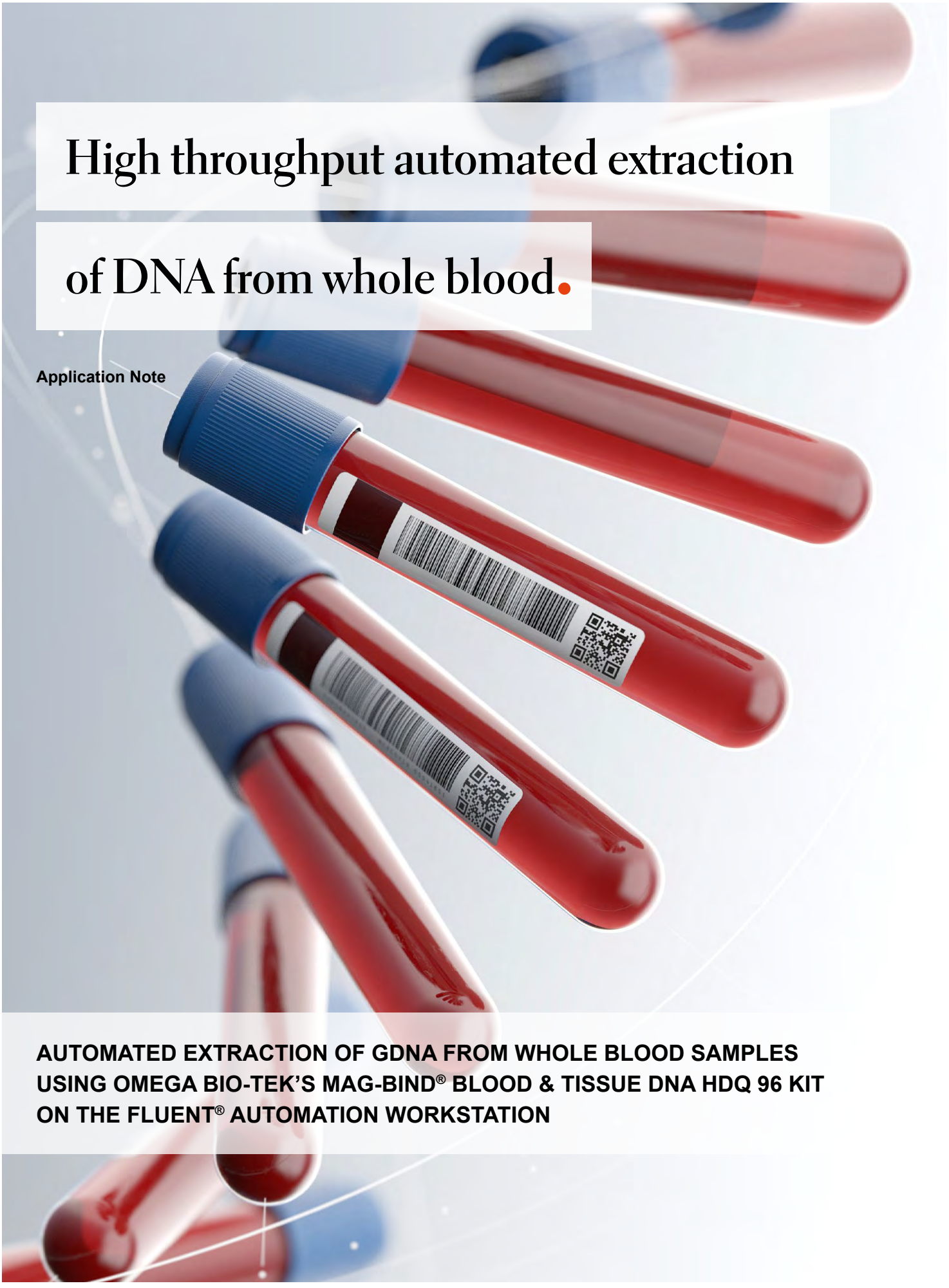
High throughput automated extraction of DNA from whole blood.

Application Note



AUTOMATED EXTRACTION OF GDNA FROM WHOLE BLOOD SAMPLES USING OMEGA BIO-TEK'S MAG-BIND® BLOOD & TISSUE DNA HDQ 96 KIT ON THE FLUENT® AUTOMATION WORKSTATION

TECAN.

INTRODUCTION

Blood is the most common biospecimen used to obtain genomic DNA (gDNA) for use in many genomics-based downstream analyses. For a successful downstream implementation, it is not only crucial to extract high quality, high yielding DNA, but also to meet the criteria of throughput, reliability, and reproducibility.

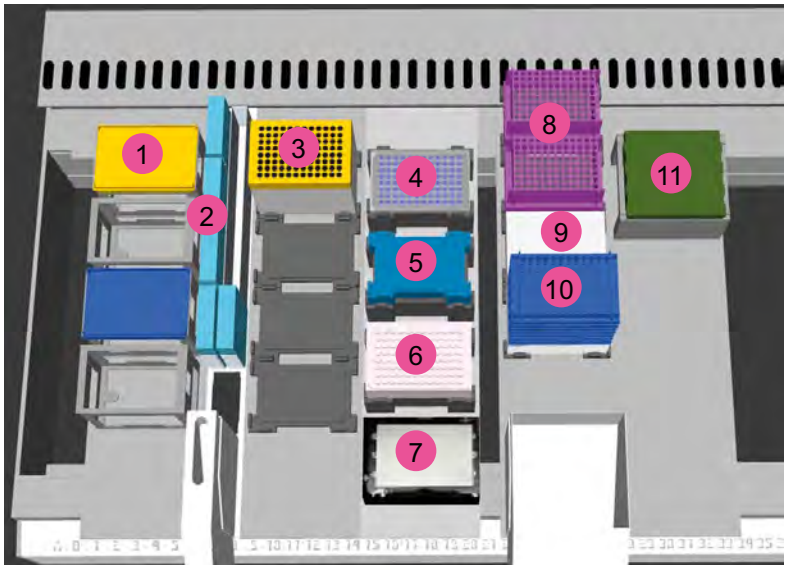
Omega Bio-tek has developed an automated solution by using its Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399) on the Tecan Fluent 780 Automation Workstation to extract DNA from 250 µl blood samples in a high throughput fashion, with minimal manual intervention.

The performance of the automated system was evaluated based on how closely it reflected the manual approach in terms of yield, purity, and integrity of DNA extracted. The results indicate that the automated workflow is capable of extracting high quality, high molecular weight DNA from 96 250 µl whole blood samples in under 75 minutes. This application note presents this automated solution along with data from internal testing to demonstrate its performance.

MATERIALS AND METHODS

The workflow to extract and purify gDNA from 1-96 250 µl aliquots of human whole blood was automated using a Fluent 780 workstation equipped with an eight-channel Air Flexible Channel Arm™ (Air FCA), a MultiChannel Arm™ (MCA) with the Extended Volume Adapter (EVA) head adapter, a Robotic Gripper Arm™ (RGA), a BioShake™ D30-T elm (QInstruments) for heating and shaking, and a Magnum FLX® Enhanced Universal Magnet Plate (Alpaqua), as well as all the consumables required to process a batch of 96 samples (deck layout shown in Figure 1).

Eight 250 µl aliquots from the same lot of human whole blood were transferred to a 96-well DeepWell™ plate and placed on the Fluent workstation for automated gDNA extraction and purification using the Mag-Bind Blood & Tissue DNA HDQ 96 Kit. DNA was eluted in 100 µl of 10 mM Tris-HCl (pH 8.5). All consumables and carriers were placed onto the Fluent workdeck (Figure 1). The workflow was fully automated, from preparation and extraction of the samples to elution of the final product. Aliquots of the same lot of human whole blood were manually extracted in parallel, and the results of the two workflows were compared to validate the automated methodology and instrument set-up.



1. 1,000 µl hanging DiTis
2. 100 ml reagent troughs
3. 1,000 µl SLAS, filtered DiTis
4. 96-well standard microplate
5. Magnum FLX plate
6. Nunc 96-well DeepWell plate
7. BioShake D30-T elm
8. 500 µl MCA filtered DiTis
9. Liquid waste trough
10. 200 µl MCA filtered DiTis, nested 8 stack
11. Extended Volume Adapter

Figure 1: Fluent deck layout for extraction of 96 blood samples with 250 µl input volumes. DiTi = disposable tips.

Fluent 780 example deck layout

1. FCA 1000 µl hanging disposable filtered conductive tips
2. 100 ml reagent troughs
3. FCA 1000 µl SLAS-format filtered disposable tips
3. Standard 96-well elution microplate
4. Magnum FLX Enhanced Universal Magnet Plate
5. 2 ml Nunc® 96 DeepWell plate (sterile)
6. BioShake D30-T elm heater/shaker
7. MCA 500 µl filtered disposable tips
8. 300 ml trough for liquid waste
9. 200 µl nested eight stack MCA disposable tips
10. Extended Volume Adapter

The purified DNA was quantified using a Thermo Scientific™ NanoDrop™ 2000c spectrophotometer, and absorbance measurements were taken at 230, 260 and 280 nm wavelengths to assess quality and check for any RNA/protein or salt contamination. DNA was also quantified using the QuantiFluor® dsDNA System (Promega), to enable specific quantification of double-stranded DNA (dsDNA) without interference from single-stranded DNA (ssDNA) and RNA. The size and integrity of the isolated gDNA was analyzed on a 2200 TapeStation® system (Agilent) using Genomic DNA ScreenTape. The suitability of the extracted DNA for downstream applications was evaluated by real-time PCR using human-specific primers on 10-fold and 100-fold dilutions of the purified DNA. Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent) was used as the master mix, following a standard amplification protocol on an AriaMx Real-Time PCR system (Agilent).

RESULTS AND DISCUSSION

The DNA yields from the whole blood samples – determined using the NanoDrop 2000c system and the QuantiFluor dsDNA System – are shown in Figure 2. The average DNA yield from manual extraction was found to be comparable, and not significantly different from, the average DNA yield obtained using the automated protocol (Tukey's post-hoc analysis; $p>0.05$). These results validate both the instrument set-up and the automated purification protocol.

Comparable DNA Yield - Manual vs Automated

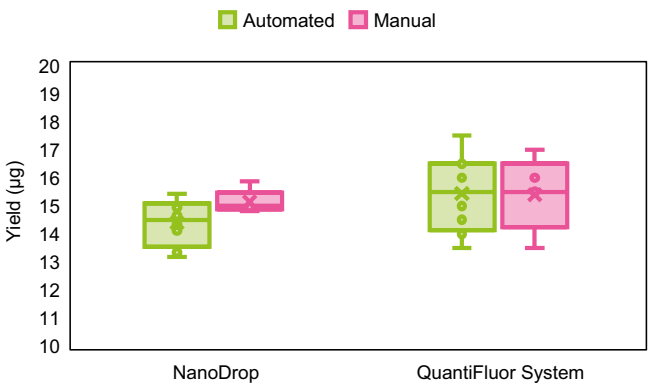


Figure 2: DNA was extracted from 250 µl of whole blood samples and eluted into 100 µl of 10 mM Tris-HCl (pH 8.5). The average DNA yield from the manual and automated extractions was found to be comparable, with no significant difference (Tukey's post-hoc analysis; $p>0.05$).

DNA purity and quality were analyzed by looking at the A260/A280 and A260/A230 ratios obtained post spectrophotometric analysis (Figure 3). For both manual and automated protocols, the A260/A280 absorbance ratio was consistently between 1.80 and 1.84, indicating pure DNA free of contaminating RNA and proteins. The A260/A230 ratios were all greater than 2.0 following either of the protocols, implying low contamination due to carryover. Both ratios indicate high quality DNA, typically considered suitable for a variety of downstream applications.

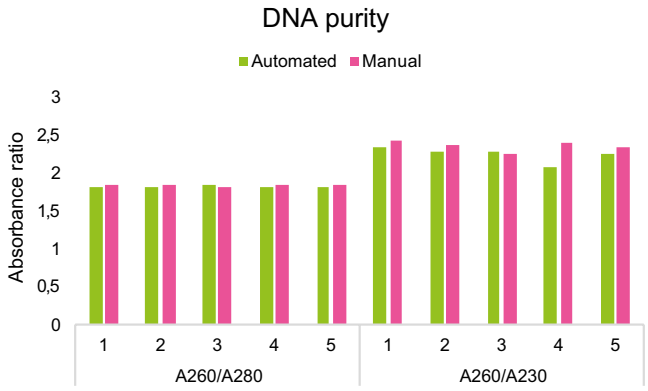


Figure 3: The purity of the DNA isolated using manual and automated protocols was analyzed using spectrophotometry, focusing on A_{260}/A_{280} and A_{260}/A_{230} ratios. Data corresponding to the first five samples are shown.

The purified DNA was also analyzed on the 2200 TapeStation to verify the size and integrity of the gDNA. A DNA Integrity Number (DIN) was determined using the 2200 TapeStation Analysis Software; typically, DNA with a DIN of 10 is considered intact and of the highest integrity. Figure 4 shows the analysis of DNA extracted from the first three samples by the automated and manual protocols. The purified DNA is of high molecular weight and migrated as a well-defined band above the largest ladder peak (48,500 bp), which was determined to be >60 kb for both the automated and manual protocols, with DIN values of 8.3, 8.5 and 8.5, and 7.8, 7.9 and 8.2, respectively. Overall, the DIN values are all >7.7, suggesting that the DNA was highly intact and of high quality, regardless of the extraction methodology.

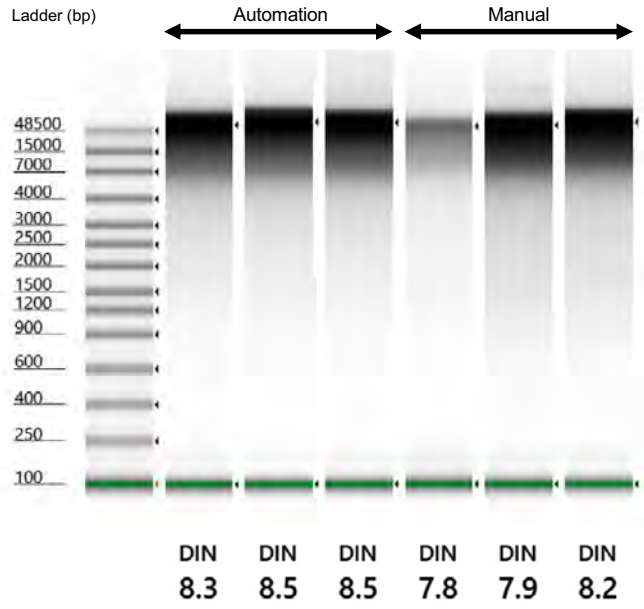


Figure 4: TapeStation analysis performed on the DNA extracted from 250 µl of blood following the automated and manual protocols. Data corresponding to the first three samples are shown.

Real-time PCR was performed on a representative set of the first four samples following manual and automated extraction using human-specific primers. The average Ct values of 10-fold and 100-fold dilutions of the purified DNA are shown in Figure 5. The Ct values across all the dilutions indicated positive amplification and were comparable, irrespective of the extraction methodology. The average ΔCt values between the 100-fold and 10-fold dilutions were 3.14 ± 0.19 and 3.80 ± 0.35 for the manual and automated protocols, respectively. Typically, the Ct values of samples where concentration differs by a factor of 10 are ~3.3 cycles apart. The results not only indicate good PCR efficiency without inhibition, but also endorse the downstream suitability of the extracted DNA, regardless of the extraction approach.

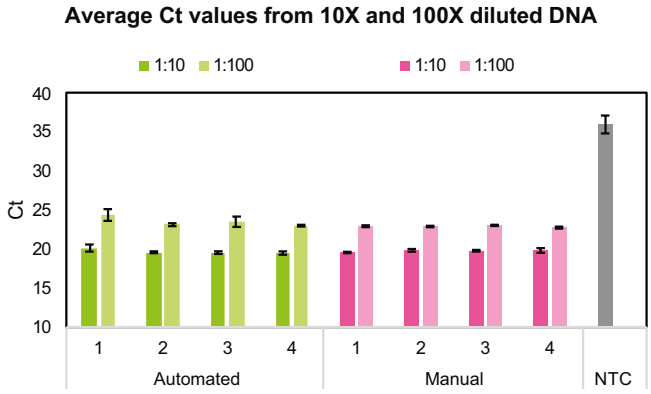


Figure 5: Average Ct values obtained from amplification of the purified DNA following the automated and manual protocols. Data corresponding to the first four samples are shown.

CONCLUSION

The combination of the Mag-Bind Blood & Tissue DNA HDQ 96 Kit and the Fluent 780 demonstrates an automated, high throughput example for the extraction and purification of gDNA from whole blood samples. The automated approach matches the quality of manually extracted DNA and, using this workflow, 96 250 µl blood samples can be processed in under 75 minutes. The deck configuration for this workflow has the capacity for four microplates, but this can easily be adapted and scaled up to different Fluent configurations according to liquid handling arm availability and size. The high molecular weight and quality of the purified DNA supports its use in various downstream applications and makes it particularly attractive for next generation sequencing technologies, including those by Pacific Biosciences and Oxford Nanopore that require long single-molecule DNA fragments.

ACKNOWLEDGEMENTS

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Kiranmai Durvasula (PhD) *is a Product Manager at Omega Bio-tek, Inc., leading the efforts to develop and deliver novel nucleic acid extraction products supporting the fields of basic and translation research, diagnostics, pharmacogenomics, etc. Previously, she served as Biological Scientist at Small Business Innovation Research (SBIR/STTR) grants-funded start-up (OpenCell Technologies Inc.) developing an innovative intracellular delivery and transfection device. Kiranmai obtained her PhD in Chemical Engineering from University of Florida, Gainesville, and received postdoctoral training in cell-based therapies for treatment of insulin-dependent diabetes in Dr Athanassios Sambanis's laboratory at Georgia Institute of Technology. She has authored several peer-reviewed journal papers and is passionate about translating fundamental research platforms into practical technologies.*

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