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## **VIRGINIA DEPARTMENT OF FORENSIC SCIENCE**

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

**Prepared in July, 2020**

#### **PURPOSE**

This validation study compared the performance of a modified version of the robotic setup with the previous version for the Promega PowerQuant® DNA Quantification System assay. This robotic setup was designed to be used in conjunction with the Applied Biosystems® QuantStudio™ 5 Real-Time PCR Instrument for quantifying DNA samples. This performance check was performed after the following modifications were made to the robot method: elimination of on-deck mixing step prior to aspiration of master mix from tube, and transfer of master mix into the optical reaction plate using p50 tips rather than p250 tips.

#### **MATERIALS AND METHODS**

The modified robotic PowerQuant® system (Promega Corp., Madison, WI) method was compared with the previous method, using six male/female mixture samples, previously purified robotically using the DNA IQ™ System (Promega Corp.), according to the Virginia Department of Forensic Science Procedures Manual.<sup>1</sup>

The PowerQuant® method was performed following the manufacturer's recommendations.<sup>2</sup> The same PowerQuant® kit lot number was used for the previous and modified robotic and plate setups. The samples were quantified in duplicate using the modified robotic method for one plate setup, and using the previous robotic method for the other. Both setups used 2 µL of DNA extract added to 18 µL of PowerQuant® amplification cocktail. For one of the two replicates using the modified robotic setup, 2 µL of DNA extract was added to the optical reaction plate manually. All other steps were performed robotically. 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 DNA 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. 1.5.1, and analyzed using Promega's PowerQuant® Analysis Tool software, ver. 1.0.0.0.

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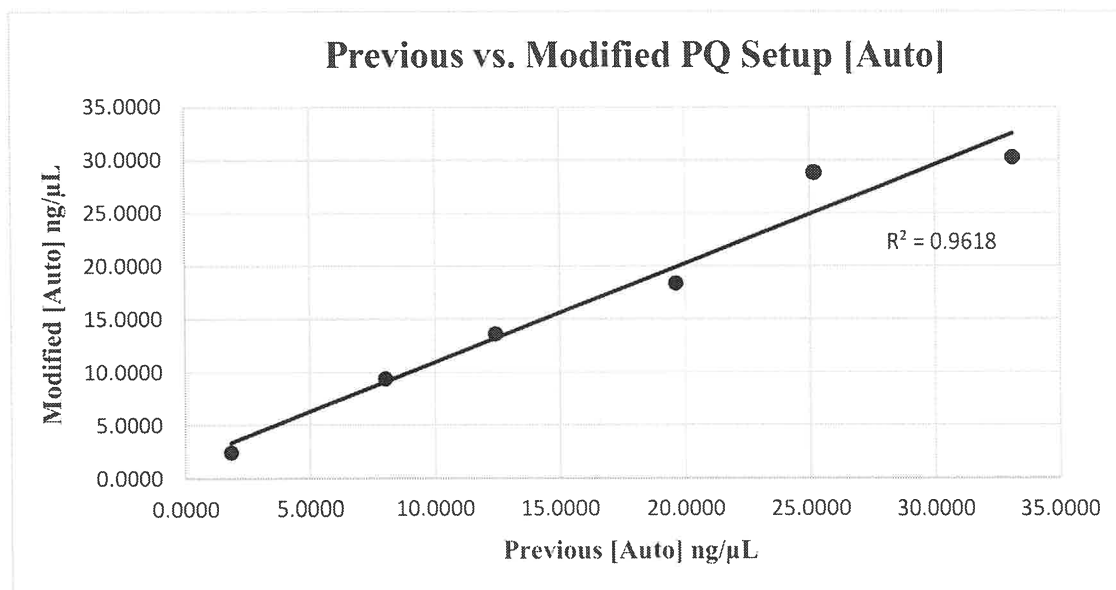
<sup>1</sup> Forensic Biology Extraction of DNA, Procedures Manual. Virginia Department of Forensic Science. June 30, 2020.

<sup>2</sup> Promega PowerQuant® System Technical Manual, revised 1/20.

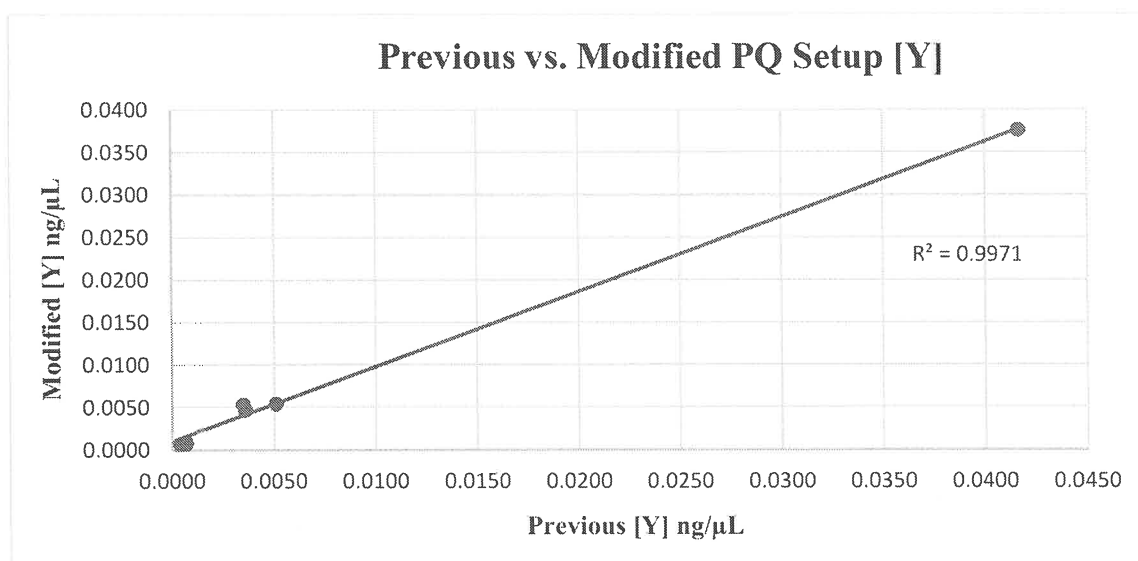
## RESULTS

An automated PowerQuant® system method for serial dilution of the male standard and reaction plate setup was previously developed for use with the Beckman Coulter Biomek® NX<sup>P</sup> Automation Workstation (Fullerton, CA). During the aspiration of master mix small droplets were observed to form on the outside of the p250 tips during the mixing step for the reagent master mix when aspirating with multiple tips from a single tube. Those droplets could lead to variability in volume of master mix dispensed into each well of the optical reaction plate thereby increasing the potential for less accurate quantitation results. Two modifications were made to decrease this potential for variability. The robotic mixing step prior to master mix aspiration was removed (mixing will be performed manually prior to placing the tube on the deck) and the smaller volume p50 tip was used for this step.

The modified robotic setup of the PowerQuant® system assay was evaluated for suitability by comparing the previous and modified robotic plate setups using the same set of samples and quantifying them on the same qPCR instrument. Estimated autosomal and male DNA concentrations were similar for both robotic setups of the standards and samples (Figures 1 and 2). For the robotic samples quantified in duplicate, the average concentration was plotted. The correlation coefficient demonstrated high correlation ( $>0.9$ ) between autosomal quantitation estimates (Figure 1) and Y-chromosome estimates (Figure 2) for both previous and modified robotic methods.



*Figure 1.* A comparison of autosomal DNA concentration estimates generated by PowerQuant® using the old and modified robotic reaction plate setups.



*Figure 2.* A comparison of male DNA concentration estimates generated by PowerQuant® using the previous and modified robotic reaction plate setups.

Estimated degradation target concentrations as well as the autosomal to Y-DNA (A/Y) ratios were also similar between the robotic methods (data not shown). The software did not flag any samples as inhibited. All samples were flagged as male/female mixtures with one exception, where a sample that was robotically set up using the modified method displayed a very low concentration of the male target in the first replicate ( $[Y]=0.0008$  ng/μL) and a high male/female index ( $[Auto]/[Y]>10,000$ ), whereas for the second replicate, the  $[Auto]/[Y]$  was 'Undetermined', since no amplified DNA was detected for the male target. Similar degradation flags were observed between the robotic setups of the same DNA extracts, with two samples showing slight degradation ( $[Auto]/[D]$  ratios between 2 and 3) in each replicate, and 1 sample showing slight degradation ( $[Auto]/[D]$  ratios=2.1) in the modified robotic method replicates and no degradation flag ( $[Auto]/[D]$  ratio=1.9) in the previous robotic setup. No contamination in the no-template controls was observed using either method.

## CONCLUSION

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