Validation of the InnoQuant™ DNA Quantification & Degradation Assessment kit and the InnoTyper™ 21 PCR Amplification Kit for Use on Forensic Samples

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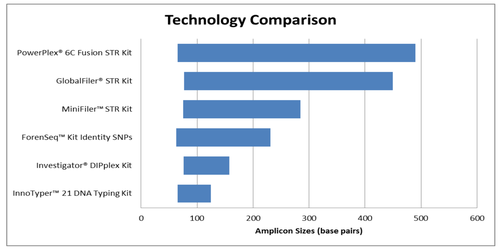
Abstract
The availability of robust, sensitive DNA testing systems that enable the analysis of highly degraded and/or inhibited samples continues to be an issue for forensic practitioners. Recently, InnoGenomics introduced two systems specifically developed for degraded, low level and/or inhibited sample analysis that have been useful in generating probative results from human remains samples and rootless hair samples.



Introduction
This poster will provide a summary of the validation of the InnoQuant™ and InnoTyper™ 21 kits performed by DNA Solutions in compliance with QAS and ISO17025:2005 requirements, highlighting their utility for testing challenging samples such as human remains and rootless hair shafts. A total of 90 samples and 200 replicates were evaluated during this validation study. An updated version of the InnoQuant™ kit was utilized that is compatible with the BioRad CFX Real-Time PCR system in use at DNA Solutions.

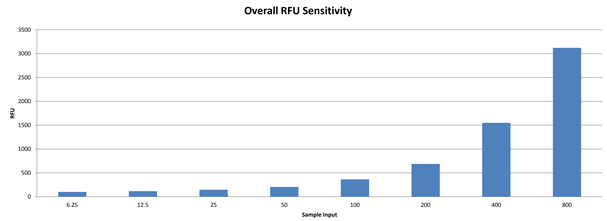
Minimum Threshold and Contamination
•InnoQuant™ kit did not contain or introduce contaminating DNA
•Analytical threshold determined and used throughout the validation:
•Blue 69, Green 90, Yellow and Red 110
•Stochastic threshold calculated and used throughout the validation:
•278 RFU
•POP7 artifacts observed:

Dye	Avg. base pair size	Avg. RFU	Label and Marker Range
Blue	91.236	772	"OL"/ALU79712
Green	77.106	71	"OL"/RG148
Green	87.954	628	"OL"/NBC13
Yellow	85.467	136	Between Amel and AC2305
Yellow	108.929	39	"OL" or "I"/HSA4.69
Yellow	111.147	96	"OL"/HS4.69

Standard Curve and Control Metrics Evaluation
•Three standard dilution series were prepared using InnoQuant™.
•Two standard dilution series were run on the five quantification plates used in this validation.
•Short and long target standard curves were examined and quality metrics were recorded.
•All standard curves showed acceptable PCR efficiencies, R^2 values, and y-intercept values.

NIST Inter-laboratory Study
An inter-laboratory study was preformed using NIST components 2391c. Samples were amplified using InnoTyper™ 21 kit at DNA Solutions and InnoGenomics. The profiles produced at DNA Solutions were fully concordant with the profiles produced at InnoGenomics. Profiles produced at DNA solutions had slightly higher peak heights and contained POP7 artifacts. However, this artifacts did not interfere with interpretation.

Sensitivity
•Human male gDNA was quantified with InnoQuant™, yielding a short target value of 0.1 ng/µL.
•DNA was diluted to a range of 6.25 pg/µL to 800 pg/µL and amplified in triplicate with InnoTyper ™21 kit
•Complete profiles were observed for samples containing DNA concentrations of 200pg and above
•Partial profiles were observed at 100pg and below.



Precision and Reproducibility
The InnoQuant™ kit provided quantification results with relative standard deviations below 22% in samples containing at least 0.2pg/µL DNA.
All 48 ladders produced standard deviations less than 0.15bp, which meets recommendations. The replicate amplifications showed standard deviations less than 0.0721%. Bases on these results, the kit yields accurate and reproducible results.

Mixtures
• Two DNA samples (one male and one female) of known quantities were used to prepare the mixture series
• Approximately 0.4ng (4µL) of each mixture dilution was amplified
• DNA profiles were correctly identified in all of the mixture dilutions, except for 15:1 where a partial mixture profile was obtained
• It should be noted that in bi-allelic kits such as InnoTyper ™ 21, peaks heights cannot always determine the major and minor contributor
• Mixtures of more than two contributors would provide some complications during analysis and should be analyzed with caution

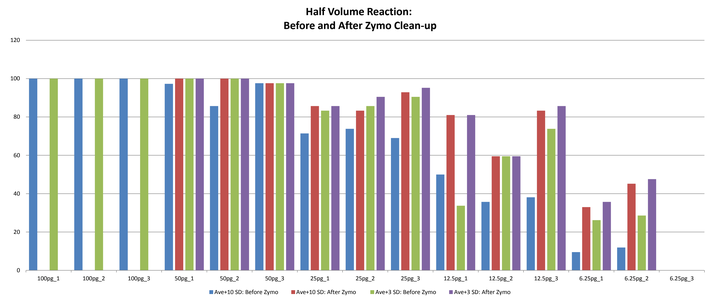
InnoTyper™ 21 profile 1:1 mixture.

InnoTyper™ 21 profile 15:1 mixture.

Forensic Samples
Twenty-eight non-probative samples of varying types (buccal swabs, hair shafts, blood, bone, and semen) were quantified with InnoQuant™ and amplified with InnoTyper ™21. Samples with a quantity of less than 100 pg were concentrated using post-PCR clean up columns (Zymo D4013).

Sample Number	Sample Type	InnoQuant ™ Short Concentration (ng/µL)	IPC CT	DI	Total DNA Input for InnoTyper™ 21 (ng)	InnoTyper™ 21 Result
NP1	Buccal swab	0.02213	18.98	0.97	0.35408	Full Profile
NP2	Hair	0.00026	18.63		0.00416	Partial Profile
NP3	Buccal swab	0.38390	18.59	1.16	0.42229	Full Profile
NP4	Buccal swab	0.06473	18.27	1.72	0.40133	Full Profile
NP5	Buccal swab	0.25188	18.44	1.07	0.40301	Full Profile
NP6	Hair	0.01184	18.47	15.71	0.18944	No Profile
NP7	Buccal swab	0.49556	18.37	0.84	0.49556	Full Profile
NP8	Hair	0.00032	18.68		0.00512	No Profile
NP9	Hair	0.00811	18.80	1.42	0.12976	Partial Profile
NP10	RB- hair samples	0.00007	18.50		0.00112	No Profile
NP11	RB-swab samples	-	18.90		0	No Profile
NP12	Bone	0.06862	18.54	1.38	0.41172	Full Profile
NP13	Bone	0.00172	18.38	2.63	0.02752	No Profile
NP14	Bone	0.01768	18.43	4.25	0.28288	Partial Profile
NP15	Bone	0.00531	18.47	2.72	0.08496	No Profile
NP16	Bone	0.00210	18.93	2.49	0.0336	No Profile
NP17	Bone	0.00409	18.87	11.18	0.6544	No Profile
NP18	RB- for NP13	0.00003	18.64		0.00048	No Profile
NP19	RB- for NP14	0.00003	18.56		0.00048	No Profile
NP20	RB- for NP15	0.00004	18.40		0.00064	No Profile
NP21	RB- for NP16	0.00003	18.43		0.00048	No Profile
NP22	RB- for NP17	0.00002	18.81		0.00032	No Profile
NP23	Semen	0.40051	18.29	9.26	0.40051	Full Profile
NP24	Blood	0.00114	18.62	0.75	0.01596	Partial Profile
NP25	Blood	0.04156	18.56	0.94	0.12468	Full Profile
NP26	Blood	0.00290	18.68	1.06	0.0464	Partial Profile
NP27	Hair	0.00009	18.92		0.00144	No Profile
NP28	Hair	0.00396	18.61	1.29	0.06336	Partial Profile

Protocol Modifications
The last five dilutions of the sensitivity series (100, 50, 25, 12.5, and 6.25 pg) were amplified in triplicate using a 12.5µL (half) reaction volume with and without post-PCR clean up.



Conclusion
The validations of InnoQuant™ H-Dye DNA Quantification Kit and InnoTyper™ 21 were successful. The data shows that both kits provide results for a variety of samples with accuracy and precision. Sample quality can be determined by the InnoQuant ™with the use of the degradation index while InnoTyper ™is able to obtain profiles from compromised samples.
Based on the results from the validation of InnoQuant™ and InnoTyper™, DNA Solutions has established procedures and interpretation guidelines for these kits. The validation requirements for these kits are in compliance with QAS and ISO17025:2005 and can be used in forensic casework.

Acknowledgements- Thank you to InnoGenomics™ for providing InnoTyper™ and InnoQuant™ kits along with technical support for their use.