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VALIDATION OF THE APPLIED BIOSYSTEMS® QUANTSTUDIO™ 5 REAL-TIME PCR SYSTEM: PERFORMANCE CHECK OF ROBOTIC STR NORMALIZATION AND PCR SETUP

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PURPOSE

This study was designed to check the suitability of the existing PowerPlex® Fusion analysis template in the Promega STR Normalization Manager software for use with Promega PowerQuant® DNA Quantification System data. The Biomek® NX^P Automation Workstation was used in conjunction with the 3500xL capillary electrophoresis instrument to prepare and analyze samples processed with the template.

MATERIALS AND METHODS

Nine of the samples were non-sperm or sperm fractions of mock sexual assault swabs, previously differentially extracted and purified using the DNA IQ™ System (Promega Corp., Madison, WI) on the Biomek® NX^P Automation Workstation (Biomek; Beckman Coulter, Fullerton, CA), as described in the VDFS Procedures Manual, and one sample was a sperm fraction reagent blank, previously prepared using an automated DNase I enzyme draft method on the Biomek.¹ All extracts were previously quantified with the Promega PowerQuant® System, following the manufacturer's recommendations, with the exception that the PQ Dilution Buffer (used to prepare the standards) was also used for the no-template control.² Automated serial dilution of the male standard and reaction plate setup was performed using a robotic method developed specifically for the PQ system on the Biomek.

Quantitative PCR (qPCR) amplification and detection were performed using the QuantStudio™ 5 Real-Time PCR System (QS5; Thermo Fisher Scientific, Waltham, MA), which was calibrated for the following dyes: FAM for the autosomal target (84-base-pair amplicon), CAL Fluor® Gold 540 for the male targets (81bp and 136bp), TMR for the internal positive control (IPC) (435bp), Quasar® 670 for the degradation target (294bp), and CXR for the passive reference dye. The raw data were collected with QuantStudio™ Design and Analysis Software (Thermo Fisher Scientific) v.1.5.1, and analyzed using the PowerQuant® Analysis Tool (Promega) v.1.0.0.0.

Samples were selected to include those that required normalization prior to Fusion amplification (dilution within tip or within plate) to meet the target DNA template amount of

¹ Forensic Biology Procedures Manual. Extraction of DNA. Virginia Department of Forensic Science. Issued May 28, 2021.

² PowerQuant® System Technical Manual. Promega. Revised 1/2020.

0.5ng (per 5uL), those that did not meet the target amount, and one that was defined as a control ('RB' contained in sample name).

The Biomek was used for the normalization and PowerPlex® Fusion (Fusion; Promega Corp.) amplification setup of the samples, as described in the VDFS procedures manual.³ A Sample Dilution Tracking report was created using both versions of the STR Normalization Manager software (v1.1.0.0 and v1.1.0.1) in use at the Central Laboratory. Since identical reports were generated, a single normalization and PCR setup was performed using software v1.1.0.0.

Amplification was performed using a GeneAmp™ 9700 Thermal Cycler (Thermo Fisher Scientific). All samples were separated and detected on the 3500xL Genetic Analyzer (Thermo Fisher Scientific) using Data Collection Software v3.1, according to the VDFS Procedures Manuals.⁴ Samples and allelic ladders were prepared by mixing 9.5 µL Hi-Di formamide (Thermo Fisher Scientific), 0.5 µL WEN ILS size standard (Promega), and 0.5 µL or 2 µL sample, or 1 µL allelic ladder (Promega). The sample plate was heated at 95°C for 3 minutes and snap-cooled at 0°C for three minutes. The amplification products were separated using the manufacturer's recommended settings, including a 24s, 1.2 kV injection, 1800s, 15 kV separation, 36 cm (length), 50 µm i.d. capillary, and POP-4 polymer (Thermo Fisher Scientific). Analysis and interpretation of electropherogram data was completed as directed in the VDFS Procedure Manuals using the GeneMapper® ID-X v1.4 analysis software (Thermo Fisher Scientific).^{5,6}

RESULTS

Consistent Sample Dilution Tracking reports were created from both versions of the STR Normalization Manager software and all sample and diluent volumes listed were correct, based on autosomal DNA concentrations provided in the PQ data input file. The reagent blank was correctly recognized as a control, such that the maximum volume (5 µL) would be added to the amplification tube. The normalization and sample addition steps for all samples and controls were monitored closely and all volumes transferred looked appropriate.

Additionally, the success of the DNA typing (profile completeness) was compared to the expected outcome based on the autosomal and male DNA concentrations estimated for each sample. For all but one sample, the extent of the DNA profile detected from each contributor in the mixture reflected the amount of total human and male DNA estimated (data not shown). For the remaining sample, the amount of DNA estimated was 0.0070 ng/µL for the male target and

³ Forensic Biology Procedures Manual. PowerPlex® Fusion Amplification and Long Term Storage. Virginia Department of Forensic Science. Issued June 30, 2020.

⁴ Forensic Biology Procedures Manual. CE for PowerPlex® Fusion. Virginia Department of Forensic Science. Issued December 30, 2019.

⁵ Forensic Biology Procedures Manual. Analysis of CE Results Using GeneMapper® ID-X. Virginia Department of Forensic Science. Issued December 13, 2019.

⁶ Forensic Biology Procedures Manual. Interpretation of PowerPlex® Fusion CE Data. Virginia Department of Forensic Science. Issued December 13, 2021.

0.0097 ng/μL for the degradation target; however, the autosomal DNA target was not detected and the IPC shift value was higher than 0.3, which may indicate inhibition in the qPCR assay. Despite the low values measured in the well (F2) during quantitation, complete DNA profiles from both the male and female contributors were obtained for this sample, and these STR results were similar to those obtained for a duplicate swab sample, which contained approximately 0.03 ng/μL DNA for all targets measured. The low DNA quantity measured for this sample was subsequently attributed to the QS5 instrument well (F2), which required cleaning and additional maintenance to improve performance and was not related to the PowerQuant system or the STR normalization method. The positive and negative amplification controls produced the expected DNA typing results.

CONCLUSION

A test with the existing Fusion analysis template in the Promega STR Normalization Manager software confirmed that the automated method performed as expected for STR normalization and Fusion amplification setup of samples that have been quantitated using the PowerQuant system in conjunction with the QuantStudio™ 5 Real-Time PCR Instrument.