

B. C. J.   
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## **VIRGINIA DEPARTMENT OF FORENSIC SCIENCE**

### **VALIDATION OF THE APPLIED BIOSYSTEMS® QUANTSTUDIO™ 5 REAL-TIME PCR SYSTEM: PERFORMANCE CHECK OF ROBOTIC POWERQUANT® SYSTEM SETUP**

**Prepared in December, 2019**

#### **PURPOSE**

This validation study compared the performance of the robotic and manual setup of the Promega PowerQuant® DNA Quantification System assay, in conjunction with the Applied Biosystems® QuantStudio™ 5 Real-Time PCR Instrument for quantifying DNA samples.

#### **MATERIALS AND METHODS**

An automated PowerQuant® (Promega Corp., Madison, WI) method for serial dilution of the male standard and reaction plate setup was developed for use with the Beckman Coulter Biomek® NX<sup>P</sup> Automation Workstation (Fullerton, CA). The automated PowerQuant® method was compared with the manual method, using five male and three female buccal samples, previously extracted and purified manually using the DNA IQ™ System (Promega Corp.), according to the Virginia Department of Forensic Science Procedures Manual.<sup>1</sup>

Robotic and manual PowerQuant® reaction plate setups were performed following the manufacturer's recommendations, with the exception of the PowerQuant® Dilution Buffer used to prepare the standards, which was also used for the no-template control.<sup>2</sup> One robotic and one manual plate setup were prepared on the same day using the same male gDNA standard, dilution buffer and manually prepared master mix. The samples were quantified in duplicate for each method using 2 µL of DNA extract added to 18 µl of PowerQuant® amplification cocktail. Amplification and detection were performed using the Applied Biosystems® QuantStudio™ 5 Real-Time PCR Instrument (Thermo Fisher Scientific, Waltham, MA), which was calibrated for the following dyes: FAM for the autosomal target, CAL Fluor® Gold 540 (CFG540) for the male target, TMR for the internal positive control (IPC), Quasar® 670 for the degradation target, and CXR for the passive reference dye. The raw data was collected with Applied Biosystems® QuantStudio™ Design and Analysis Software ver. 1.4.3, and analyzed using Promega's PowerQuant® Analysis Tool software, ver. 1.0.0.0.

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<sup>1</sup> Forensic Biology Extraction of DNA, Procedures Manual. Virginia Department of Forensic Science. December 28, 2018.

<sup>2</sup> Promega PowerQuant® System Technical Manual, revised 3/18.

## RESULTS

The robotic setup of the PowerQuant® assay was evaluated for suitability by performing a manual plate setup and a robotic plate setup of the same set of single source samples and quantifying them on the same qPCR instrument. Estimated autosomal and male DNA concentrations were similar for the robotic and manual setup of the standards and samples (Figures 1 and 2). The correlation coefficient demonstrated high correlation ( $>0.9$ ) between the manual and robotic autosomal quantitation estimates (Figure 1) and Y-chromosome estimates (Figure 2).

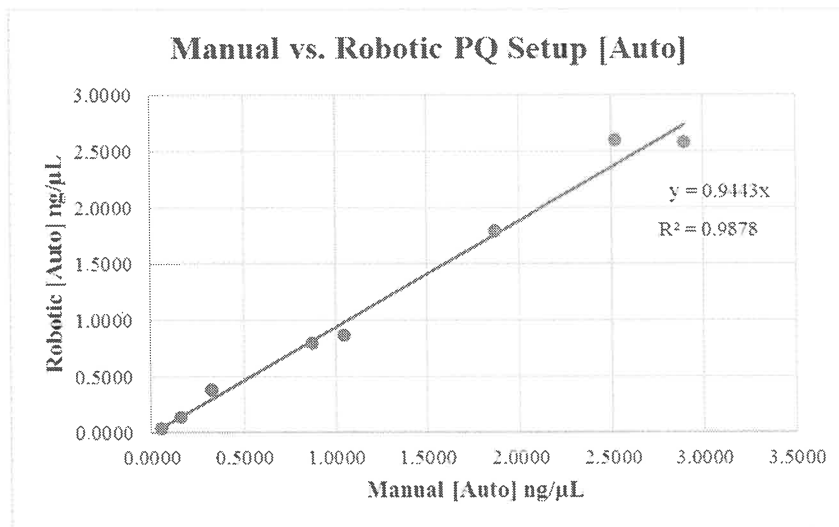


Figure 1. A comparison of average autosomal DNA concentration estimates generated by PowerQuant® using a manual versus robotic reaction plate setup.

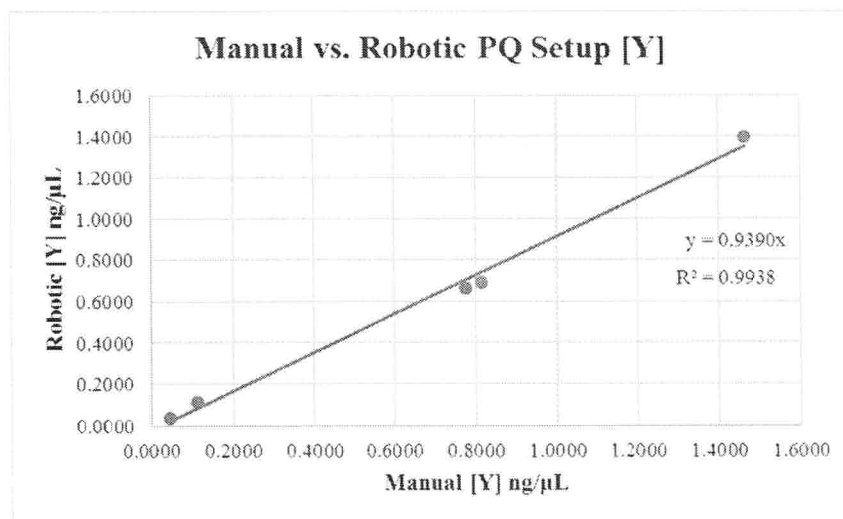


Figure 2. A comparison of average male DNA concentration estimates generated by PowerQuant® using a manual versus robotic reaction plate setup.

Estimated degradation target concentrations were also similar between the robotic and manual methods (data not shown). The software did not flag any samples as inhibited or as male/female mixtures. Similar degradation flags were observed between the robotic and manual setups of the same DNA extracts, with three male samples showing slight degradation ([Auto]/[D] ratios between 2 and 3) in all replicates, and 1 female sample showing moderate degradation ([Auto]/[D] ratios between 10 and 13) in all replicates. A fifth degraded male sample that was manually set up had a very low concentration of the degradation target in the first replicate ([D]=0.0004 ng/μL) and a high degradation index ([Auto]/[D]>100), whereas for the second replicate, the [Auto]/[D] was 'Undetermined', since no amplified DNA was detected for the degradation target. No contamination in the no-template controls was observed using either method.

## **CONCLUSION**

A test of the automated PowerQuant® assay setup versus a manual setup confirmed that the Biomek® NX<sup>P</sup> Automation Workstation method produced similar quantitation data when testing single source samples of varying quantity and quality. Therefore, the automated method for PowerQuant® setup, in conjunction with the QuantStudio™ 5 Real-Time PCR Instrument, is a reliable alternative to the manual setup for estimating the amount of human DNA present in forensic samples.