Development of a Highly Sensitive Human and Male Quantification System for Assessing DNA Quality in Forensic Samples

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Introduction

A qPCR quantification system which can simultaneously provide human and male quantitative information, as well as assess the extent of degradation in a forensic sample, will be a useful tool for DNA analysts. 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[1]. The advantage of an *Alu* system is the presence of a large number of fixed insertions[2] (a total of ~1800 copies/genome of Yb8 and ~1700 full length copies/genome of SVA).

This system (InnoQuant-HY™) utilizes two independent genomic targets and a male specific target in a multiplex to simultaneously obtain quantification of an 80 bp fragment and a 207 bp fragment, as well as male DNA (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, SVA[3].

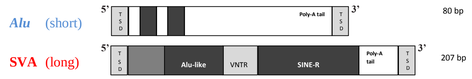


Figure 1. Illustration of *Alu* and SVA (full-length retrotransposons not drawn to scale). As represented, the REs have a target site duplication (TSD) consisting of identical DNA sequences at the beginning and end.

Materials and methods

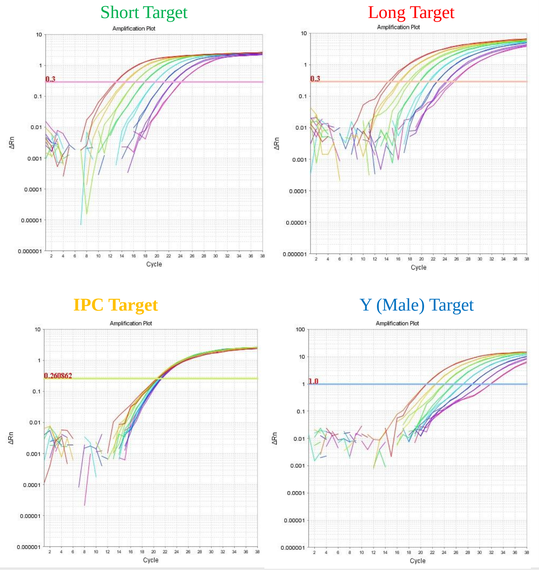
Primers and TaqMan probes were designed using two independent intra *retrotransposon* insertions targets, and a male target. Use of a synthetic 172 bp target as an IPC provides an additional assessment for the presence of PCR inhibitors in the sample.

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: 10 min at 95°C, followed by 38 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".

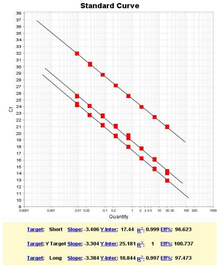
$$QI = \frac{[short]}{[long]}$$

Results

Sample amplification plots and PCR efficiencies:



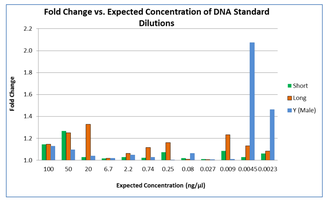
Highly PCR efficiencies in all three targets:



Sensitivity:

Low variation from expected values observed in the range of the standards.

Greater variation observed in Y target near the system's LOD.



Correlation between autosomal and male targets:

The sensitivity dilutions (std DNA and NIST SRM), were analyzed for correlation between the autosomal and Y target quantitation values. The average fold change between the long and Y target quantity values was ***1.21*** and between the short and Y target quantity values was ***1.23***.

Additionally, 60 semen samples were run in duplicate and autosomal short/long and male quantitation values were analyzed. The average fold change between the long and Y target quantity values was 1.5 and between the short and Y target quantity values was 1.76.

High correlation between autosomal and male targets

In both experiments, as with other multi-copy target real-time PCR systems, the averages between data sets are well within twofold.

Reproducibility:

Highly reproducible

60 semen samples were run in duplicate on separate plates and standard deviations between the two readings were analyzed.

	Long Target	Short Target	Y Target
Average SD	0.091	0.049	0.060
Min SD	0.000	0.000	0.000
Max SD	0.883	0.327	0.325

NIST SRM reproducibility:

Five runs, different days, duplicate dilutions of NIST SRM 2372 Human DNA Quantitation Standard at 2 ng/ul:

Average percent variation from expected [NIST]: ***Highly accurate***

Short	Long	Y
14%	***8 %***	***21%***

Conclusions

- A DNA based qualitative/quantitative/inhibition assessment system can be a valuable tool when processing forensically compromised samples.
- The Quality Index (ratio of quantity of short target to long target) strongly correlates to STR profiling success.
- The use of retrotransposon targets significantly improves both sensitivity and reproducibility compared to currently commercially available kits.
- InnoQuant™–HY allows a forensic DNA analyst to:
  - Accurately and simultaneously obtain quantitation values for total human DNA as well as male DNA
  - Improve sample success rate
  - Eliminate unnecessary rework by increasing first pass success rates
  - Streamline sample workflow

Literature cited

1. Walker JA, Hedges DJ, Perodeau BP, Landry KE, Stoilova N, Laborde ME, Shewales J, Sinha SK, Batzer MA. Multiplex polymerase chain reaction for simultaneous quantitation of human nuclear, mitochondrial, and male Y-chromosome DNA: application in human identification. Analytical Biochemistry 2005:337, 89-97.
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3. Damert A, Raiz J, Horn AV, Lower J, Wang H, Xing J, Batzer MA, Lower R, and Schumann GG. 5'-Transducing SVA retrotransposon groups spread efficiently throughout the human genome. Genome Research 2009:19, 1992-2008.

Acknowledgments

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