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

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Introduction

Currently, several different systems are used for fluorescence-based real-time PCR quantification of forensic samples. However, none of the systems enable an assessment of the quality of a sample. A system which can assess the extent of degradation in a forensic sample is a useful tool for forensic 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).

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, SVA[3].

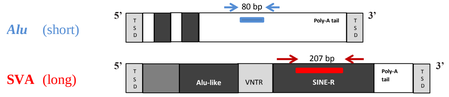


Figure 1. Illustration of *Alu* and SVA (full-length retrotransposons are not drawn to scale). As represented, the REs have a target site duplication (TSD) consisting of identical DNA sequences at the beginning and end.

Materials and Methods

Primers and TaqMan probes were designed using two independent intra *retrotransposon* insertions targets. Primers and probes for the two targets were selected such that they have no interaction among themselves and are completely independent. 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.

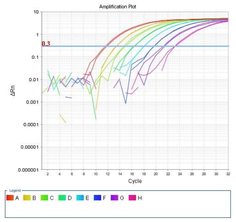
Real-time PCR reactions were processed on the Applied Biosystems 7500 Real-Time PCR System using Agilent Technologies' Brilliant Multiplex QPCR Master Mix with the following parameters: 10 min at 95°C, followed by 32 cycles of: 15 sec at 95°C, and 2 min at 61°C. The ratio between the DNA quantity of 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".

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

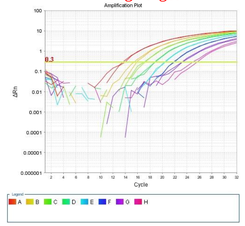
Results

Sample amplification plots and PCR efficiencies:

Short Target



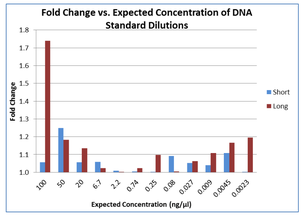
Long Target



	Short Target	Long Target
PCR efficiency:	96.689%	98.153%
Slope:	-3.404	-3.367
R2:	0.998	0.996

Reproducible PCR efficiencies higher than 90%

Sensitivity:



NIST Serial Dilution

Expected ng/uL	Observed Short Qty	Observed Long Qty	Short Qty Fold change	Long Qty Fold change
5	4.1543	6.8428	1.20	1.37
1.25	1.2454	1.2499	1.00	1.00
0.3125	0.3201	0.2849	1.02	1.10
0.0781	0.0765	0.0608	1.02	1.28
0.0195	0.0202	0.0165	1.04	1.18
0.0049	0.0055	0.0042	1.12	1.17
0.0012	0.0013	0.0010	1.06	1.18
		Average Fold Change	1.07	1.18

Less than 20% variation from expected values as low as 1 picogram of DNA

NIST reproducibility and concordance with Quantifiler Human:

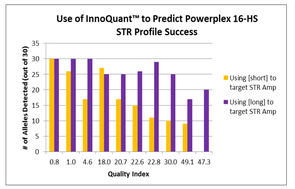
Concordance between InnoQuant™ Short and Quantifiler® Human:
20% variation

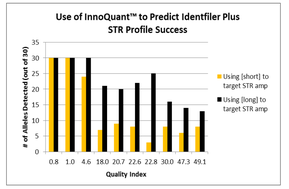
NIST DNA standard reproducibility:
Seven runs, different days, triplicate dilutions of NIST SRM 2372 Human DNA Quantitation Standard between 0.5 ng/ul and 5 ng/ul:

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

Highly accurate

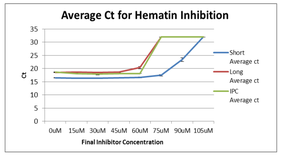
Quality Index as predictor of STR success:

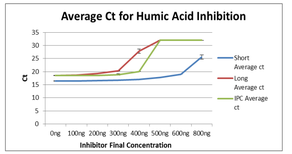




Can use the Quality Index to decide target DNA amount for STR amp
Increasing target DNA increased allele calls by average of 130%

Inhibition:





True Zero Test:

<1.0 pg/uL =
No informative STR profile*
*Dependent on STR kit

Sample Name	IDP Allele Count	IDP Average RFU	Sample Name	IDP Allele Count	IDP Average RFU
Sample 1_2pg	11	78	Sample 2_2pg	6	71
Sample 1_2pg	17	86	Sample 2_2pg	0	--
Sample 1_1pg	4	65	Sample 2_1pg	3	67
Sample 1_1pg	2	64	Sample 2_1pg	2	63
Sample 1_0.5pg	0	--	Sample 2_0.5pg	1	58
Sample 1_0.5pg	0	--	Sample 2_0.5pg	0	--
Sample 1_0.25pg	0	--	Sample 2_0.25pg	0	--
Sample 1_0.25pg	0	--	Sample 2_0.25pg	0	--

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.
- 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%.
- The use of retrotransposon targets significantly improves both sensitivity and reproducibility compared to currently commercially available kits.
- InnoQuant™ allows a forensic DNA analyst to:
  - 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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