InnoQuant® as a tool to Determine Profile Suitability and Improve Profile Success Rates for High Throughput Property Crime Specimens

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Introduction

Varying degrees of degradation as a result of environmental insults, storage conditions, or age are typically observed in forensic casework DNA specimens. These types of specimens may need to be excessively re-worked since their level of degradation may not be known until after amplification and detection, at which time adjustments may be made to the amount of input DNA in the amplification reaction in order to produce a more acceptable STR profile (i.e. gain more alleles from degraded amplicons).

Next-generation kits for quantitation of human DNA aim to provide information on the extent of sample degradation prior to STR amplification in order to reduce rework, and associated reagent and processing costs, of degraded forensic DNA specimens. We present here results of testing performed on property crime casework samples using one of these next-generation quantification systems, InnoQuant®.

InnoQuant®[1] uses two independent genomic targets to provide an assessment of the level of degradation of a forensic sample:

- A "short" Alu based target of 80 bp in size
- A "long" target from a separate retrotransposon of 207 bp in size
- An Internal Positive Control (IPC) to assess PCR inhibition

The purpose of the study was to determine if InnoQuant® could provide better information to identify samples unlikely to produce profiles and provide more accurate prediction of the optimal PCR target to obtain the most profile data.

Materials and Methods

- 215 property crime samples primarily consisting of touch DNA swab samples with a few blood samples, were tested using Cellmark Forensics' standard methods in the Biotracks™ high throughput section, including the Quantifiler® Human DNA Quantification kit, STR typing with Identifiler® Plus 12.5 µL reactions with a 500pg input DNA target, and local database searching. The input DNA used in the amplification reaction was determined by the Quantifiler® data.
- In this study, after being tested and reported using standard methods described above, the 215 property crime samples were quantified with InnoQuant®. For samples that did not obtain any STR data, a quantification threshold was evaluated to determine how successfully each quantification assay (Quantifiler® Human and InnoQuant®) could be used as a screening test to identify samples with insufficient DNA for STR profiling.
- Degradation indices were determined by: $DI_{80/207}$ = [short]/[long]
- AB 3130xl Genetic Analyzer was utilized. Data analysis was performed with GeneMapper® *ID* using an analytical threshold of 75 RFU and a stochastic threshold of 130 RFU.

Results

Samples with "undetermined" quants:

System	Samples w/ no Quant Value	Pct
InnoQuant (Short)	5	2.3%
InnoQuant (Long)	45	20.9%
Quantifiler Human	66	30.7%

InnoQuant® kit, particularly the short target, has very high sensitivity, greatly reducing false negatives

Degradation Indices: $DI_{80/207}$ = [short] / [long]

DI	# Samples	Pct
<3	27	15.8%
3-5	68	39.8%
5-10	61	35.7%
10-15	7	4.1%
15-20	3	1.8%
>20	4	2.3%

The vast majority of property crime samples (75%) exhibit sufficient degradation (DI of 3-10) to cause issues in obtaining optimal DNA profiles

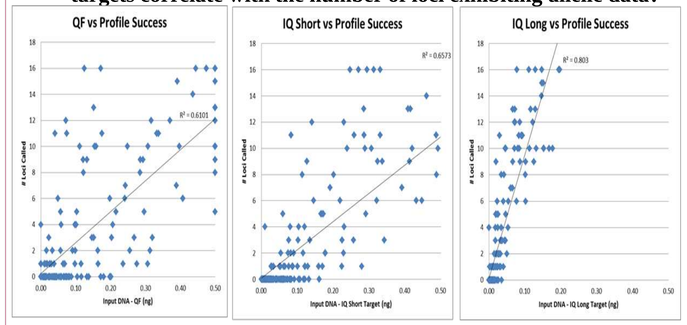
Can quantification values accurately predict which samples would not produce STR profiles?

Out of the 121 samples that produced no STR profile data above the analytical threshold of 75 RFU (considered "true negatives"), which quantification value threshold provides most accuracy in identifying true negatives while minimizing potentially missing profiles?

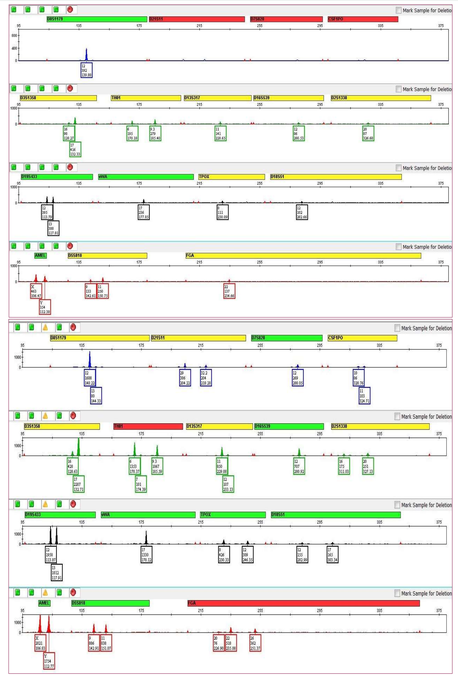
Quant Marker	Cutoff (ng/µL)	Quant Neg.	Profile Neg.	% True Neg. Identified	Potentially Missed Profiles
Quantifiler Human	0.003	79	121	65%	3 – 1 allele profiles 1 – 3 allele profile
InnoQuant Short	0.015	113	121	93%	4 – 1 allele profiles 2 – 2 allele profiles 1 – 3 allele profiles 1 – 6 allele profile
InnoQuant Long	0.003	112	121	93%	4 – 1 allele profiles 1 – 3 allele profile

Overall, the InnoQuant® Long target is shown to be an accurate predictor of true negative samples, identifying 93% of samples with no allelic data (as compared to 65% with Quantifiler® Human) with minimal "potentially missed" profiles, none of which are informative profiles.

How well does the predicted input DNA from different quant targets correlate with the number of loci exhibiting allelic data?



Input DNA estimated by InnoQuant Long target was the best predictor of profile success with a much stronger correlation than Quantifiler or the InnoQuant Short targets. This is likely due to the fact that the InnoQuant Long target is 207 bp in size which is in the range of a typical STR marker.



*Using **Quantifiler® Human** to target input DNA in Identifiler® Plus*

8 loci > stochastic threshold

*Using **InnoQuant® Long** to target input DNA in Identifiler® Plus*

15 loci > stochastic threshold

Conclusions

- Overall, this study demonstrates that InnoQuant® can be a very effective tool in the processing of high throughput property crime specimens by:
  - Using it as a screening test to identify samples that will not produce informative DNA profiles, and
  - By providing more reliable quantification data to obtain optimal STR profiles
- If InnoQuant® Long quant value is used to target STR amplification, significantly more allelic data can be obtained from degraded samples.
- InnoQuant® Long quant data (R^2=0.80) correlated with profile success significantly better than Quantifiler® Human quant data (R^2=0.61).
- Using InnoQuant® can help improve first pass success rates and minimize sample reprocessing.
- The vast majority of forensic samples in this study exhibited degradation to an extent where an adjustment to target DNA input based on DI values can be helpful in obtaining optimal DNA profiles.

Literature cited

1. G.M. Pineda et al. *Development and validation of InnoQuant®, a sensitive human DNA quantitation and degradation assessment method for forensic samples using high copy number mobile elements Alu and SVA*. Forensic Science International: Genetics 13 (2014) 224–235.

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