Automated Method for Improved DNA Isolation from Whole Blood with Novel Silica-Based MicroPorous Xtraction (MPX) Technology

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HIGHLIGHTS: Fully automated, fast (< 30 min), reproducible

MPX

INTRODUCTION

DNA isolation by silica binding is a robust and efficient technique used to recover high-quality genomic DNA from complex biological samples. Magnetic silica bead technology has been widely adopted for these extractions; however, bead capture time, bead carryover, bead resuspension and requirements for magnets on automation platforms complicate the process. DPX Technologies has developed a novel MicroPorous Xtraction (MPX) Technology that is incorporated into a pipette tip and eliminates magnetic beads and magnets for a fully automated, high throughput solution. This method uses the Hamilton® Robotics platform and a 1 mL MPX Tip from DPX Technologies for rapid bind, wash and elution steps. The MPX technology design and automated method minimizes cross-contamination and enhances reproducibility.

- DNA extraction from 250 µL of whole blood using the 1 mL MPX Tip was compared to the leading bead-based and column-based technologies using their protocols.
- DNA yield (ng/µL), purity (260/280 ratio), and overall quality (gel electrophoresis) were compared.
- The MPX method can process 96 samples from lysate to purified DNA in only 18 minutes and obtained higher yields of DNA with similar or better purities.

MATERIALS AND METHODS

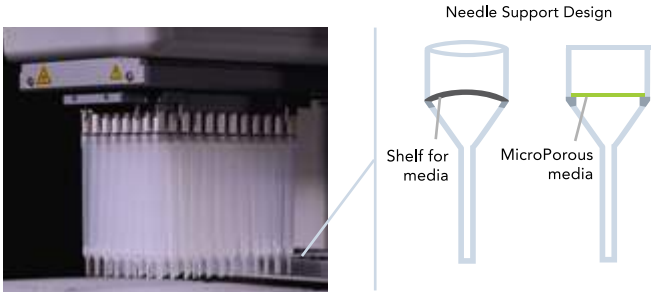


Figure 1. *The 1 mL MPX Tip is comprised of a needle support which contains a proprietary silica media attached to a 1 mL wide bore Hamilton tip.*

DNA was extracted from 250 µL of whole blood collected in K-EDTA tubes (Innovative Research, Novi, MI). The novel MPX technology from DPX Technologies was compared to leading bead-based and column-based DNA extraction kits. The 1 mL MPX Tip was utilized with the accompanying room-temperature stable buffer system which includes lysis buffer, proteinase K, wash 1 buffer, wash 2 buffer and elution buffer (DPX Technologies, Columbia, SC). Isopropanol (IPA) is also utilized for the method but not provided in the MPX Tip kit. For the MPX protocol, 250 µL of whole blood was lysed with 250 µL of lysis buffer and 25 µL of proteinase K at 56°C for 10 minutes. The lysates were transferred to a 2 mL well plate and loaded onto the Hamilton® NIMBUS automated liquid handler (ALH). Lysis and accessioning can be performed on the larger Hamilton robots as well. Well plates were loaded with 500 µL wash 1, 1 mL wash 2, and 55 µL elution buffer. IPA was added to a reservoir. Blank 1 mL transfer tips and the MPX Tips were also loaded onto the deck **Figure 3**. The steps for sample preparation are listed in **Table 1** and shown in **Figure 2**. These steps are optimized for speed to minimize shearing.

For the bead- and column-based extraction kits, the recommended protocols provided by the manufacturer were followed. DNA yield and purity (260/280 ratio) were measured using a Thermo Fisher Scientific NanoDrop One Microvolume UV-Vis Spectrophotometer (Waltham, MA) and/or a Molecular Devices FilterMax F5 Microplate Reader (San Jose, CA). The quality of the DNA was confirmed by performing real-time PCR on a QuantStudio™ 3 (Thermo Fisher Scientific) and electrophoresis on an Agilent 5200 Fragment Analyzer (Santa Clara, CA). The PCR master mix used was TaqMan™ Genotyping MM from Thermo Fisher Scientific.

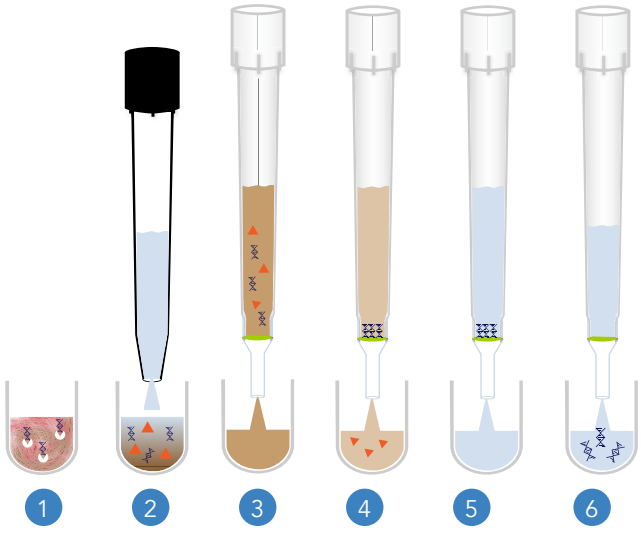
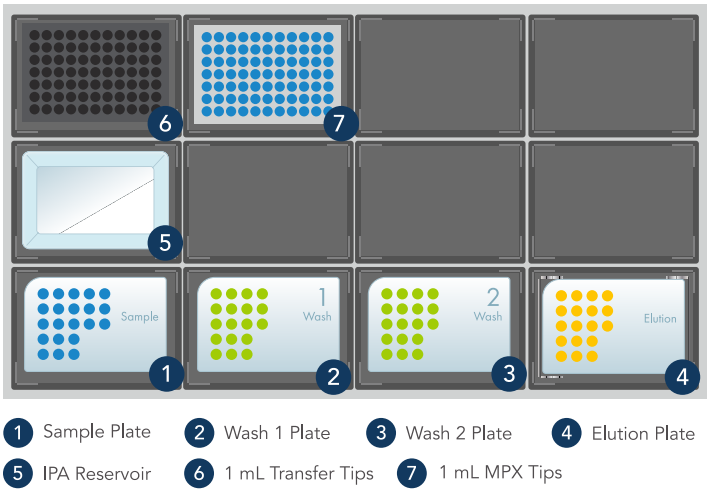


Figure 2. *Schematic of the 1 mL MPX Tip workflow outlined in Table 1.*

1 Lyse	Combine 250 µL whole blood, 250 µL lysis buffer, and 25 µL pro K. Incubate at 56°C for 10 min.
2 Precipitate	Add 350 µL of IPA to lysate with transfer tips. Aspirate and dispense 25X.
3 Bind	Aspirate and dispense lysate with 1 mL MPX Tips 3X.
4 Wash 1	Aspirate and dispense 500 µL of wash 1 buffer.
5 Wash 2	Aspirate 500 µL wash 2 buffer, dispense to waste (wash 1 well plate location). Aspirate and dispense second aliquot of 500 µL wash 2 buffer. Dry by aspirating and dispensing air 4X.
6 Elute	Aspirate and dispense 55 µL of elution buffer at 56°C 2X.

Table 1. *Method for DNA extraction from whole blood with 1 mL MPX Tip.*



1 Sample Plate 2 Wash 1 Plate 3 Wash 2 Plate 4 Elution Plate
5 IPA Reservoir 6 1 mL Transfer Tips 7 1 mL MPX Tips

Figure 3. *Deck layout for DNA isolation method with 1 mL MPX Tips. Reservoirs of each solution can also be added to the deck to utilize the ALH for transferring appropriate volumes to well plates.*

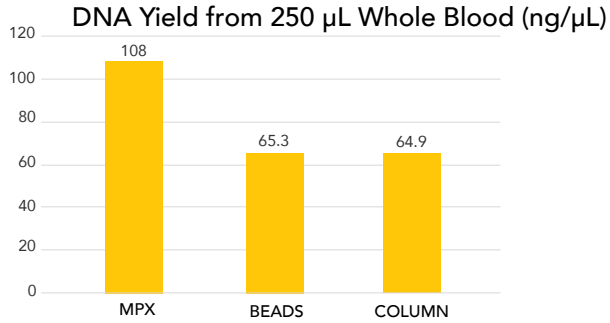


Figure 4. *Direct comparison of average DNA yields from 250 µL of blood with DPX MPX isolation versus the leading bead-based and column-based isolation competitors.*

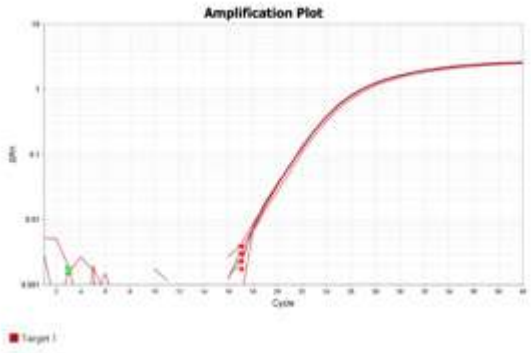


Figure 5. *PCR was performed on 5 replicates of genomic DNA isolated from whole blood using MPX technology.*

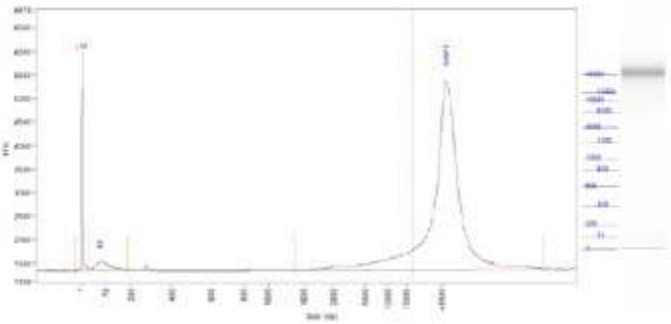


Figure 6. *An Agilent 5200 Fragment Analyzer was used to determine the size distribution of the isolated genomic DNA. Over 80% of the isolated DNA was above 20 kbp in size.*

RESULTS

The 1 mL MPX Tip produced the highest DNA yield (108 ng/µL) compared to the magnetic bead (65.3 ng/µL) and spin column (64.9 ng/µL) methods, with a 260/280 ratio of 1.76, indicating high purity. Reproducibility was confirmed across five replicate extractions, yielding an average %RSD of 3.4%, a notable improvement over the column method (6.6%) and the magnetic bead competitor (5.6%). (**Figure 4**)

The quality of the DNA was confirmed by real-time PCR with a mean Ct value of 22.47 ± 0.13 (**Figure 5**) and by electrophoresis with 80% of DNA < 20 kbp and the average fragment size of 48 kbp (**Figure 6**).

CONCLUSIONS

DPX Technologies' automated bind-wash-elute system is fast, reliable, and reproducible with minimal hands-on time. The 1 mL MPX Tip offers a rapid, high-yield (5-10 µg total genomic DNA) and high-purity DNA extraction suitable for applications like PCR and Next Generation Sequencing. The MPX Tip takes advantage of traditional silica technology in a modern way by automating the entire process without the use of laborious bead equipment and protocols. Automation platforms provide the capability to control critical variables like flow rates to minimize shearing for high quality, high molecular weight DNA. Therefore, this technology is compatible with both short-read and long-read sequencing applications.