

Evaluation of the PowerQuant[®] System on the QuantStudio[™] 5 Real-Time PCR System

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PowerQuant[®] System (Cat.# PQ5002)

The ability of the PowerQuant[®] System to detect mixed, degraded and inhibited samples on the QuantStudio[™] 5 System is comparable to that on the 7500 System.

Introduction

Quantifying the amount of human DNA extracted from a biological evidence sample is an essential step in the forensic DNA typing workflow. Quantification provides critical information needed to perform further downstream analysis, such as amplification of short tandem repeats (STR). Accurate quantification is also necessary to normalize the DNA template amount so that it falls within the optimal range of a PCR assay (1).

The latest commercial quantification kits, such as the Promega PowerQuant[®] System, also provide information about the quality of DNA extracted from a sample. The PowerQuant[®] System includes targets that assess DNA degradation and PCR inhibitors (2). The initial validation of the PowerQuant[®] System was performed on an Applied Biosystems[®] 7500 Real-Time PCR System (2).

The Applied Biosystems[®] QuantStudio[™] 5 Real-Time PCR System contains a six-color optical filter set for fluorescence-based quantitative and qualitative detection of nucleic acid targets (3). The experiments in this White Paper address the performance of the QuantStudio[™] 5 Real-Time PCR System used in conjunction with the PowerQuant[®] System for the quantification of forensic samples. The experiments are based on requirements listed in the Federal Bureau of Investigation (FBI) Quality Assurance Standards for Forensic DNA Testing Laboratories (4) and guidelines outlined by the Scientific Working Group on DNA Analysis Methods (5).

Materials and Methods

Samples

Human genomic DNA extracts were collected from male and female volunteer donors. The hematin and humic acid inhibitor stock chemicals were purchased from Sigma-Aldrich (MO, USA). All other chemicals used in this study were of analytical grade.

DNA Quantification and Real-Time PCR Method

All DNA extracts were quantified using the PowerQuant® System following the protocol detailed in the *PowerQuant® System Technical Manual #TMD047* (6). Experiments were performed on both the Applied Biosystems® 7500 Real-Time PCR System (7500) and the QuantStudio™ 5 Real-Time PCR System (QuantStudio™ 5). The 7500 System was used in combination with Applied Biosystems® 7500 Software ver. 2.3. The QuantStudio™ 5 System was used in combination with the QuantStudio™ Design and Analysis Software ver. 1.4.3. Spectral calibrations using the PowerQuant® Calibration Kit were completed on both instruments. The PCR thermal cycling method from the *PowerQuant® System Technical Manual #TMD047* was applied to both instruments. The ramp rate for the 7500 System was previously optimized to 100%. The ramp rate for the QuantStudio™ 5 System was tested and a rate of 2.44°C/second was chosen.

Data Analysis

All data were analyzed initially on the 7500 Software ver. 2.3 and QuantStudio™ Design and Analysis Software ver. 1.4.3, respectively. The results were imported into the PowerQuant® Analysis Tool ver. 1.0.0.0 for further calculations and evaluation. The following sample quality flags were monitored throughout the studies: 1) the ratio of autosomal DNA target concentration to that of the target for assessing DNA degradation ([Auto]/[Deg]); 2) the ratio of autosomal DNA target concentration to that of the Y-chromosomal target ([Auto]/[Y]); and 3) the Internal PCR Control (IPC) C_q. These quality flags provide information about the sample and probable STR profile quality. A threshold of 2 was used for the [Auto]/[Deg] and [Auto]/[Y] ratios to flag a sample as possibly degraded or as a potential mixture, respectively. A shift of 0.3 for the IPC C_q was used to flag a sample for possible inhibition. The analyzed data produced by the QuantStudio™ 5 System were compared to the equivalent experimental data produced by the 7500 System.

Sensitivity Study

To evaluate the sensitivity of the PowerQuant® System on the QuantStudio™ 5 System, a male genomic DNA sample was diluted to the following concentrations: 0.625, 0.125, 0.025, 0.005, 0.001, 0.0002 and 0.00004ng/μl. Additionally,

the linearity of the assay within the dynamic range of the standard curve was tested with a female genomic DNA sample diluted to the following concentrations: 50, 10, 2.0, 0.4, 0.08, 0.016 and 0.0032ng/μl. Each DNA concentration was tested in duplicate. The initial DNA concentration of the male and female samples was determined by A₂₆₀ measurement.

Repeatability Study

The repeatability of DNA quantity and quality obtained from replicate samples was assessed on the QuantStudio™ 5 System. The PowerQuant® Male genomic DNA Standard was diluted to the following concentrations: 45, 10, 0.40 and 0.016ng/μl. The samples were tested in triplicate to compare replicates within a quantification run. The setup was performed on an additional amplification plate to compare replicates among quantification runs.

Mixture Study

A mixture study was performed to show that extracts containing DNA from mixed donors in a variety of ratios can be reliably detected by the PowerQuant® System on the QuantStudio™ 5 System. Two mixture series were prepared by combining DNA extracts to obtain M:F ratios of 1:1, 1:5, 1:10, 1:15, 1:20, 1:35, 1:50, 1:65 and 1:80. The mixtures were designed to keep the male DNA amount constant in the presence of increasing amounts of female DNA. Mixture samples were tested in duplicate.

Degradation Study

A study was performed to assess the ability of the PowerQuant® System on the QuantStudio™ 5 System to detect degraded DNA. Two male genomic DNA samples were exposed to increasing amounts of ultraviolet (UV) energy utilizing a Stratlinker 1800 instrument (Stratagene, CA, USA). The samples were exposed to the following amounts of UV radiation: no exposure, 50, 100 and 300mJ. The degraded samples were tested in duplicate.

Inhibition Study

A study was performed to show that DNA containing common PCR inhibitors can be detected by the PowerQuant® System on the QuantStudio™ 5 System. A male genomic DNA sample was prepared at three different concentrations: 2.5, 1.0 and 0.1ng/μl. Each DNA



concentration had an appropriate volume of inhibitor solution (humic acid or hematin) added to the extract. The concentrations of humic acid tested were: 20, 40 and 60ng/μl. The concentrations of hematin tested were: 41.7, 83.3 and 125.0μM. Additionally, each sample was run with no inhibitor as a control. All samples were tested in duplicate.

Contamination Study

To assess the potential for sample-to-sample contamination on the QuantStudio™ 5 System, no-template controls (NTC) were processed along with high-quantity samples (≥ 50 ng/μl) in an alternating pattern on the instrument. An additional four NTCs were also included with each amplification plate throughout the evaluation. NTCs were negative controls that contained only PowerQuant® reagents and no DNA.

Standard Curve Reproducibility Study

The standard curves generated for each amplification plate throughout the evaluation were assessed for consistency

between each run. Variability of the amplification efficiency, slope, Y-intercept and R² for each target were considered.

Results and Discussion

Sensitivity Study

Quantifiable levels of DNA were detected for every male DNA sample at every PowerQuant® target down to the 0.001ng/μl concentration on both instruments. No DNA was detected with the autosomal target at the 0.0002ng/μl concentration on the QuantStudio™ 5 System; however, DNA was detected at this concentration with the degradation and Y targets. No DNA was detected with any target at the 0.00004ng/μl concentration on either instrument. The range of detection for low-level DNA concentrations measured on the QuantStudio™ 5 System was comparable with the 7500 System (Table 1). A linear relationship between the expected quantity and measured concentration was observed (Figure 1). IPC C_q flags were not detected with the PowerQuant® System in any of the samples (data not shown).

Table 1. Average concentration and coefficient of variation (CV) across PowerQuant® targets on the 7500 and QuantStudio™ 5 Systems for the male DNA sensitivity sample. The standard deviation is shown for each concentration. 7500, 7500 Real-Time PCR System; QS5, QuantStudio™ 5 Real-Time PCR System.

Sample Expected Quantity (ng/μl)	[Auto] (ng/μl)		[Auto] CV (%)		[Deg] (ng/μl)		[Deg] CV (%)	
	7500	QS5	7500	QS5	7500	QS5	7500	QS5
0.625	0.57 ± 0.01	0.81 ± 0.03	2.4%	3.2%	0.50 ± 0.01	0.88 ± 0.01	2.6%	0.7%
0.125	0.103 ± 0.010	0.156 ± 0.014	9.5%	8.7%	0.107 ± 0.017	0.194 ± 0.009	16.1%	4.6%
0.025	0.0189 ± 0.0016	0.0278 ± 0.0002	8.6%	0.6%	0.0171 ± 0.0021	0.0367 ± 0.0003	12.4%	0.8%
0.005	0.0042 ± 0.0006	0.0068 ± 0.0012	14.1%	18.1%	0.0038 ± 0.0001	0.0074 ± 0.0009	2.6%	12.8%
0.001	0.00045 ± 0.00003	0.00134 ± 0.00002	7.2%	1.2%	0.00065 ± 0.00001	0.00111 ± 0.00009	2.0%	8.3%
0.0002	0.00033 ± 0.00000	0.00000 ± 0.00000	0.0%	0.0%	0.00063 ± 0.00000	0.00028 ± 0.00000	0.0%	0.0%
0.00004	0.00000 ± 0.00000	0.00000 ± 0.00000	0.0%	0.0%	0.00000 ± 0.00000	0.00000 ± 0.00000	0.0%	0.0%

Sample Expected Quantity (ng/μl)	[Y] (ng/μl)		[Y] CV (%)	
	7500	QS5	7500	QS5
0.625	0.55 ± 0.02	0.77 ± 0.03	3.4%	4.4%
0.125	0.103 ± 0.017	0.153 ± 0.002	16.8%	1.5%
0.025	0.0170 ± 0.0027	0.0266 ± 0.0002	15.9%	0.7%
0.005	0.0048 ± 0.0004	0.0052 ± 0.0008	8.0%	14.8%
0.001	0.00105 ± 0.00017	0.00099 ± 0.00017	15.8%	16.8%
0.0002	0.00037 ± 0.00000	0.00027 ± 0.00000	0.0%	0.0%
0.00004	0.00000 ± 0.00000	0.00000 ± 0.00000	0.0%	0.0%



The quantity of DNA detected for each female DNA sample with PowerQuant® was similar between the QuantStudio™ 5 and 7500 Systems for all targets (Table 2). The DNA concentrations of the samples processed on the QuantStudio™ 5 System showed less variability among runs than the samples processed on the 7500 System.

A linear relationship was observed between the expected quantity and actual concentration within the dynamic range of the PowerQuant® standard curve (Figure 2). IPC C_q flags were not detected with the PowerQuant® System in any of the samples (data not shown).

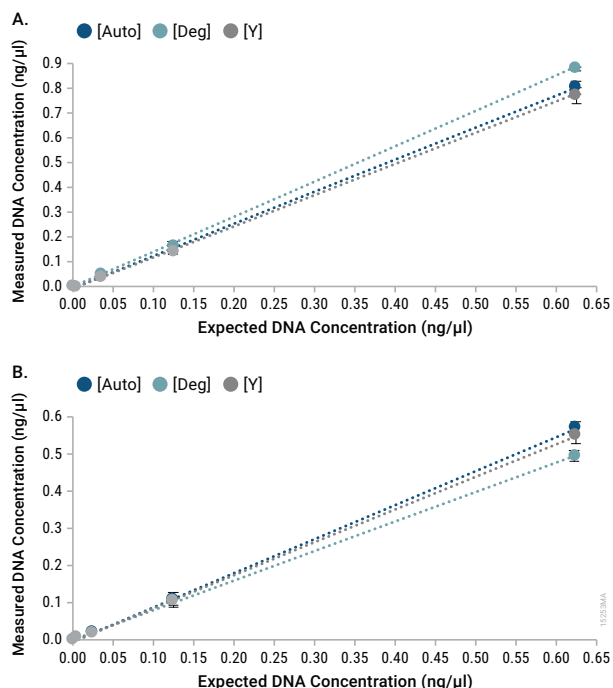


Figure 1. Linearity of DNA concentration obtained from the male DNA sensitivity sample on the QuantStudio™ 5 (Panel A) and the 7500 (Panel B) Systems. The X and Y axes represent the expected and measured DNA concentrations, respectively, for the autosomal [Auto], degradation [Deg] and Y-chromosomal [Y] targets. Error bars represent ± 1 standard deviation. Samples were quantified using the PowerQuant® System.

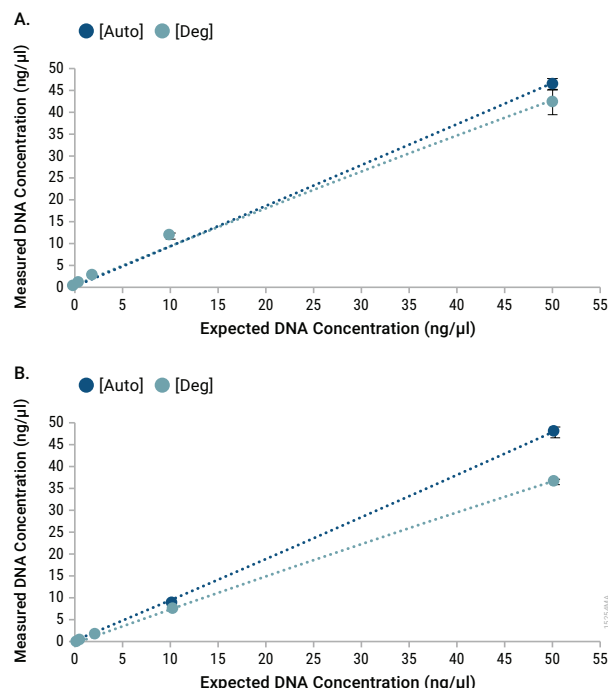


Figure 2. Linearity of DNA concentration obtained from the female DNA sample on the QuantStudio™ 5 (Panel A) and the 7500 (Panel B) Systems. The X and Y axes represent the expected and measured DNA concentrations, respectively, for the autosomal [Auto] and degradation [Deg] targets. Error bars represent ± 1 standard deviation. Samples were quantified using the PowerQuant® System.

Table 2. Average concentration and coefficient of variation (CV) across PowerQuant® targets on the 7500 and QuantStudio™ 5 Systems for the female DNA sensitivity sample. The standard deviation is shown for each concentration. 7500, 7500 Real-Time PCR System; QS5, QuantStudio™ 5 Real-Time PCR System.

Sample Expected Quantity (ng/μl)	[Auto] (ng/μl)		[Auto] CV (%)		[Deg] (ng/μl)		[Deg] CV (%)	
	7500	QS5	7500	QS5	7500	QS5	7500	QS5
50	53.55 ± 1.41	46.55 ± 1.23	2.6%	2.6%	40.95 ± 0.62	42.35 ± 2.81	1.5%	6.6%
10	10.51 ± 0.25	11.53 ± 0.43	2.4%	3.7%	8.75 ± 0.34	11.57 ± 0.72	3.9%	6.2%
2	2.22 ± 0.01	2.43 ± 0.11	0.3%	4.5%	1.98 ± 0.03	2.43 ± 0.11	1.3%	4.5%
0.4	0.494 ± 0.016	0.557 ± 0.022	3.1%	3.9%	0.416 ± 0.004	0.527 ± 0.016	1.1%	3.1%
0.08	0.0947 ± 0.0022	0.1080 ± 0.0016	2.3%	1.5%	0.0729 ± 0.0003	0.1015 ± 0.0039	0.5%	3.8%
0.016	0.0180 ± 0.0027	0.0253 ± 0.0031	14.9%	12.2%	0.0144 ± 0.0011	0.0265 ± 0.0011	7.7%	4.1%
0.0032	0.00425 ± 0.00087	0.00416 ± 0.00003	20.5%	0.8%	0.00364 ± 0.00030	0.00348 ± 0.00007	8.3%	2.1%

Repeatability Study

The quantification results were consistent within and between runs for all tested DNA concentrations on the QuantStudio™ 5 System (Table 3). The DNA concentrations of the samples processed on the QuantStudio™ 5 System showed less variability between runs than the samples processed on the 7500 System (Table 3 and Figure 3). The QuantStudio™ 5 and 7500 Systems were compared using

replicate reactions of varying DNA concentrations (Figure 3). IPC C_q flags were not detected with the PowerQuant® System in any of the samples (data not shown).

Mixture Study

The second replicate of the 1:80 ratio sample was removed from calculated averages for both mixture series 1 and 2 for the QuantStudio™ 5 System data. These samples

Table 3. Average concentration, standard deviation and coefficient of variation (CV) across PowerQuant® targets on the 7500 and QuantStudio™ 5 Systems for replicates within and between runs. 7500, 7500 Real-Time PCR System; QS5, QuantStudio™ 5 Real-Time PCR System.

Sample Expected Quantity (ng/μl)		[Auto] (ng/μl)		[Auto] CV (%)		[Deg] (ng/μl)		[Deg] CV (%)	
		7500	QS5	7500	QS5	7500	QS5	7500	QS5
45	Run #1 (N=3)	46.92 ± 5.16	46.00 ± 1.21	11.0%	2.6%	45.68 ± 5.58	46.39 ± 4.83	12.2%	10.4%
	Run #2 (N=3)	31.38 ± 0.38	51.28 ± 5.17	1.2%	10.1%	32.87 ± 1.22	52.88 ± 6.75	3.7%	12.8%
	Inter-Run (N=6)	39.15 ± 8.59	48.64 ± 4.59	21.9%	9.4%	39.27 ± 7.57	49.64 ± 6.71	19.3%	13.5%
10	Run #1 (N=3)	10.77 ± 0.18	10.89 ± 1.07	1.7%	9.9%	11.04 ± 0.33	12.26 ± 1.55	3.0%	12.6%
	Run #2 (N=3)	6.99 ± 0.67	11.13 ± 0.66	9.6%	5.9%	7.70 ± 0.81	13.51 ± 1.08	10.5%	8.0%
	Inter-Run (N=6)	8.88 ± 1.95	11.01 ± 0.90	22.0%	8.2%	9.37 ± 1.78	12.89 ± 1.47	19.0%	11.4%
0.4	Run #1 (N=3)	0.45 ± 0.04	0.49 ± 0.02	8.1%	4.5%	0.44 ± 0.04	0.49 ± 0.02	9.3%	3.3%
	Run #2 (N=3)	0.31 ± 0.008	0.41 ± 0.01	2.4%	2.9%	0.33 ± 0.008	0.47 ± 0.02	2.3%	3.9%
	Inter-Run (N=6)	0.38 ± 0.07	0.45 ± 0.04	19.6%	10.0%	0.38 ± 0.06	0.48 ± 0.02	16.4%	4.2%
0.016	Run #1 (N=3)	0.015 ± 0.0013	0.022 ± 0.0021	9.0%	9.9%	0.018 ± 0.0019	0.023 ± 0.0024	10.9%	10.6%
	Run #2 (N=3)	0.011 ± 0.0006	0.014 ± 0.0005	5.1%	3.6%	0.014 ± 0.0007	0.019 ± 0.0013	5.0%	7.0%
	Inter-Run (N=6)	0.013 ± 0.0022	0.018 ± 0.0041	16.6%	22.5%	0.016 ± 0.0023	0.021 ± 0.0028	14.8%	13.7%

Sample Expected Quantity (ng/μl)		[Y] (ng/μl)		[Y] CV (%)		IPC C _q		IPC C _q CV (%)	
		7500	QS5	7500	QS5	7500	QS5	7500	QS5
45	Run #1 (N=3)	44.32 ± 4.15	46.39 ± 1.50	9.4%	3.2%	21.34 ± 0.12	21.39 ± 0.04	0.6%	0.2%
	Run #2 (N=3)	32.81 ± 0.41	48.93 ± 3.54	1.2%	7.2%	21.35 ± 0.11	21.32 ± 0.04	0.5%	0.2%
	Inter-Run (N=6)	38.57 ± 6.47	47.66 ± 3.00	16.8%	6.3%	21.34 ± 0.11	21.36 ± 0.05	0.5%	0.2%
10	Run #1 (N=3)	10.16 ± 0.08	10.41 ± 1.25	0.8%	12.0%	21.23 ± 0.05	20.86 ± 0.02	0.2%	0.1%
	Run #2 (N=3)	7.24 ± 0.66	11.03 ± 0.82	9.2%	7.5%	21.20 ± 0.08	20.81 ± 0.03	0.4%	0.1%
	Inter-Run (N=6)	8.70 ± 1.54	10.72 ± 1.10	17.7%	10.3%	21.22 ± 0.07	20.84 ± 0.03	0.3%	0.2%
0.4	Run #1 (N=3)	0.42 ± 0.03	0.41 ± 0.01	7.0%	2.9%	21.10 ± 0.05	20.74 ± 0.05	0.2%	0.2%
	Run #2 (N=3)	0.31 ± 0.01	0.40 ± 0.02	3.6%	4.6%	21.04 ± 0.04	20.71 ± 0.08	0.2%	0.4%
	Inter-Run (N=6)	0.36 ± 0.06	0.41 ± 0.02	15.7%	4.1%	21.07 ± 0.05	20.73 ± 0.07	0.3%	0.3%
0.016	Run #1 (N=3)	0.017 ± 0.0016	0.017 ± 0.0012	10.0%	7.2%	21.09 ± 0.02	20.71 ± 0.03	0.1%	0.1%
	Run #2 (N=3)	0.012 ± 0.0014	0.014 ± 0.0010	11.5%	6.7%	20.96 ± 0.18	20.53 ± 0.05	0.8%	0.2%
	Inter-Run (N=6)	0.014 ± 0.0026	0.016 ± 0.0015	18.4%	9.7%	21.03 ± 0.14	20.62 ± 0.10	0.7%	0.5%



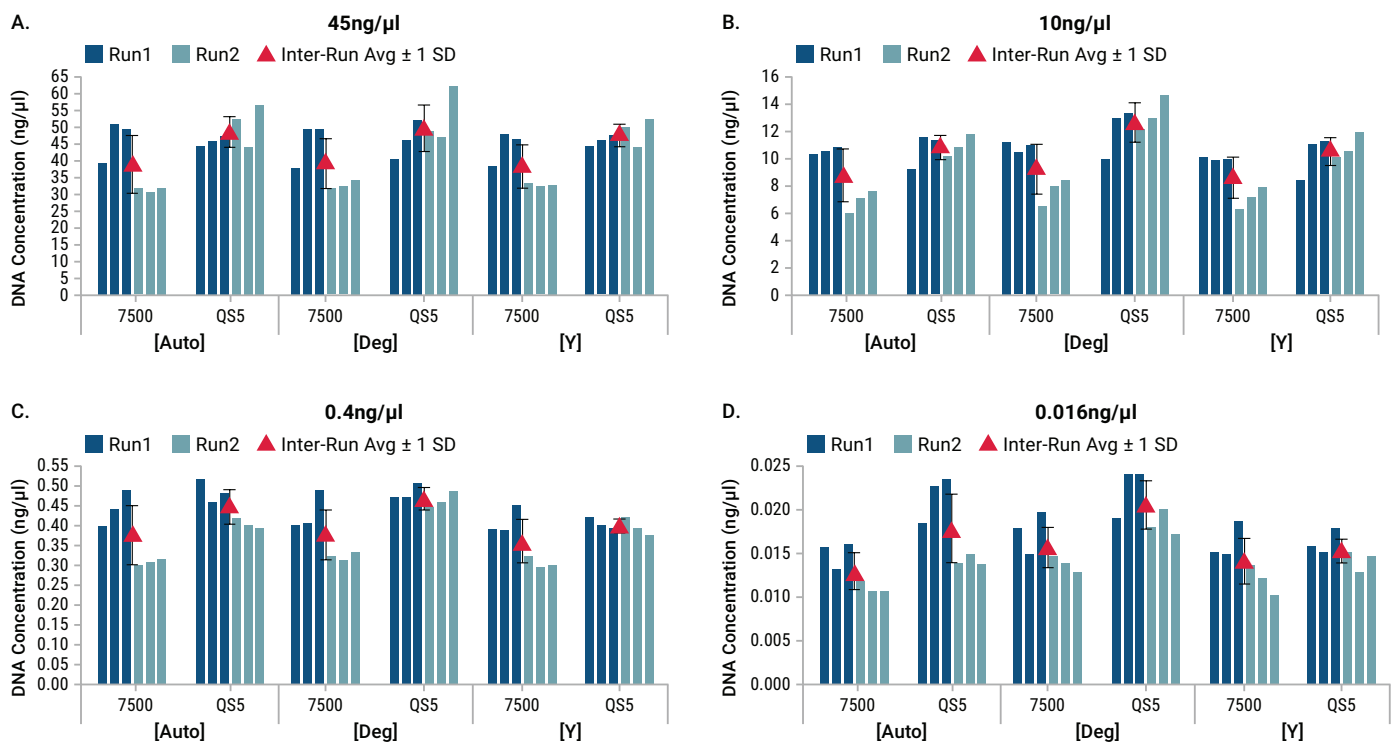


Figure 3. Individual replicate quantification results and average among-run variability for different DNA concentrations on the QuantStudio™ 5 and 7500 Systems. The X axis represents a sample replicate run on each instrument for each target (autosomal [Auto], degradation [Deg] and Y-chromosomal [Y]); the Y axis represents the DNA concentration. Run #1 is indicated by dark blue bars and #2 by light blue bars. The inter-run average for each instrument is shown by triangles. The error bars represent ± 1 standard deviation. Samples were quantified using the PowerQuant® System.

were considered outliers due to their low autosomal DNA concentration. The male and female DNA components of all remaining mixture samples were successfully quantified. The detected concentration values correlated to the expected [Auto]/[Y] values for all tested ratios. The results showed a correlation between an increase in the [Auto]/[Y] ratio and an increase in the amount of female DNA in a mixture sample (Figure 4). The [Auto]/[Y] ratios

calculated from mixture samples on the QuantStudio™ 5 System were comparable to those calculated from the 7500 System (Table 4). The [Auto]/[Y] ratio thresholds were appropriately flagged by the PowerQuant® Analysis Tool for analogous samples on each instrument. IPC C₁ flags were not detected with the PowerQuant® System in any of the samples (data not shown).

Table 4. Average concentration and standard deviation across PowerQuant® targets on the 7500 and QuantStudio™ 5 Systems for Mixture Series 1 and 2. Average [Auto]/[Y] ratios are shown. Orange values indicate a threshold flag. 7500, 7500 Real-Time PCR System; QS5, QuantStudio™ 5 Real-Time PCR System.

Expected [Auto]/[Y] Ratio	[Auto] (ng/μl)		[Deg] (ng/μl)		[Y] (ng/μl)		[Auto]/[Y]	
	7500	QS5	7500	QS5	7500	QS5	7500	QS5
Mix1 1:1	0.4060 ± 0.0173	0.4016 ± 0.0261	0.3734 ± 0.0245	0.3228 ± 0.0379	0.2272 ± 0.0038	0.2141 ± 0.0198	1.79 ± 0.05	1.88 ± 0.05
Mix1 1:5	1.2693 ± 0.0891	1.3452 ± 0.0146	1.1793 ± 0.0721	1.2377 ± 0.0335	0.2334 ± 0.0127	0.2561 ± 0.0052	5.44 ± 0.09	5.25 ± 0.16
Mix1 1:10	2.4668 ± 0.0085	2.2829 ± 0.1511	2.3558 ± 0.0080	2.1501 ± 0.1167	0.2617 ± 0.0052	0.2466 ± 0.0109	9.43 ± 0.15	9.26 ± 0.20
Mix1 1:15	3.5382 ± 0.2373	3.2740 ± 0.1125	3.2073 ± 0.1882	3.0289 ± 0.1231	0.2636 ± 0.0084	0.2531 ± 0.0129	13.42 ± 0.47	12.94 ± 0.22
Mix1 1:20	4.1515 ± 0.4265	4.4912 ± 0.1036	3.5678 ± 0.3607	3.7653 ± 0.0991	0.2371 ± 0.0359	0.2624 ± 0.0071	17.51 ± 0.87	17.11 ± 0.07
Mix1 1:35	7.0469 ± 0.1673	7.7593 ± 0.2751	6.6272 ± 0.2996	7.4868 ± 0.0531	0.2382 ± 0.0185	0.2900 ± 0.0047	29.59 ± 1.60	26.76 ± 0.51
Mix1 1:50	9.9613 ± 0.2556	8.1973 ± 0.5346	9.2527 ± 0.1703	8.1684 ± 0.6574	0.2393 ± 0.0027	0.2231 ± 0.0210	41.62 ± 0.59	36.75 ± 1.08
Mix1 1:65	11.8770 ± 0.1510	13.0536 ± 0.6507	9.8118 ± 0.1735	11.5259 ± 0.1271	0.2209 ± 0.0060	0.2776 ± 0.0048	53.76 ± 0.78	47.03 ± 1.53
Mix1 1:80	15.6521 ± 0.2422	15.3184 ± 0.0000	13.6421 ± 0.1354	14.6694 ± 0.0000	0.2386 ± 0.0038	0.2657 ± 0.0000	65.60 ± 0.03	57.66 ± 0.00
Mix2 1:1	0.3582 ± 0.0269	0.4290 ± 0.0065	0.3072 ± 0.0268	0.3796 ± 0.0019	0.1611 ± 0.0080	0.1922 ± 0.0019	2.22 ± 0.06	2.23 ± 0.01
Mix2 1:5	1.1197 ± 0.0348	1.1327 ± 0.0802	1.0431 ± 0.0274	1.0702 ± 0.0205	0.2025 ± 0.0066	0.1947 ± 0.0127	5.53 ± 0.01	5.82 ± 0.03
Mix2 1:10	1.7312 ± 0.0987	2.0613 ± 0.0371	1.4344 ± 0.0391	1.9014 ± 0.0258	0.1706 ± 0.0038	0.2157 ± 0.0027	10.15 ± 0.35	9.56 ± 0.05
Mix2 1:15	2.8167 ± 0.0107	2.7202 ± 0.1179	2.5410 ± 0.0489	2.7839 ± 0.1552	0.1835 ± 0.0028	0.1893 ± 0.0140	15.35 ± 0.18	14.37 ± 0.44
Mix2 1:20	3.2065 ± 0.0319	3.7683 ± 0.0647	2.7959 ± 0.0275	4.1796 ± 0.0456	0.1600 ± 0.0068	0.2089 ± 0.0013	20.04 ± 0.65	18.04 ± 0.20
Mix2 1:35	5.9445 ± 0.4007	5.4626 ± 0.6519	4.8799 ± 0.2940	5.1999 ± 0.7808	0.1772 ± 0.0119	0.1808 ± 0.0186	33.55 ± 0.00	30.21 ± 0.50
Mix2 1:50	9.0445 ± 0.2159	9.3589 ± 0.5715	8.0001 ± 0.4950	9.5028 ± 0.2312	0.1772 ± 0.0094	0.1979 ± 0.0022	51.03 ± 3.93	47.29 ± 2.35
Mix2 1:65	11.4163 ± 0.3303	11.6401 ± 0.1841	9.6437 ± 0.2733	12.9281 ± 0.0224	0.1885 ± 0.0060	0.2073 ± 0.0023	60.55 ± 0.16	56.15 ± 0.26
Mix2 1:80	13.9859 ± 0.2915	13.2472 ± 0.0000	11.6277 ± 0.1694	15.3273 ± 0.0000	0.1897 ± 0.0053	0.2052 ± 0.0000	73.72 ± 3.59	64.54 ± 0.00

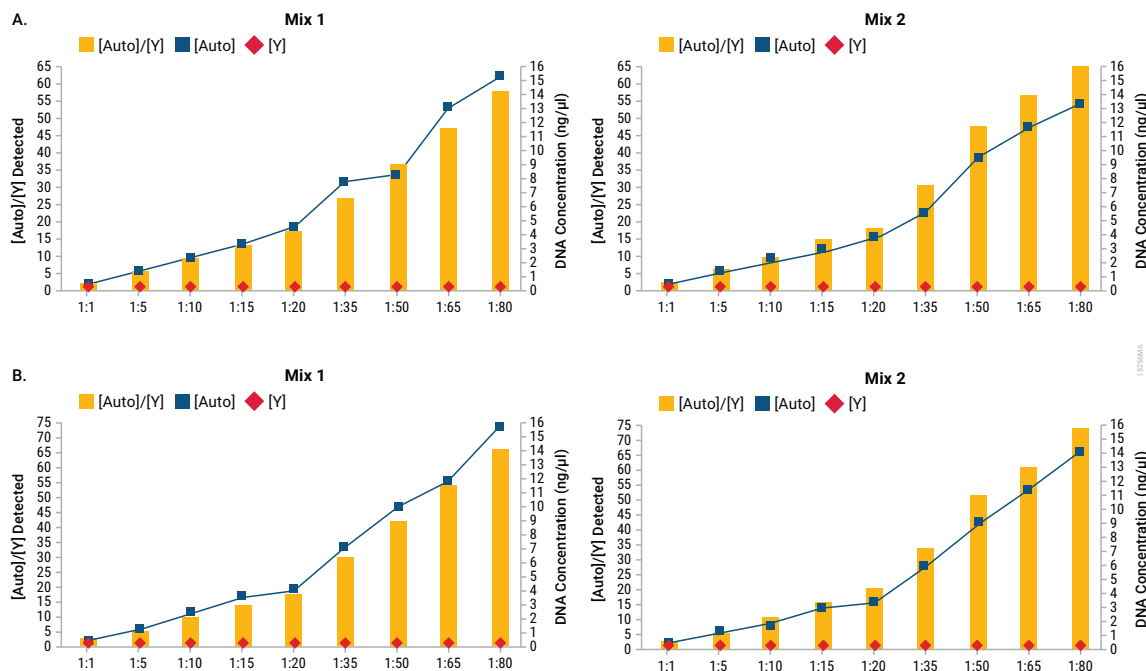


Figure 4. Quantification results for two male/female mixture sets on the QuantStudio™ 5 (Panel A) and the 7500 (Panel B) Systems. The X axis represents the ratio of male to female DNA that was used in the sample. The left Y axis represents the average ratio of autosomal DNA [Auto] to Y-chromosomal DNA [Y] concentrations (indicated by the bars). The right Y axis represents the average autosomal DNA concentration (indicated by the line) and average Y-chromosomal DNA concentration (indicated by the diamonds). Samples were quantified using the PowerQuant® System.



Degradation Study

The [Auto]/[Deg] ratios calculated from samples on the QuantStudio™ 5 System were consistent with those calculated from the 7500 System (Figure 5). The [Auto]/[Deg] ratio thresholds were appropriately flagged by the PowerQuant® Analysis Tool for analogous samples on each instrument. The results showed a correlation between an increase in the [Auto]/[Deg] ratio and an increase in the amount of UV exposure (Figure 5). A correlation between a decrease in DNA concentration and an increase in amount of UV exposure was also observed (data not shown). IPC C_q flags were not detected with the PowerQuant® System in any of the samples (data not shown).

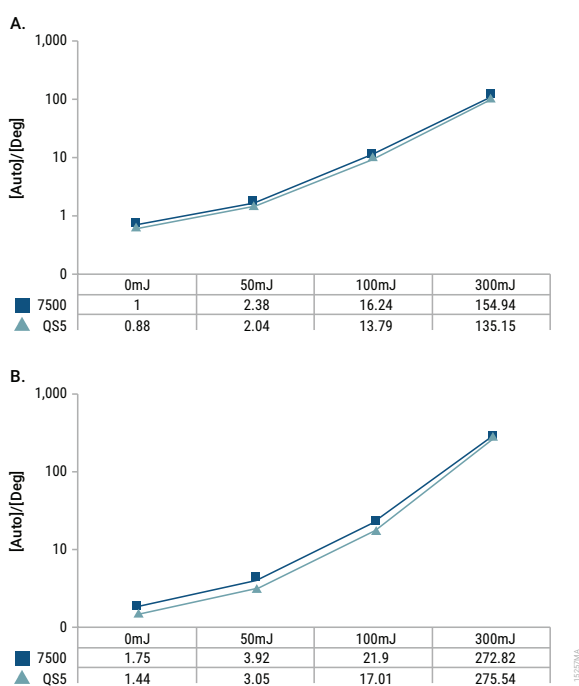


Figure 5. Autosomal to degradation target ratio [Auto]/[Deg] results from DNA 1 (Panel A) and DNA 2 (Panel B) samples exposed to increasing amounts of UV radiation on the 7500 and QuantStudio™ 5 Systems. The X axis represents the amount of UV exposure (mJ). The Y axis represents the average [Auto]/[Deg] ratio in logarithmic scale. The values in the data table represent the actual average [Auto]/[Deg] ratio for each sample. Samples were quantified using the PowerQuant® System.

Inhibition Study

The IPC C_q shifts calculated from inhibitor samples on the QuantStudio™ 5 System were similar to those calculated from the 7500 System with both humic acid and hematin (Tables 5 and 6). The IPC C_q shift thresholds were appropriately flagged by the PowerQuant® Analysis Tool for the humic acid and hematin samples on the QuantStudio™ 5 System. The samples containing humic acid displayed greater overall increases for the IPC C_q shift than samples containing hematin. The results showed a correlation between an increase in the IPC C_q shift and an increase in the amount of inhibitor (Figures 6 and 7).

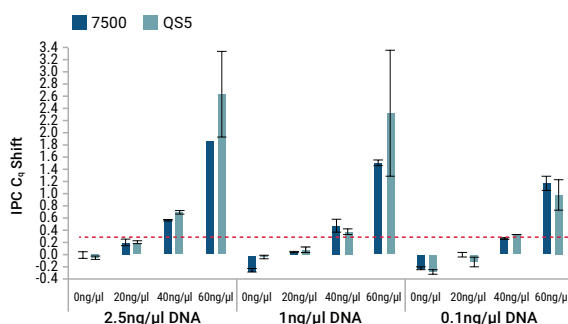


Figure 6. IPC C_q shift results from DNA samples exposed to increasing amounts of humic acid on the 7500 and QuantStudio™ 5 Systems. The X axis represents the DNA sample and amount of humic acid. The Y axis represents the average IPC C_q shift. The red dashed line represents the default IPC C_q shift flag threshold of 0.3. Error bars represent ± 1 standard deviation. Samples were quantified using the PowerQuant® System.

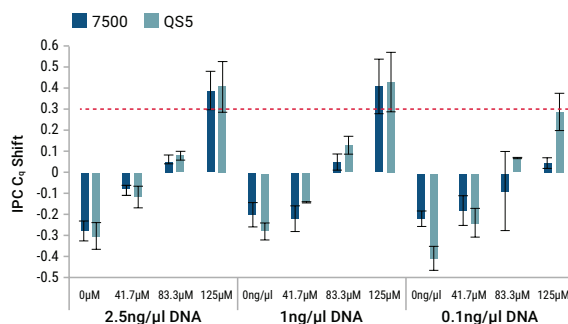


Figure 7. IPC C_q shift results from DNA samples exposed to increasing amounts of hematin on the 7500 and QuantStudio™ 5 Systems. The X axis represents the DNA sample and amount of hematin. The Y axis represents the average IPC C_q shift. The red dashed line represents the default IPC C_q shift flag threshold of 0.3. Error bars represent ± 1 standard deviation. Samples were quantified using the PowerQuant® System.

Table 5. Average concentration, IPC C_q and IPC C_q shift using the PowerQuant® System on the 7500 and QuantStudio™ 5 Systems for the humic acid inhibitor series. The standard deviation is shown. [DNA] and [Inhibitor] are the final concentrations in the PowerQuant® reaction. The asterisk (*) indicates one undetermined replicate that was not averaged into the data. Orange values indicate a threshold flag. 7500, 7500 Real-Time PCR System; QS5, QuantStudio™ 5 Real-Time PCR System.

Humic Acid Sample		[Auto] (ng/μl)		[Deg] (ng/μl)		[Y] (ng/μl)	
[DNA]	[Inhibitor]	7500	QS5	7500	QS5	7500	QS5
2.5ng/μl	0ng/μl	2.5131 ± 0.1707	2.2811 ± 0.0091	1.3348 ± 0.0056	1.3639 ± 0.0551	1.7583 ± 0.0492	1.9306 ± 0.0276
	20ng/μl	2.8338 ± 0.0854	2.3604 ± 0.0546	1.6668 ± 0.0145	1.9165 ± 0.0691	1.9508 ± 0.0309	2.1444 ± 0.0349
	40ng/μl	2.8098 ± 0.0186	2.3922 ± 0.0245	1.6929 ± 0.0140	2.1877 ± 0.0242	1.9386 ± 0.0348	2.1650 ± 0.0090
	60ng/μl	2.4959 ± 0.2672	2.2238 ± 0.1031	1.4868 ± 0.0404	2.2063 ± 0.1131	2.0744 ± 0.1020	2.2815 ± 0.0649
1ng/μl	0ng/μl	1.0545 ± 0.1292	0.7668 ± 0.0256	0.5668 ± 0.0169	0.5575 ± 0.0467	0.7211 ± 0.0043	0.7346 ± 0.0050
	20ng/μl	1.1466 ± 0.0592	1.0089 ± 0.0261	0.6476 ± 0.0030	0.8030 ± 0.0385	0.7549 ± 0.0052	0.8447 ± 0.0182
	40ng/μl	1.2639 ± 0.0133	1.0106 ± 0.0025	0.6853 ± 0.0151	0.9449 ± 0.0059	0.7774 ± 0.0092	0.8966 ± 0.0011
	60ng/μl	1.0086 ± 0.1207	0.9363 ± 0.0033	0.6214 ± 0.0083	0.9048 ± 0.0041	0.8275 ± 0.0029	0.9279 ± 0.0068
0.1ng/μl	0ng/μl	0.0677 ± 0.0024	0.0578 ± 0.0027	0.0330 ± 0.0003	0.0367 ± 0.0019	0.0399 ± 0.0011	0.0474 ± 0.0022
	20ng/μl	0.1215 ± 0.0208	0.1057 ± 0.0031	0.0657 ± 0.0015	0.0851 ± 0.0010	0.0806 ± 0.0010	0.0897 ± 0.0004
	40ng/μl	0.1473 ± 0.0003	0.1050 ± 0.0000	0.0755 ± 0.0012	0.1043 ± 0.0003	0.0856 ± 0.0047	0.0951 ± 0.0000
	60ng/μl	0.1247 ± 0.0025	0.1009 ± 0.0083	0.0655 ± 0.0012	0.0898 ± 0.0038	0.0853 ± 0.0005	0.0926 ± 0.0027

Humic Acid Sample		IPC C _q		IPC C _q Shift	
[DNA]	[Inhibitor]	7500	QS5	7500	QS5
2.5ng/μl	0ng/μl	21.12 ± 0.06	20.76 ± 0.04	-0.01 ± 0.06	-0.04 ± 0.04
	20ng/μl	21.32 ± 0.05	20.99 ± 0.02	0.20 ± 0.05	0.20 ± 0.02
	40ng/μl	21.70 ± 0.01	21.49 ± 0.03	0.57 ± 0.01	0.70 ± 0.03
	60ng/μl	22.99*	23.43 ± 0.70	1.86	2.64 ± 0.70
1ng/μl	0ng/μl	20.86 ± 0.01	20.75 ± 0.03	-0.28 ± 0.00	-0.03 ± 0.03
	20ng/μl	21.18 ± 0.01	20.87 ± 0.04	0.05 ± 0.01	0.08 ± 0.04
	40ng/μl	21.59 ± 0.10	21.16 ± 0.04	0.47 ± 0.10	0.38 ± 0.04
	60ng/μl	22.63 ± 0.03	23.10 ± 1.03	1.50 ± 0.04	2.32 ± 1.03
0.1ng/μl	0ng/μl	20.92 ± 0.02	20.50 ± 0.04	-0.22 ± 0.02	-0.28 ± 0.04
	20ng/μl	21.13 ± 0.03	20.66 ± 0.07	0.00 ± 0.03	-0.12 ± 0.07
	40ng/μl	21.39 ± 0.01	21.11 ± 0.00	0.26 ± 0.01	0.33 ± 0.00
	60ng/μl	22.31 ± 0.11	21.76 ± 0.24	1.17 ± 0.11	0.98 ± 0.24

Table 6. Average concentration, IPC C_q and IPC C_q shift using the PowerQuant® System on the 7500 and QuantStudio™ 5 Systems for the hematin inhibitor series. The standard deviation is shown. [DNA] and [Inhibitor] are the final concentrations in the PowerQuant® reaction. Orange values indicate a threshold flag. 7500, 7500 Real-Time PCR System; QS5, QuantStudio™ 5 Real-Time PCR System.

Hematin Sample		[Auto] (ng/μl)		[Deg] (ng/μl)		[Y] (ng/μl)	
[DNA]	[Inhibitor]	7500	QS5	7500	QS5	7500	QS5
2.5ng/μl	0μM	2.5914 ± 0.0946	2.3416 ± 0.0316	1.1733 ± 0.0121	1.5124 ± 0.0401	1.5150 ± 0.0329	1.8554 ± 0.0521
	41.7μM	2.8144 ± 0.0089	2.3039 ± 0.0486	1.5511 ± 0.0656	2.1674 ± 0.0296	1.9704 ± 0.0570	2.1681 ± 0.0419
	83.3μM	2.8173 ± 0.1152	2.2850 ± 0.0087	1.9221 ± 0.1124	2.9110 ± 0.0312	2.2289 ± 0.1013	2.3710 ± 0.0070
	125μM	2.3600 ± 0.0505	2.4392 ± 0.0846	1.8853 ± 0.1639	3.4168 ± 0.1667	2.1335 ± 0.1694	2.6502 ± 0.0155
1ng/μl	0μM	1.0139 ± 0.0045	0.9124 ± 0.0030	0.4382 ± 0.0107	0.6050 ± 0.0152	0.6064 ± 0.0295	0.7279 ± 0.0032
	41.7μM	1.1493 ± 0.0882	0.9440 ± 0.0257	0.6436 ± 0.0084	0.9074 ± 0.0473	0.7922 ± 0.0123	0.8975 ± 0.0097
	83.3μM	1.2050 ± 0.0179	0.9624 ± 0.0503	0.7998 ± 0.0245	1.1866 ± 0.0132	0.9168 ± 0.0399	0.9959 ± 0.0265
	125μM	1.0340 ± 0.0637	0.9053 ± 0.0159	0.7936 ± 0.0784	1.3468 ± 0.0700	0.9448 ± 0.0304	1.0337 ± 0.0030
0.1ng/μl	0μM	0.0628 ± 0.0053	0.0558 ± 0.0017	0.0284 ± 0.0008	0.0393 ± 0.0043	0.0379 ± 0.0022	0.0476 ± 0.0022
	41.7μM	0.1158 ± 0.0038	0.0895 ± 0.0018	0.0582 ± 0.0029	0.0923 ± 0.0068	0.0742 ± 0.0007	0.0908 ± 0.0012
	83.3μM	0.1509 ± 0.0076	0.0961 ± 0.0069	0.0880 ± 0.0083	0.1209 ± 0.0035	0.0995 ± 0.0084	0.1049 ± 0.0033
	125μM	0.1466 ± 0.0073	0.1106 ± 0.0011	0.1207 ± 0.0025	0.1474 ± 0.0068	0.1309 ± 0.0018	0.1239 ± 0.0073

Hematin Sample		IPC C _q		IPC C _q Shift	
[DNA]	[Inhibitor]	7500	QS5	7500	QS5
2.5ng/μl	0μM	20.84 ± 0.05	20.49 ± 0.07	-0.28 ± 0.05	-0.31 ± 0.07
	41.7μM	21.04 ± 0.03	20.68 ± 0.06	-0.08 ± 0.03	-0.12 ± 0.06
	83.3μM	21.18 ± 0.03	20.87 ± 0.02	0.05 ± 0.03	0.08 ± 0.02
	125μM	21.51 ± 0.09	21.20 ± 0.12	0.39 ± 0.09	0.41 ± 0.12
1ng/μl	0μM	20.94 ± 0.06	20.50 ± 0.04	-0.2 ± 0.06	-0.28 ± 0.04
	41.7μM	20.91 ± 0.06	20.65 ± 0.00	-0.22 ± 0.06	-0.14 ± 0.00
	83.3μM	21.18 ± 0.04	20.91 ± 0.04	0.05 ± 0.04	0.13 ± 0.04
	125μM	21.54 ± 0.14	21.22 ± 0.14	0.41 ± 0.13	0.43 ± 0.14
0.1ng/μl	0μM	20.92 ± 0.04	20.38 ± 0.06	-0.22 ± 0.04	-0.41 ± 0.06
	41.7μM	20.95 ± 0.07	20.55 ± 0.07	-0.18 ± 0.07	-0.24 ± 0.07
	83.3μM	21.05 ± 0.19	20.85 ± 0.00	-0.09 ± 0.19	0.07 ± 0.00
	125μM	21.18 ± 0.03	21.07 ± 0.09	0.04 ± 0.03	0.29 ± 0.09

Contamination Study

Thirty-six NTC samples were assessed throughout the study. No DNA was detected in any of the NTC samples using the PowerQuant® System. The average IPC C_q value for all NTCs processed on the QuantStudio™ 5 System was 20.60 (± 0.16). The average IPC C_q value for all NTCs processed on the 7500 System was 21.07 (± 0.13). IPC C_q flags were not detected with the PowerQuant® System for any of the NTCs (data not shown).

Standard Curve Reproducibility Study

Five pairs of standard curves from each instrument were assessed. The data showed that the PowerQuant® standard curve is reproducible across different runs on the QuantStudio™ 5 System. The observed standard curve values were within the recommended R² and slope thresholds outlined in the *PowerQuant® System Technical Manual #TMD047* (6). The values calculated from standard curves on the QuantStudio™ 5 System were consistent with those calculated from the 7500 System for all targets (Table 7).

Conclusion

A series of experiments was conducted to evaluate the performance of the PowerQuant® System on a QuantStudio™ 5 Real-Time PCR System as compared to the 7500 Real-Time PCR System. The results demonstrate that the precision and dynamic range of the PowerQuant® System on the QuantStudio™ 5 System is consistent with that of the 7500 System. The ability of the PowerQuant® System to detect mixed, degraded and inhibited samples on the QuantStudio™ 5 System is comparable to that on the 7500 System. Additionally, no sample-to-sample contamination was observed on the QuantStudio™ 5 System. The QuantStudio™ 5 Real-Time PCR System is a suitable instrument to use in conjunction with the Promega PowerQuant® System to generate predictive, quantitative and qualitative information about forensic DNA extracts.

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4. Federal Bureau of Investigation. (2011) Quality Assurance Standards for Forensic DNA Testing Laboratories.
5. Scientific Working Group on DNA Analysis Methods. (2016) Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits.
6. *PowerQuant® System Technical Manual #TMD047*, Promega Corporation.

Table 7. Average R², Slope, Y-intercept and amplification efficiency for standard curves across PowerQuant® targets on the 7500 and QuantStudio™ 5 Systems. Standard deviation and coefficient of variation (CV) are shown. 7500, 7500 Real-Time PCR System; QS5, QuantStudio™ 5 Real-Time PCR System.

Target Parameter	Autosomal							
	R ²		Slope		Y-Intercept		Amp. Efficiency	
	7500	QS5	7500	QS5	7500	QS5	7500	QS5
Curve 1	1.000	0.998	-3.35	-3.41	27.04	26.48	98.83%	96.53%
Curve 2	0.999	0.999	-3.32	-3.25	26.81	26.03	100.10%	102.92%
Curve 3	1.000	1.000	-3.47	-3.33	25.86	25.01	94.04%	99.60%
Curve 4	1.000	1.000	-3.33	-3.33	26.80	25.88	99.78%	99.80%
Curve 5	0.999	1.000	-3.32	-3.33	25.80	24.92	99.91%	99.60%
Average	1.000	0.999	-3.36	-3.33	26.46	25.66	98.53%	99.69%
St Dev	0.0002	0.0008	0.0583	0.0488	0.5233	0.6055	0.0229	0.0202
CV%	0.02%	0.1%	1.7%	1.5%	2.0%	2.4%	2.3%	2.0%

Target Parameter	Y							
	R ²		Slope		Y-Intercept		Amp. Efficiency	
	7500	QS5	7500	QS5	7500	QS5	7500	QS5
Curve 1	0.998	1.000	-3.36	-3.39	26.72	27.00	98.50%	97.16%
Curve 2	1.000	1.000	-3.37	-3.35	26.53	26.72	97.88%	98.87%
Curve 3	0.999	0.999	-3.37	-3.37	25.69	25.78	97.87%	98.02%
Curve 4	0.999	0.999	-3.37	-3.44	26.53	26.61	98.09%	95.16%
Curve 5	1.000	1.000	-3.33	-3.40	25.67	25.71	99.49%	96.95%
Average	0.999	1.000	-3.36	-3.39	26.23	26.37	98.37%	97.23%
St Dev	0.0008	0.0002	0.0149	0.0315	0.4519	0.5211	0.0061	0.0124
CV%	0.1%	0.0%	0.4%	0.9%	1.7%	2.0%	0.6%	1.3%

Target Parameter	Degradation							
	R ²		Slope		Y-Intercept		Amp. Efficiency	
	7500	QS5	7500	QS5	7500	QS5	7500	QS5
Curve 1	1.000	0.996	-3.43	-3.46	27.07	27.45	95.77%	94.59%
Curve 2	0.999	0.999	-3.46	-3.32	26.88	27.13	94.59%	99.90%
Curve 3	1.000	1.000	-3.45	-3.31	25.94	25.72	94.97%	100.58%
Curve 4	1.000	1.000	-3.45	-3.39	26.88	26.98	95.04%	97.24%
Curve 5	1.000	0.999	-3.38	-3.43	25.93	25.72	97.58%	95.84%
Average	1.000	0.999	-3.43	-3.38	26.54	26.60	95.59%	97.63%
St Dev	0.0002	0.0013	0.0276	0.0577	0.4995	0.7356	0.0107	0.0230
CV%	0.0%	0.1%	0.8%	1.7%	1.9%	2.8%	1.1%	2.4%

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