

Introduction

- Quantitation of human DNA is critical to forensic DNA casework. Commercial multiplex kits such as Quantifiler® Duo are used to provide information on the quantity of DNA, presence of inhibitors, and male to female ratio. Newer commercial kits, such as Quantifiler® Trio, also provide information on the extent of DNA degradation, reflecting the quality of the sample. DNA analysts use acquired quantitation information to determine the how to best process each evidence sample through amplification and development of a DNA profile.
- Quantifiler® Duo and Quantifiler® Trio cost approximately \$9 and \$6 per evidence sample, respectively. Reduction in reaction volume holds a large potential for cost savings for academic and caseworking forensic DNA labs.

Objectives

- Evaluate the suitability of reduced reaction volume for Quantifiler® Duo and Quantifiler® Trio by assessing reproducibility and comparison of results to manufacturer's protocol
- Assess reduced reaction volume analysis of degraded and inhibited samples

Materials and Methods

- Manufacturer's instructions and protocol were used for all experiments. The reagent volumes were modified as in Table 1. In all experiments, samples were run in full and reduced volumes simultaneously using Applied Biosystems 7500 Real-Time PCR System and HID Real-Time PCR Analysis Software v 1.2. Purchased DNA from immortal cell lines was used (9947A, 007, 2800M).
- Reproducibility of the protocol was assessed by quantifying two samples in triplicate three times by at least two researchers.
- Inhibited samples containing a constant concentration of DNA and 0-750ng/μL humid acid were created and analyzed. 1ng of selected samples was amplified in triplicate using Globalfiler® amplification kit. *
- Degraded samples were created with exposure to 100°C for 0-30 minutes.

	Quantifiler® Duo		Quantifiler® Trio	
	Full	Reduced	Full	Reduced
Reaction Mix	12.5	3.125	10	2.5
Primer Mix	10.5	2.625	8	2
Water	0	0.25	0	0
Sample	2	2	2	2
Total Volume	25	8	20	6.5

Table 1. Reaction Volumes

Volumes (in μL) used in manufacturer's protocols and our modified reduced volume protocols. Sample volume remained constant (2.0 μL).

*Veriti Thermal Cycler, 3500 Genetic Analyzer, and GeneMapper® ID-X v. 1.4 were used according to manufacturer's recommendations

Results

Reproducibility Study - Quantifiler® Duo and Quantifiler® Trio

- Replicate analysis of samples in reduced reaction volumes demonstrated reproducible results
- Reduced reaction volumes of Quantifiler® Duo and Quantifiler® Trio returned quantitation results for all targets that are comparable to the full volumes

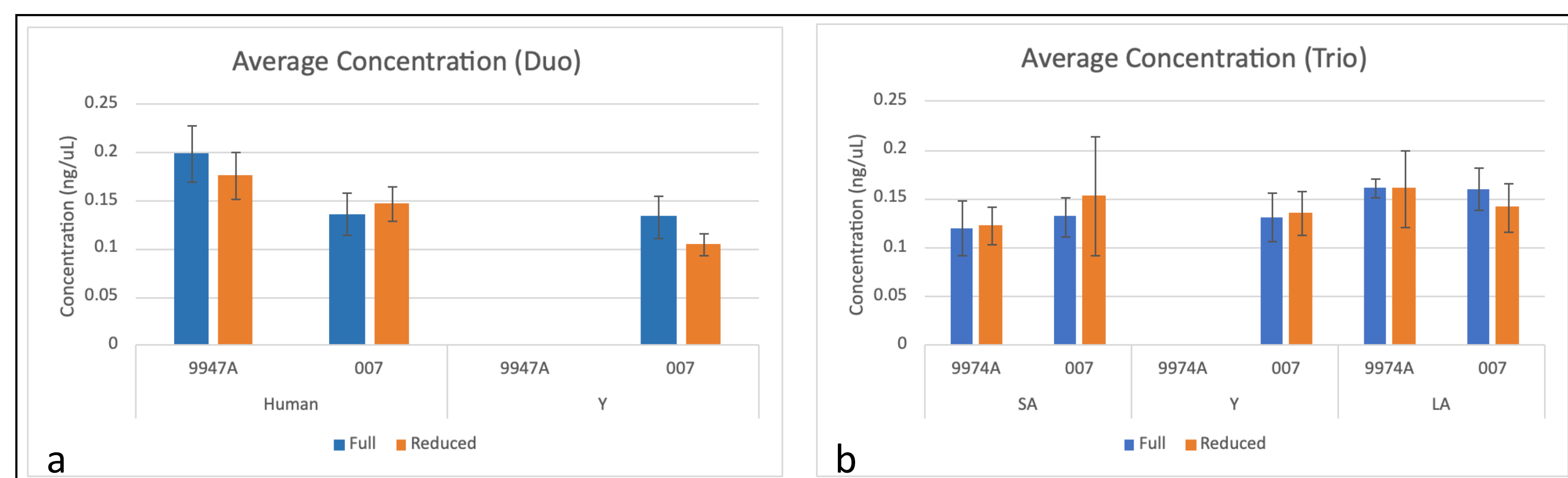


Figure 1. Quantitation Results in Full and Reduced Volume Reactions

Reproducibility: results of replicates in reduced reaction volume protocols illustrate reproducibility. Analysis of all targets in full and reduced volume reactions returned comparable concentrations using Quantifiler® Duo (a) and Quantifiler® Trio (b). SA = small autosomal target; LA = large autosomal target; Y = male target

Results continued

Inhibition Study - Quantifiler® Duo

- Reduced reaction volume demonstrated elevated and undetected IPC C_T with lower concentrations of inhibitor. Given the increased proportion of sample in the reaction, these results were expected. Similar levels of inhibitor in the reaction between the two conditions generated similar IPC C_T results.
- The 60ng/μL and 30ng/μL humid acid samples (undetected and elevated IPC C_T in the reduced volume protocol) were successfully amplified in Globalfiler® and demonstrated no decrease in average peak height. Elevated or undetected IPC C_T in reduced volume is not predictive of inhibition during amplification.

In sample	Humic Acid Conc (ng/μL)		Globalfiler Average Peak Height
	In full reaction	In reduced reaction	
750	60	187.5	2
187.5	15	46.875	NA
100	8	25	NA
60	4.8	15	3185
40	3.2	10	NA
30	2.4	7.5	2496
20	1.6	5	NA
0	0	0	2953

Table 2. Quantifiler® Duo Inhibition Results

Columns 2 and 3 show the concentration of inhibitors in the full reaction and reduced reactions, respectively. IPC C_T results are similar with respect to concentration of inhibitor in each reaction. Yellow represents samples with elevated IPC C_T. Red represents samples with undetected IPC C_T. Orange represents samples with some replicates elevated IPC C_T and others undetected. Gray represents samples not analyzed. Column 4 shows the average heterozygote peak height for amplified samples. Samples with different inhibitor levels generating undetected IPC C_T during quantitation exhibit extreme differences in peak heights upon amplification. NA = samples not amplified in Globalfiler®

Degradation Study - Quantifiler® Trio

- Degraded samples returned decreased quantitation values for both small and large autosomal targets
- Degraded samples demonstrated increased ratio between small autosomal and large autosomal targets, also known as Degradation Index

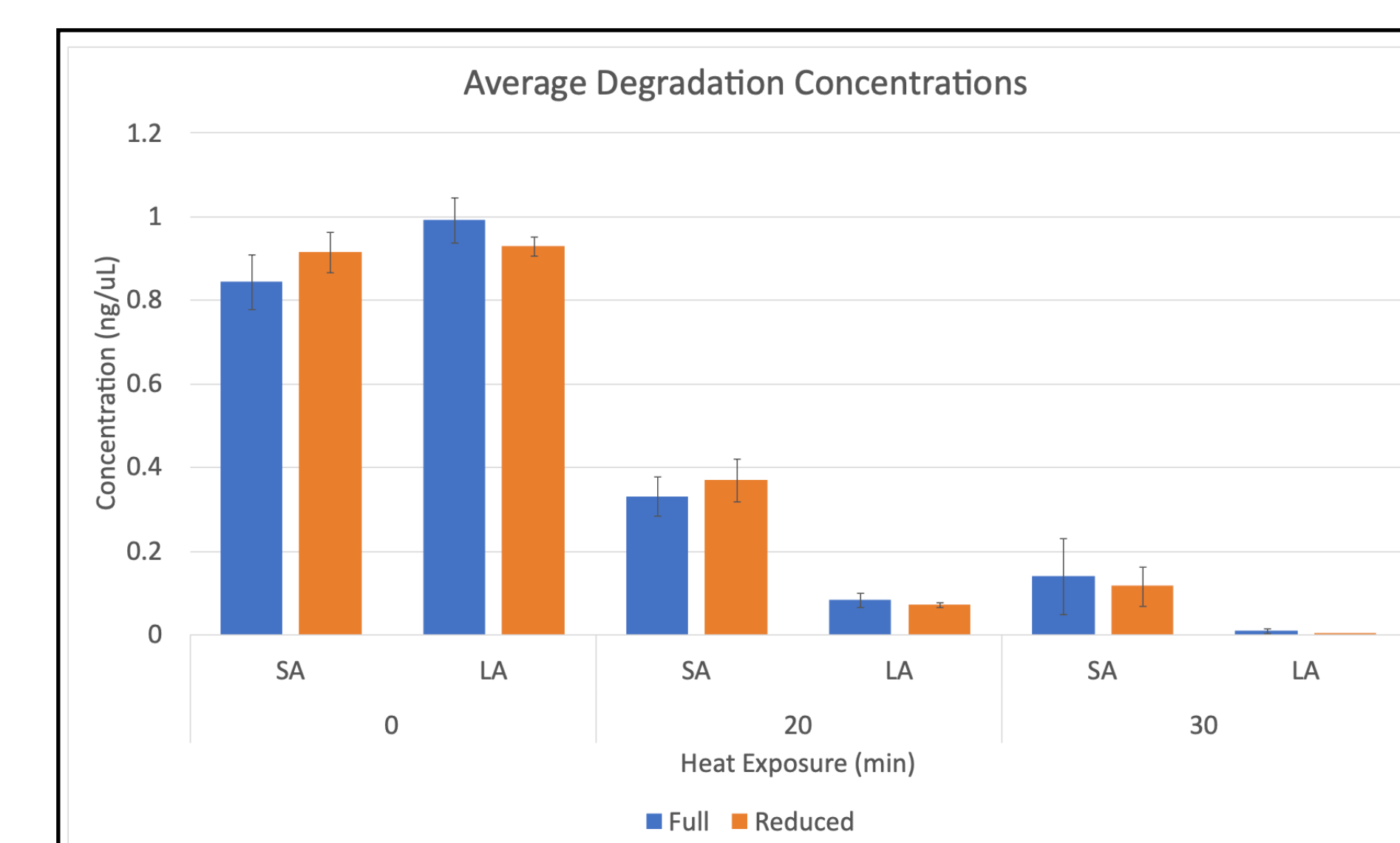


Figure 3. Quantitation Results of Degraded Samples in Full and Reduced Volume Reactions

2800M was exposed to 100°C for 0-30 minutes. Full and reduced volume reactions returned similar results in both quantitation targets. SA = small autosomal target; LA = large autosomal target

Conclusions and Future Work

- Reduced reaction volume demonstrates comparable results to full volume with respect to degradation in Quantifiler® Trio.
- Reduced reaction volumes are more sensitive to inhibitors due to the proportional increase of inhibitor concentration (Quantifiler® Duo). Partial or complete inhibition in reduced reaction volume does not correlate to failure in STR amplification. Samples that return undetected IPC C_T with these parameters should be re-quantified, either using the full volume reaction or a diluted sample. Samples that return elevated IPC C_T with these parameters should be amplified as normal.
- Inhibited samples will be evaluated in Quantifiler® Trio.
- Additional studies on mixtures of male and female DNA will be completed before implementation.
- Mock and real casework samples should be evaluated to determine suitability in forensic laboratories.

References

- Westring CG, Kristinsson R, Gilbert DM, Danielson PB. Validation of reduced-scale reactions for the Quantifiler Human DNA kit. *J Forensic Sci.* 2007 Sep; 52(5):1035-1043.
- Cho Y, Kim HS, Kim MH, Park M, Kwon H, Lee YH, Lee DS. Validation of Reduced Reagent Volumes in the Implementation of the Quantifiler® Trio Quantification Kit. *J Forensic Sci.* 2018 Mar; 63(2):517-525.
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- ThermoFisher Scientific. Quantifiler HP and Trio DNA Quantification Kits User Guide. Revision H. 09 Oct 2018.
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