Developmental validation of InnoQuant® HY, a sensitive human and male DNA real-time PCR quantitation and degradation assessment system for forensic samples

Andrew Loftus, Ph.D., Gina Murphy, M.S., Anne H. Montgomery, M.S., Jonathan Tabak, B.A., and Sudhir K. Sinha, Ph.D.

InnoGenomics Technologies, LLC; 1441 Canal St, Suite 307, New Orleans, LA 70112

Introduction

The InnoQuant® HY DNA Quantification Kit is designed to quantify the total amount of amplifiable human and male DNA in a sample, and provides indication of a sample's quality and integrity (level of degradation). This multiplexed four target real-time qPCR assay contains an internal positive control (IPC) and two different intra-Retrotransposon targets, high copy number Alu (80 bp) and SVA (207 bp), as well as a sensitive multi-copy Y-chromosome specific male target for additional information about a sample's male DNA composition. The use of high copy number (>1000 copies/genome) retrotransposable element targets provides excellent sensitivity ranging 0.001–100 ng/µL, with a limit of detection < 1 pg/µL. The large copy number of these human-specific DNA assays also minimizes the effect of variation between individuals, resulting in highly reproducible quantitation values from InnoQuant HY.

InnoQuant® HY uses two independent autosomal targets to provide an assessment of the level of degradation of a forensic sample, and employs a multi-copy target on the Y chromosome, with an IPC for PCR inhibition assessment:

Target	Genomic Location	Amplicon Length (bp)	Reporter Dye
Short	Yb8 autosomal RE	80	HEX
Long	SVA autosomal RE	207	Cy5
Male	Y chromosome	79	FAM
IPC	Synthetic sequence	172	TAMRA*

* For instruments not requiring the use of a reference dye, a kit version with IPC labeled in ROX is available

This study describes the results of the development and validation of the InnoQuant® HY assay.

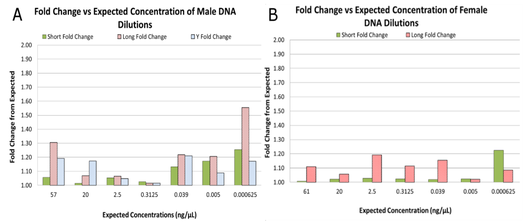
Materials and Methods

- The assays were run on an Applied Biosystems 7500 Real Time PCR instrument and data collection utilizing the HID Real-Time PCR Analysis Software v 1.2.
- PCR conditions were one denaturation cycle for 10 min at 95ºC, followed by 40 cycles of 2-step qPCR (15 sec at 95ºC and 2 min at 61ºC combined annealing/extension time). Brilliant QPCR Master Mix and ROX-labeled passive reference dye that is included in the InnoQuant HY kit was used (from Agilent Technologies) per manufacturer instructions.
- The 7500 was calibrated for FAM and ROX dyes using the dye calibration plates from Applied Biosystems following manufacturer instructions. For HEX, Cy5, and TAMRA dyes, calibration plates were prepared using the InnoQuant® Spectral Calibration kit (InnoGenomics, New Orleans, LA) according to manufacturer instructions.
- Degradation indices were determined by: $DI_{80/207}$ = [short]/[long]
- AB 3130 Genetic Analyzer was utilized. Data analysis was performed with GeneMapper® *ID-X* using an analytical threshold of 50 RFU.

Results

Sensitivity & Accuracy Study

Serial dilutions of NIST A and NIST B samples from SRM 2372 (Human DNA Quantitation Standard) within the standard curve (20 ng/µL to 0.005 ng/µL) showed average variations from the expected values of 5% for the short target, 11% for the long target, and 11% for the Y target, with fold changes increasing as template DNA decreased.



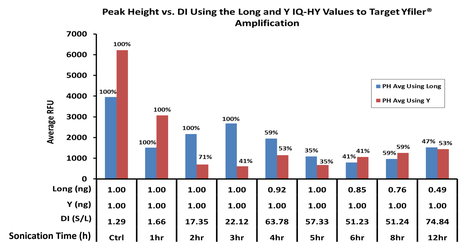
InnoQuant® HY kit consistently and reliably detected all samples at as little as 0.625 pg/µL of total DNA

Degradation & DNA Input Target Study

Mechanically degraded genomic DNA via sonication produced higher DI as sonication time increased:

Sonication Time (hours)	Short Qty. (ng/µL)	Long Qty. (ng/µL)	Y Qty. (ng/µL)	DI (S/L)	S/Y
0	14.805	11.447	13.869	1.29	1.07
1	16.779	10.087	12.021	1.66	1.40
2	7.341	0.423	4.531	17.35	1.62
3	8.480	0.383	4.695	22.12	1.81
4	5.876	0.092	3.446	63.78	1.70
5	5.978	0.104	3.556	57.33	1.68
6	4.361	0.085	2.906	51.23	1.50
8	3.899	0.076	2.788	51.24	1.40
12	3.645	0.049	2.476	74.84	1.47

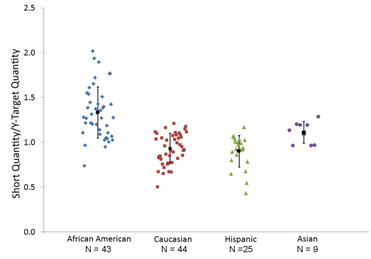
Sonicated samples were amplified with Yfiler® using the Y target and Long quantity values to target the DNA input amount for the amplification. This was done to determine if the quantitation result of the Long target could be used to more effectively target the STR input amount in degraded samples.



Overall, the InnoQuant® HY Long target is shown to be an accurate indicator of STR profile success for degraded samples, showing improved allele recovery and peak heights compared to the Y target.

Population Study

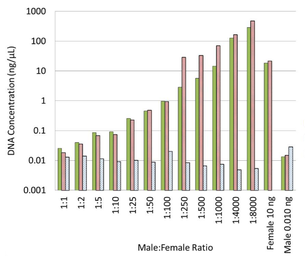
A system designed with such high copy targets in the human genome is more resistant to individual sequence variations. Therefore, even in the presence of slight variations among individuals, the system is highly reproducible among populations. Short/Y (S/Y) ratios for the 120 male DNA samples were analyzed:



The average ratio of the quantification values for the Short/Y target among the four populations tested is 1.082 +/-0.290.

Mixture Study

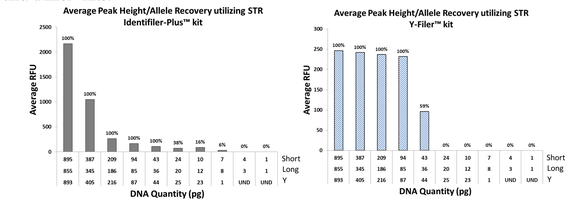
A series of samples were prepared with mixtures of human male NIST A and human female NIST B DNA. The male DNA was kept at a constant low level of 10 pg/µL while the female DNA was added at increasing levels to create the male-to-female ratio range from 1:1 to 1:8000. The aim of the study was to demonstrate that the male DNA could be accurately detected in samples with large female DNA concentrations.



Y target quantification results in the mixture samples with 10 pg/µL of male DNA yielded an average value of 9.4 +/- 4.2 pg/µL. The InnoQuant® HY kit was able to detect male DNA up to the highest male:female ratio of 1:8000 tested.

True-Zero Study

To gauge the ability of the InnoQuant HY kit to establish a "true zero" value (the value at which it can be confidently determined that no usable STR profile will be obtained), titrated DNA was amplified in duplicate using the IDP (Identifiler® Plus) and Yfiler® kits.



True Zero value for this study = <7 pg total input DNA for IDP and <24 pg total input DNA for Yfiler®, using the short target

Conclusions

- The use of high copy number retrotransposon element targets significantly improves both sensitivity and reproducibility compared to previously or currently available commercial real-time PCR kits.
- Results demonstrate improved detection sensitivity with low-concentration DNA samples (to sub-picogram per microliter levels of DNA), high reproducibility across populations, and a useful tool to assess degradation in a biological sample.
- The InnoQuant® HY kit provides comprehensive sample assessment for better efficiency within the forensic casework workflow. These validation studies have demonstrated that the targets of the InnoQuant® HY kit can be efficiently used to maximize the STR information obtained from a sample in the first attempt.
  - *By using the long target to calculate the volume needed for the STR amplification as degradation increases, more alleles can be recovered in the first amplification.*
  - *A true zero value can be established using the quantitation results from InnoQuant HY and STR kits.*
- The Y chromosome target has been shown to have enhanced limit of detection and sensitivity in detecting male DNA, making it an efficient tool in screening sexual assault samples.
- InnoQuant® HY is a robust real-time PCR method, producing sensitive, reliable, and reproducible quantitation results

Acknowledgments

This material is based upon work supported by the National Science Foundation under Grant No. 1230352. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

For additional information, contact:
gpineda@innogenomics.com.