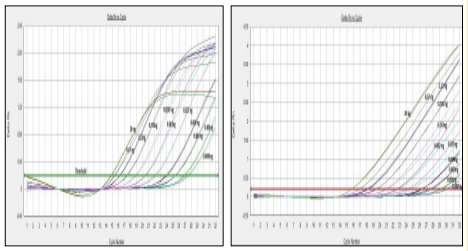

Figure 3. qPCR Amplification results demonstrating sensitivity down to 1 pg for both the short (left panel) and long (right panel) targets. A threshold of 0.2 was utilized to evaluate sensitivity.

Sensitivity down to 1 pg.

Figure 4. Concordance to NIST and Quantifiler Duo

NIST SRM 2372 Reproducibility & Concordance of Innoquant Quant

Seven runs, different days, triplicate dilutions of NIST SRM 2372 Human DNA. Quantitation Standard between 0.5 ng/ul and 5 ng/ul:

Average % variation from [NIST]:
Short 7 % Long 8 %

High Accuracy within 10% of NIST Quantification Standard Values

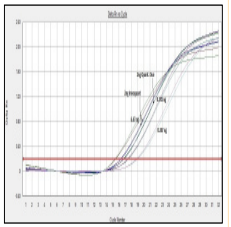

Figure 4. qPCR Amplification results of 2ng Quantifiler Duo **2ng Quantifiler standard falls within the expected range of the Innoquant 2.22ng standard**

Accurate and reproducible on NIST standard and other validated kits.

Figure 5. Predictive value of the Quality Ratio on Degraded DNA

Quality Index as predictor of STR success:

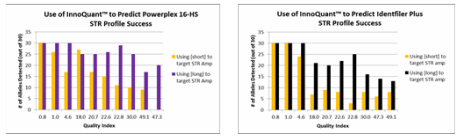

Figure 5. % Alleles Detected Using the InnoQuant to Predict Powerplex 16 HS and Identifiler Plus STR Profiling success

Use of the QI resulted in ↑ STR typing: ↑ target DNA ↑ allele calls by 130% avg.

Figure 6. Resistant to up to 400uM Humic Acid Inhibition

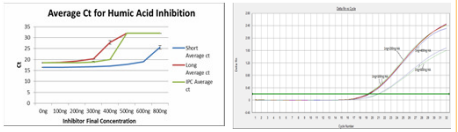

Figure 6. ct values and amplification plots of DNA spiked with increasing levels of Humic Acid.

Resistance up to 400uM Humic Acid.

Conclusion

- A DNA based qualitative/quantitative/inhibition assessment system can be a valuable tool when processing forensically compromised samples.
- Concordance with quantification values were observed using both the NIST SRM 2392 Quant standard and Quantifiler Duo Standards.
- Efficiency for both long and short target was consistently above 90%.
- Reproducible Ct values for the IPC fell between 18-22 cycles (data not shown).
- Precision and sensitivity down to 1 picogram.
- The Quality Index (ratio of quantity of short target to long target) strongly correlates to STR profiling success.
- Current quant systems significantly under-target DNA for degraded samples. Increasing DNA target for samples with a QI > 5 increased allele calls by an average of 130%.
- InnoQuant™ may allow a forensic DNA analyst to:
  - Improve sample success rate
  - Eliminate unnecessary rework by increasing first pass success rates
  - Streamline sample workflow

Future Steps

- Perform additional STR amplifications on degraded samples using the QI to estimate the optimal target DNA to verify preliminary results
- Conduct further research and testing on resistance to additional inhibitors.
- Evaluate quantification on additional DNA standards commonly utilized in other routine forensic DNA and genetic laboratories.
- Perform comparisons on the new InnoQuant™HY system.
- Expand collaborations to include additional labs, sample types and DNA extracted and stored using additional chemistries. (e.g. mixtures, blood, saliva and semen samples extracted with silica based methods, FTA paper and stored DNA samples over time).

Acknowledgements

This research was supported by the BTE program of J and J, support provided by InnoGenomics in kits, reagents, technical assistance and small research grants to SJSU, and by the Forensic Science Program at SJSU.

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