DNA Extraction from White Blood Cell Capture

HIGHLIGHT: High Reproducibility, High Throughput, Seamless Integration

PURPOSE AND OBJECTIVE

- Demonstrate the utility of patent-pending MicroPorous Xtraction (MPX) technology for providing a PCR compatible lysate for genomic DNA from whole blood or cell suspensions
- Maintain DNA concentration by utilizing a 1:1 sample to elution ratio.

MATERIALS AND METHOD

Complete DNA purification by bind-wash-elute methods may not be necessary for some downstream DNA applications (PCR, real time PCR, restriction enzyme digestion, etc.). Commercial DNA purification kits can take 45–60 minutes for completion depending on sample type, instruments and chemistries. This method utilizes a novel MPX technology with proprietary silica media to prepare lysates from 50 µL of whole blood that are PCR compatible in less than 15 minutes with the addition of our PCR enhancer. The MPX device, shown in figure 1, comes as a three-part unit comprised of an adaptor, a needle support and the microporous media housed between the two. Chemistry media within the device can come in membrane or disk format and provides the high surface area required to replace existing magnetic bead or DNA bind-wash-elute protocols.

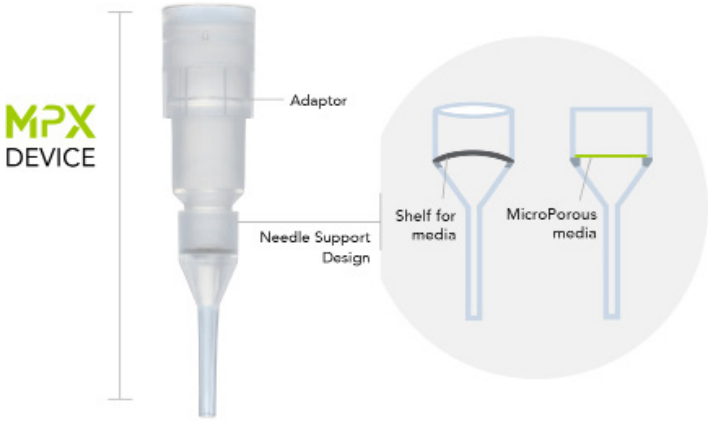


Figure 1. Anatomy of the MicroPorous Xtraction Device. Adaptor designed to work with Hamilton Robotics platforms.

As illustrated in figure 2, 50 µL of whole blood is mixed with 150 µL of dilution buffer and dispensed into the MPX Device containing a proprietary silica membrane. Intact cells are captured on the membrane and washed 2x with 225 µL of wash buffer. The captured cells are lysed in 50 µL lysis buffer and eluted into the collection plate (Protocol 1). Optionally, Proteinase K can be added to the lysate and incubated at 70°C for 10 minutes (Protocol 2).

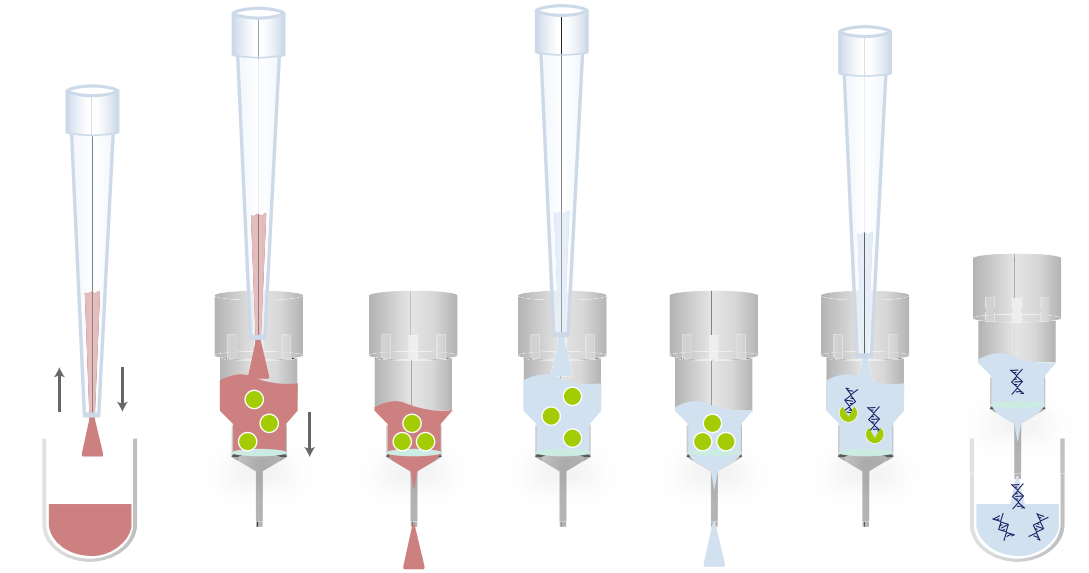
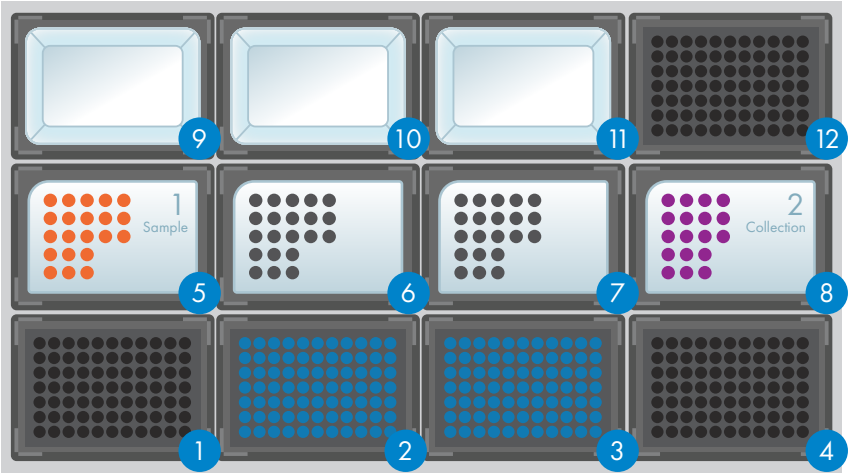


Figure 2. Schematic represents the automated MPX method for protocol 1. Proteinase K can be added to the elution (Protocol 2) which requires an additional 10-minute incubation at 70°C.

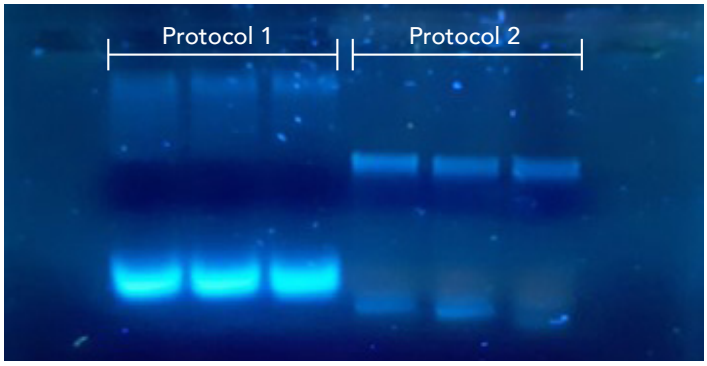
Figure 3. Nimbus96 deck layout for the automated extraction of 96 whole blood samples.



1. Loading Station
2. Membrane Device
3. Adaptor
4. 300 µL Wide Bore CO-RE Tips
5. Sample
6. Wash Plate
7. Wash 2 Plate
8. Elution Plate
9. Dilution Buffer
10. Wash Buffer
11. Lysis Buffer
12. 300 µL CO-RE Tips

RESULTS AND CONCLUSION

The data shows the results for 2 protocols for producing PCR compatible lysates from 50 µL of whole blood. The difference between the two protocols is with or without a proteinase K digestion step. Results suggest that the proteinase K digestion may increase the sensitivity of your real time PCR assay slightly, showing an average Ct value of 24.47 versus 26.33 for no proteinase K digestion (Figure 3).



RNAse P Taqman Assay

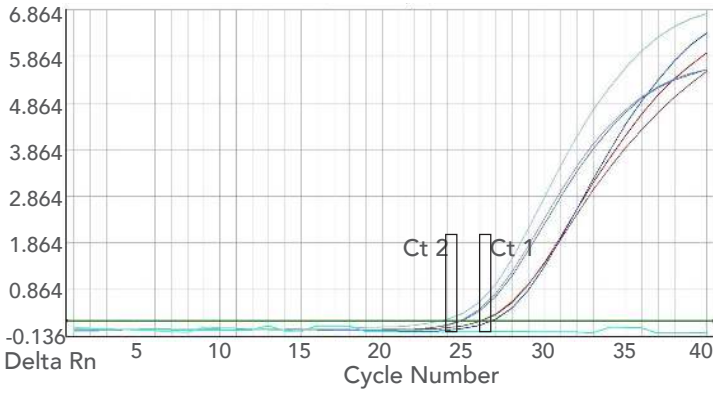
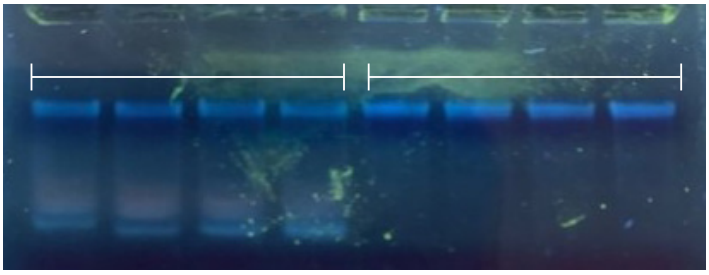


Figure 3. (A) Agarose gel electrophoresis of 3 DPX Lysates produced with protocol 1 and 3 DPX Lysates produced with protocol 2 from 50 µL of whole blood each. (B) Real time PCR Results of RNAse P Taqman assay of the 6 samples.

Figure 4 compares MPX lysates produced by protocol 2 with a commercial bind-wash-elute DNA purification kit using magnetic silica beads. No significant difference was seen for the average Ct values for the MPX lysates (24.48) vs the kit purified DNA (24.37). While these results were from whole blood samples, this chemistry would work well with cell suspensions for quick PCR assessment of cell cultures.



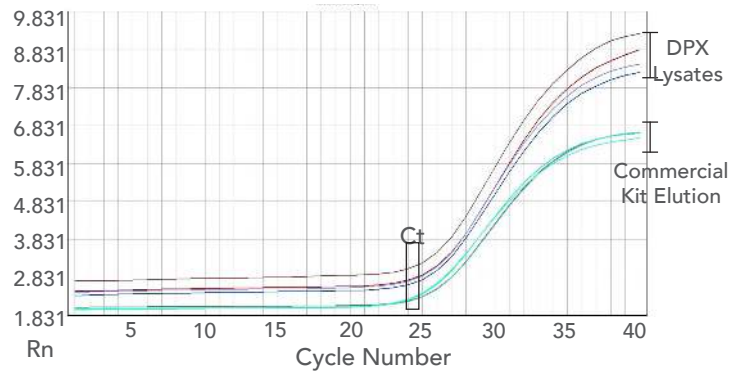


Figure 4. (A) Agarose gel electrophoresis of 4 DPX Lysates produced with protocol 2 from 50 µL of whole blood (DPX Lysates) and 4 DNA purifications from 50 µL of whole blood utilizing a commercial kit with silica beads. (B) Real time PCR Results of RNAse P Taqman assay of the 8 samples.

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