APPLICATION NOTE

Novel, Proprietary MicroPorous Xtraction Technology and Patent-pending Method for Improved DNA Isolation from Whole Blood

INTRODUCTION

DNA isolation by silica binding is a well known, powerful and efficient method used to recover genomic DNA from complex samples. This process involves a bind-wash-elute protocol by binding DNA to silica in a solution, washing away the solution to remove any contaminants, followed by eluting the DNA from the silica. Silica magnetic beads were introduced to the market as a comprable alternative to manual methods, eliminating centrifugation steps and providing compatability with automated liquid handlers. Today, most DNA isolation kits include magnetic beads that are less than 1 micron in size to increase surface area. However, the use of magnetic beads requires multiple magnetic capture steps for DNA purification, with each step requiring 1-5 minute incubations.

DPX Technologies has developed a novel, patent-pending MicroPorous Xtraction (MPX) device and patent-pending DNA isolation method that is fully automated. The DPX workflow does not require magnets or beads, therefore no magnetic capture incubation steps allows the DNA isolation process to be faster, taking less than 15 minutes from lysate to purified DNA for 96 samples. The automated method uses a high throughput vacuum process that provides rapid binding and wash steps, and allows for elution using the pipetting head of the robotic liquid handler to prevent cross-contamination and provide a very robust method.

MATERIAL AND METHODS

The method presented utilizes a MPX kit from DPX Technologies which includes the appropriate buffers for the DNA isolation method from whole blood and a DPX custom vacuum block designed to fit a Hamilton Nimbus 96 system. The MPX device (as shown in figure 1 A) contains a proprietary silica-based membrane.

Aliquots of 50 µL of whole blood were lysed with 50 µL of Lysis Buffer and 5 µL proteinase K for 5 minutes at 56°C. The lysate was placed onto the Hamilton Nimbus96 liquid handler, which adds 75 µL of Binding Buffer and mixes the solutions 25 times. The mixed lysate is transferred to

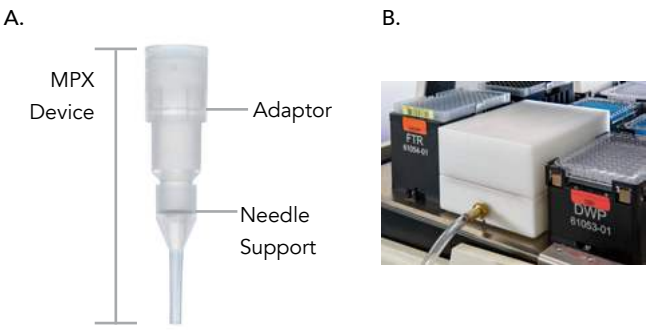

Figure 1 A. Anatomy of a MPX device. The device comes as a 3-part unit comprised of an adaptor specifically designed to be used with Hamilton robotics platform, a needle support, and microporous media housed between the two.
Figure 1 B. DPX custom vacuum block pictured on a Hamilton Nimbus 96

the vacuum block station containing the MPX devices and dispensed into the upper chamber of the device through a funnel plate. The funnel plate is essential to eliminate contamination and maintain cleanliness in the adapter rim for the liquid handler to attach to the MPX devices. Vacuum is applied and the lysate is passed through the silica membrane to bind DNA. Two 200 µL washes are performed with Wash Buffer 1, followed by three 200 µL washes with Wash Buffer 2. The membranes are then allowed to air dry under vacuum pressure for two minutes to remove any possible ethanol contamination. Heated elution buffer is transferred to the membranes and allowed to incubate for 30 seconds. The ALH picks up the MPX device (as it would a pipette tip) and dispense the elution into a well plate. This workflow is depicted in Figure 2.

Concentration data and 260/280 ratios were collected by a Thermofisher Nanodrop One and/or a Molecular Devices FilterMax F5 microplate reader. For 96 sample RSD testing, bulk lysis was performed for 120 samples for each well plate replicate of testing. Lysis incubation was performed in a 15 mL conical tube in a Labnet Vortemp 56 heater/shaker set to 56°C for 5 minutes. 105 µL of vortexed lysate was dispensed into each well of a 96 well sample plate for testing.

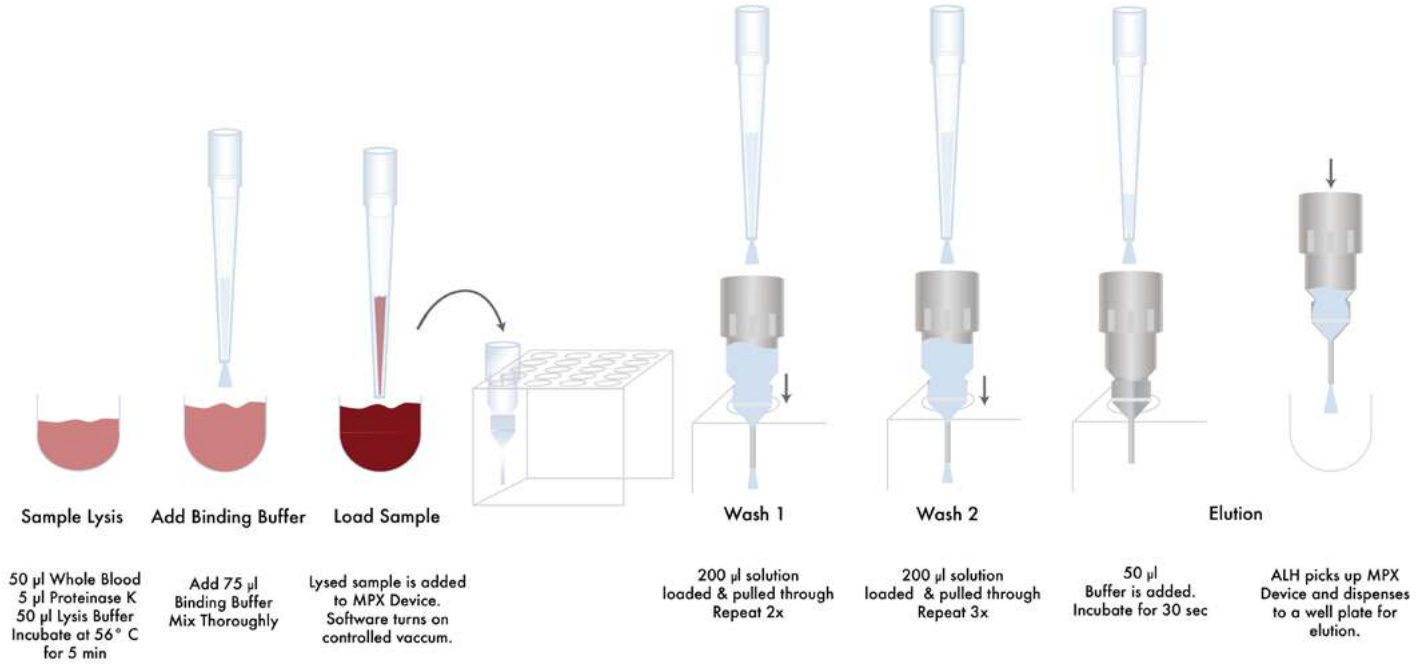

Figure 2. The schematic depicted the SNAPX workflow which processes 96 sample in under 20 minutes, including a 5 minute proteinase incubation.

RESULTS

The results compare the patent-pending DPX DNA isolation technique to standard magnetic bead and spin column-based methods available in the market. Testing was performed using 50 µL aliquots of human blood stored in K-EDTA vacuum tubes, which were lysed with lysis buffer and proteinase K prior to extraction. The DPX isolation technique was able to produce a higher yield of DNA (41.6 ng/µL) with comparable purity (with UV-Vis absorption spectra having a 260/280 ratio of 1.76), in a fraction of the time when compared to either competitor (31.2 ng/µL bead-based and 20.8 ng/µL column-based), as shown in figure 3. Most importantly, 5 separate extractions of 96 aliquots (n = 480) of a single source whole blood sample provided %RSDs that ranged from 11.0 to 19.2%, with an average of 15.8% (compared to over 21.2% for the leading spin column-based technology).

The MPX automated workflow allows for highly reproducible and contamination-free DNA isolations (as shown in figure 6 real-time PCR data) , while minimizing worker fatigue and hands on time.

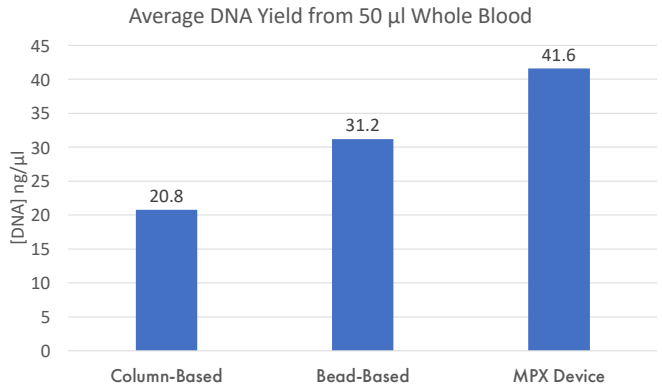

Figure 3. Direct comparison of MPX isolation versus the leading bead-based and column-based isolation products a methods in the market.

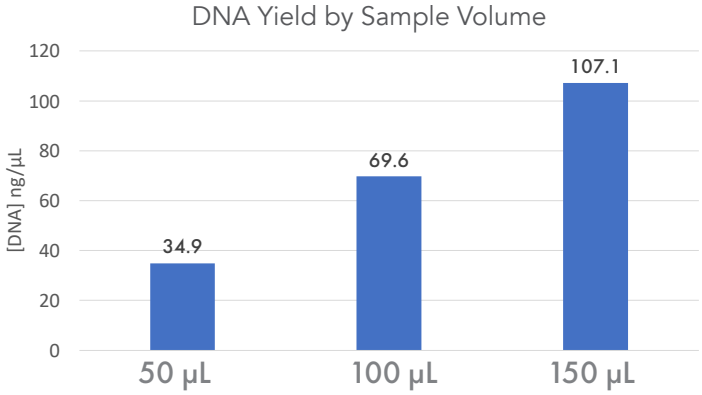

Figure 4. DNA yield across a range of sample volumes (up to 200 µL) using the MPX kit and method.

RESULTS

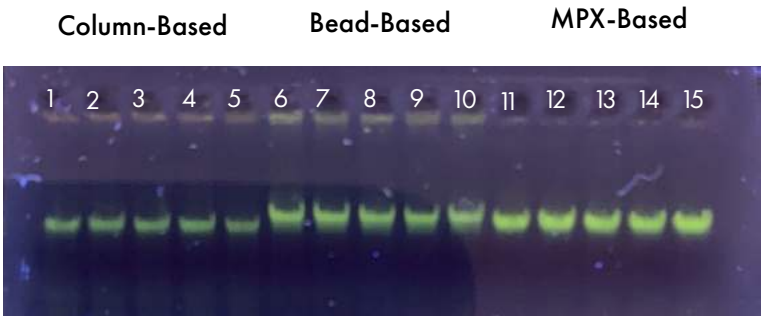

Figure 5. A 1% agarose gel image comparing the leading column based isolation company (lanes 1-5), to the leading bead based isolation company (lanes 6-10), to DPX MPX isolation method (lanes 11-15). The DPX MPX isolation method generates a higher yield of high quality genomic DNA.

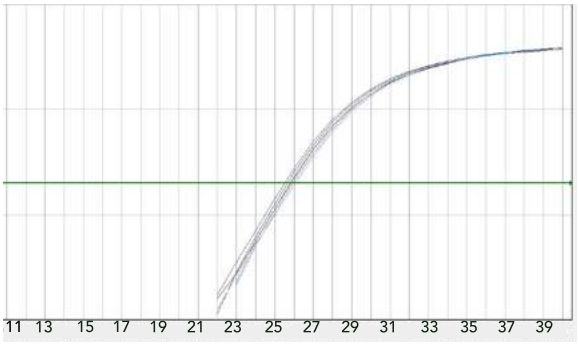

Figure 6. Real time PCR of 5 individual DPX genomic DNA isolations from a whole blood sample showing that the DNA is inhibitor free and ready for downstream applications.

CONCLUSIONS

Fast and efficient DNA isolation has become increasingly important in today's environment of genetic studies. This method prepares samples for a variety of downstream applications, including forensic testing, biotechnology, and medical research. The DPX technology allows for the isolation of 96 samples in as little as 15 minutes, the fastest available on the market today. The process is fully automated, allowing for repeatable, robust results.