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## **VIRGINIA DEPARTMENT OF FORENSIC SCIENCE**

### **VALIDATION OF THE APPLIED BIOSYSTEMS® QUANTSTUDIO™ 5 REAL-TIME PCR SYSTEM: PERFORMANCE CHECK OF POWERQUANT® POPULATABLE WORKSHEETS AND MACROS**

**Prepared in September, 2022**

#### **PURPOSE**

This performance check was conducted to test the function of the DFS populatable worksheets and macros used in conjunction with the PowerQuant® DNA Quantification System assay (PowerQuant; Promega Corp., Madison, WI) and Applied Biosystems® QuantStudio™ 5 Real-Time PCR Instrument (QuantStudio; Thermo Fisher Scientific, Waltham, MA) for quantifying DNA samples.

#### **MATERIALS AND METHODS**

Since the revisions to and functions performed using these worksheets were minor in nature, as opposed to impacting the analytical process, a functional test of these Microsoft Excel® (Excel) macro-enabled worksheets was performed using sample information on multiple validation and research runs. Each file included a different number of samples per run and configuration on the 96-well plate.

##### **Fusion Populatable Worksheets for PowerQuant® (PQ)**

This worksheet was modified to include plate maps and reagents specific for the PQ kit, calculate the master mix volumes automatically, and create a text file for importing sample names into the Applied Biosystems® QuantStudio™ Design & Analysis Software (Thermo Fisher Scientific) used on the QuantStudio to set up and run experiments using PQ. No other worksheet functions, such as those related to the CE plate map and export file for the 3500xL, were modified.

The calculations computed using the worksheet were compared to those calculated manually, according to the manufacturer's recommendations and draft VDFS Procedures Manual.<sup>1</sup> The sample-specific information entered for each run was compared to the Biomek® NX<sup>P</sup> Automation Workstation (Biomek; Beckman Coulter, Fullerton, CA) and 3500xL Genetic Analyzer (3500xL; Thermo Fisher Scientific) plate maps populated and the text file created for use with the QuantStudio.

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<sup>1</sup> PowerQuant® System Technical Manual. Promega. Revised 1/2020.

### PowerQuant® Data Export Editor

This worksheet was created to remove extraneous rows in the analyzed data file exported from the QuantStudio prior to import into the Promega PowerQuant® Analysis Tool, an Excel macro-enabled template for calculations and evaluation of standard curves and sample quality metrics developed by Promega Corp. for PQ data analysis. The rows removed by the macro were compared to those removed manually, as required by the manufacturer's instructions.<sup>2</sup>

### PowerQuant® Quantitation Worksheet

This worksheet was created to format the PowerQuant® Analysis Tool data in a quantitation report format suitable for inclusion in case file documentation. It is also enabled the creation of a tab-delineated export file that works in conjunction with the existing Promega STR Normalization Manager software and settings to perform automated DNA normalization based on the DNA quantitation data imported. The values and fields on the quantitation report (sample concentrations, Auto/Y,  $R^2$ , etc.) were compared to those in the PowerQuant Analysis Tool for accuracy, and to ensure that all sample and NTC (no-template control) results were included and the headers displayed the correct information. The Sample Dilution Tracking report created by the normalization and PCR setup method was reviewed to ensure that the correct volumes of sample were used for normalization and PCR setup based on the autosomal DNA concentrations imported.

## **RESULTS**

In every instance, the Fusion Populatable Worksheets for PowerQuant correctly populated the Biomek and 3500xL plate maps according to the information completed on the "Samples Extracted" page of the worksheet. The calculations performed by the worksheet for determination of the PowerQuant reagent volumes were accurate in all instances. Lastly, the samples names populated into the text file for importing sample names into the plate setup in the QuantStudio software were in the proper order in the "Run Sheet" tab of the worksheet. The remaining functionality of the worksheets was unaffected.

The PowerQuant Data Export Editor removed the same extraneous rows of data from the QuantStudio export file as when manually removing the rows and was used for successful import of analyzed PowerQuant data into the PowerQuant Analysis Tool for all runs.

The PowerQuant Quantitation Worksheet formatted the desired fields and quantitation data from the PowerQuant Analysis Tool accurately to produce a quantitation report. It also created a tab-delineated file, which was used to import quantitation data successfully into the existing software method for automated sample normalization and PCR setup for all runs.

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<sup>2</sup> Using the PowerQuant® System on the Applied Biosystems® QuantStudio™ 5 Real-Time PCR System. Promega. Application Note #329

## **CONCLUSION**

A functional check of the macro-enabled Excel worksheets was performed, following minor revisions to the Fusion populatable worksheets and the development of two new worksheets for use with the PQ assay and QuantStudio instrument. It showed all calculations are accurate and all processes work as expected, thus these worksheets are suitable for use with casework procedures for quantifying DNA samples and performing automated normalization and Fusion STR amplification setup.