Optimization of the Normalization and STR Setup for the Biomek® NX^P

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The Virginia Department of Forensic Science (VDFS) is currently utilizing the Biomek® 2000 to automate multiple steps of the forensic DNA analysis process. In an effort to increase efficiency and enhance workflow, VDFS is validating the Biomek® NX^P, an improvement over the Biomek® 2000 due to its more sophisticated software and its liquid-displacement pipetting, among other features. This study focuses on testing of a beta version of Promega's normalization and STR setup software specific to the Biomek® NX^P and FX platforms.

Parameters have been evaluated such as the ease with which quantitation data imports into the normalization and STR setup software, susceptibility of the entire process to contamination, accuracy of the dilution strategies employed, and the quality of the STR profiles produced. With minor adjustments resulting from trial-and-error and prompts by the user interface, the quantitation data was successfully imported into the normalization/STR setup software. Other minor glitches were identified and corrected by Promega; the user interface proved to be effective and user-friendly.

Repeated checkerboard contamination assays were performed using mock casework samples containing a wide range of concentrations in order to detect even the slightest of contamination events. Contamination was detected in four of 93 blanks when very concentrated tissue samples were tested and plates were sealed multiple times due to disruptions in work flow. Following these tests, a 32 sample tissue checkerboard test (average concentration of 72.42 ng/µl) was performed without disruptions to the work flow and no contamination was observed. The samples were successfully processed from DNA extraction to quantitation, normalization, STR setup and loaded onto the CE in a single work day, demonstrating that utilizing the Promega extraction and normalization/STR setup methods in conjunction with the Biomek® NX^P platform increases efficiency of the automated process. The clean checkerboard assays for 104 routine samples and a clean 32 sample tissue checkerboard test demonstrate that the methods on the Biomek® NX^P pose a very low susceptibility to contamination.

The accuracy and precision of creating the DNA dilutions for qPCR setup and the normalization process were evaluated in comparison to manual pipetting. When carrying out the serial dilution of qPCR standards and transferring them to the qPCR plate, the resultant standard curves were comparable to manually generated standard curves, indicating accurate and reproducible pipetting. When evaluating normalization based upon post-dilution quantitation, samples over the target concentration but directly transferred to the amplification plate with an in-tip dilution (0.16 – 0.375 ng/µl) averaged 0.157 ng/µl, those diluted through one plate (0.376 – 15 ng/µl) averaged 0.205 ng/µl, and those diluted through two plates (above 15 ng/µl) averaged 0.253 ng/µl. When looking at average peak heights, data indicated that single-plate-dilution samples had higher average peak heights than those requiring two dilution plates. Both groups exhibited average peak heights on the higher end of the ideal range of 1000 – 3000 RFU.

The Biomek® NX^P is comparable to the Biomek® 2000 and manual assay setup in levels of contamination and pipetting accuracy during the normalization and STR amplification setup. The Biomek® NX^P is more efficient due greater pipetting pod versatility, has the ability to dilute 10,000-fold versus the Normalization Wizard for the Biomek® 2000 (approximately 113-fold), and consumes less sample extract than the Biomek® 2000. Additional testing is required; however, the Biomek® NX^P is expected to be successfully validated for casework sample processing at VDFS.