Determining a stop point and optimal targeting values for downstream STR and Y-STR amplification using InnoQuant® HY Quantitation and Quality Assessment System

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Introduction

Advancements in forensic quantitation systems have greatly improved the sensitivity and reliability of sample detection in past decades. The most recently available commercial kits employ the use of two autosomal targets of different sizes to determine the level of degradation in a sample. This degradation value is known as the Degradation Index (DI), and is obtained by taking the ratio of the two target quantity values to determine the relative degradation of a sample. In addition to providing the DI, a male specific target on the Y chromosome also provides quantitation information for the male DNA present in a sample. The practical question becomes how to use the results of these next-generation quantitation kits in the everyday laboratory workflow. Previously, laboratories could simply use the single quantity value obtained from an autosomal target from each sample DNA extract to target the DNA input for downstream PCR amplification typing systems. Now, however, laboratories have additional information that may be used to more informatively target downstream typing systems, namely the long autosomal target.

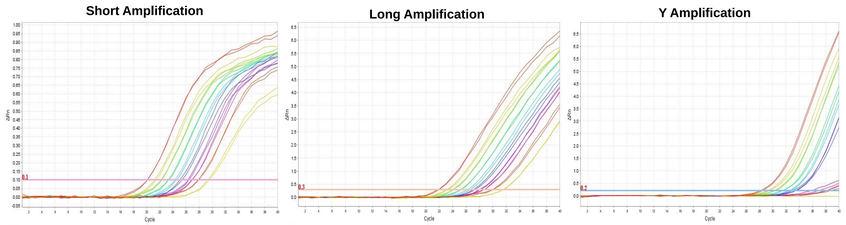
Here we describe the use of the human/male DNA quantification kit InnoQuant® HY to determine a "true zero" point value at which one can confidently identify samples that will fail to yield useful STR data. We also explore the degradation of sperm samples to determine how the degradation index (DI) and the concentration of each target correlates to STR allele recovery.

Materials and Methods

- Sample Preparation: NIST SRM 2372 A DNA sample was diluted from ~0.09 ng/µL to 0.0001 ng/µL using InnoQuant® Dilution Buffer A. A single sourced semen sample taken as is, aliquoted and used to soak a sterile cotton swab was allowed to dry overnight. Swabs were then subjected to UV light at 15 J/s at intervals from 0-6 hrs. Samples were then cut with sterilized scissors and rehydrated for extraction steps. Sonication was performed over a number of hours to mechanically degrade a separate genomic male DNA sample for degradation studies.
- DNA extraction: Semen samples on cotton were first washed and spun down to collect the sperm pellet. The samples were then subjected to a proteinase K/DTT digestion overnight followed by phenol-chloroform extraction of the DNA.
- DNA quantification: DNA extracts were quantitated using degradation assessment qPCR quantitation kit, InnoQuant HY® allowing determination of the degradation index (DI = [short]/[long]) and Y target concentration.
- PCR Amplification and typing: Samples were amplified using the Identifiler® Plus and Yfiler® PCR Amplification Kits according to manufacturer recommendations. The AB 3130 Genetic Analyzer was utilized. Data analysis was performed with GeneMapper® *ID-X* using an analytical threshold of 50 RFU.

Results

QPCR Results of Dilutions of NIST SRM 2372 A utilizing InnoQuant® HY

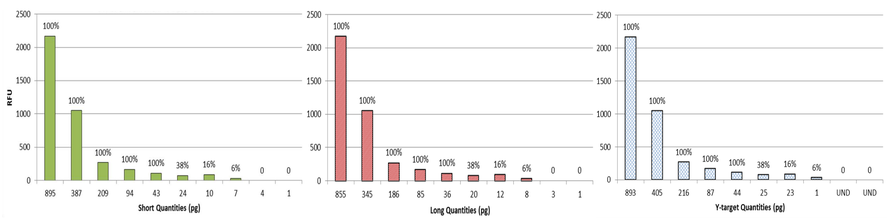


Dilutions	Short (ng/µL)	Long (ng/µL)	Y (ng/µL)	DI (Short/Long)	Short/Y Ratio
NISTA	0.0895	0.0855	0.0893	1.05	1.00
1:2	0.0387	0.0345	0.0405	1.12	0.96
1:4	0.0209	0.0186	0.0216	1.13	0.97
1:10	0.0094	0.0085	0.0087	1.10	1.08
1:20	0.0043	0.0036	0.0044	1.19	0.98
1:40	0.0024	0.0020	0.0025	1.18	0.95
1:90	0.0010	0.0012	0.0023	0.82	0.44
1:125	0.0007	0.0008	0.0001	0.94	8.04
1:225	0.0004	0.0003	UND	1.27	UND
1:1000	0.0001	0.0001	UND	1.20	UND

- **Serial Dilutions of NIST A were created and qPCR performed using the InnoQuant® HY kit primers and standards. Results show detection of each of the targets (short, long and Y) down to the lower dilution levels**

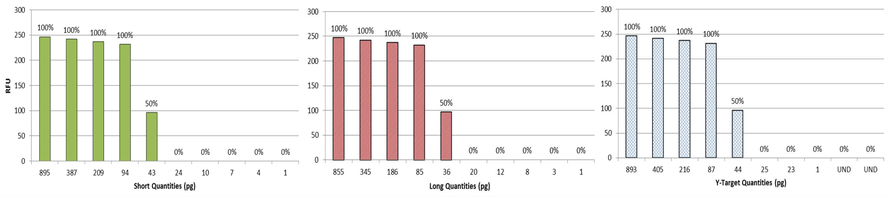
Detection of the short and long targets can be seen in the sub-pg/µL range at the lowest dilution levels.

Identifiler® Plus Allele Recovery based on InnoQuant HY qPCR Quantities



- **Identifiler® Plus correlates with IQHY qPCR concentrations providing full STR recovery results down to 43, 36 and 44 pg for Short, Long and Y targets, respectively.**
- **Partial recoveries are capable as low as 7, 8 and 1 pg of DNA as calculated from the IQHY results for Short, Long and Y targets respectively.**

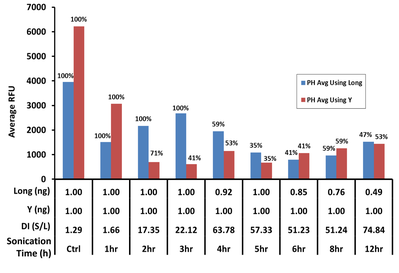
Yfiler® Allele Recovery based on InnoQuant HY Quantities



- **Yfiler® correlates with IQHY data providing full STR recovery results corresponding to 94, 85 and 87 pg for Short, Long and Y targets respectively.**
- **Partial recoveries are capable as low as 43, 36 and 44 pg of DNA as calculated from the IQHY results for Short, Long and Y targets respectively.**

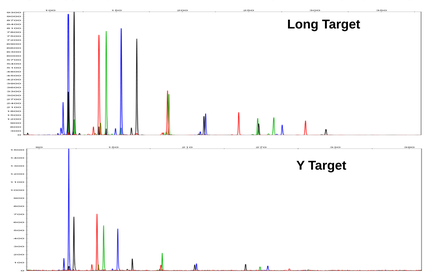
Degraded Sonication Samples: InnoQuant HY qPCR and STR Allele Recovery

Peak Height and Allele Recovery for Sonicated DNA Samples with Yfiler Amplification



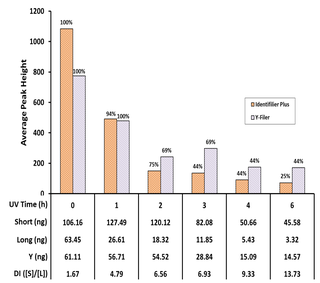
Long (ng)	1.00	1.00	1.00	1.00	0.92	1.00	0.85	0.76	0.49
Y (ng)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DI (S/L)	1.29	1.66	17.35	22.12	63.78	57.33	51.23	51.24	74.84
Sonication Time (h)	Ctrl	1hr	2hr	3hr	4hr	5hr	6hr	8hr	12hr

Yfiler electropherograms of sample with DI 22.12, showing increased PH and 100% allele recovery using DNA input based on IQHY long target (top) compared to 41% allele recovery with Y target (bottom)



- **With DNA samples degraded by sonication, sample degradation occurred as specified by the [Short]/[Long] values denoted DI.**
- **With DI calculated by IQHY as high 74.84, STR data can still be obtained**
- **InnoQuant HY long target is shown to be an accurate indicator of profile success for degraded samples, showing improved allele recovery and peak heights compared to short and Y target.**

UV Degraded Samples: IQHY qPCR and STR Allele Recovery



UV Time (h)	0	1	2	3	4	6
Short (ng)	106.16	127.49	120.12	82.08	50.66	45.58
Long (ng)	63.45	26.61	18.32	11.85	5.43	3.32
Y (ng)	61.11	56.71	54.52	28.84	15.09	14.57
DI ([S]/[L])	1.67	4.79	6.56	6.93	9.33	13.73

- **UV degraded samples show DI values increasing resulting in lower IQHY long target recovery**
- **As seen with the sonicated samples, using the long target quantitation values compared to the short or Y target will increase allele recovery with more degraded samples**

Conclusions

- Results demonstrate improved detection sensitivity with low-concentration DNA samples (to sub-picogram/µL levels of DNA) and a useful tool to assess degradation in a biological sample.
- 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.
- 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.
- A true zero value can be established using the quantitation results from InnoQuant HY and STR kits, with values for each target demonstrated for Identifiler Plus and Yfiler kits.
- The InnoQuant HY kit provides a streamlined workflow for better efficiency within forensic casework laboratories. The InnoQuant HY kit can be efficiently used to screen true negatives and successfully obtain the most STR information from a sample in the first attempt.

Acknowledgments

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