High Throughput, Automated DNA Extraction Solution from Whole Blood Samples Using Omega Bio-tek's Reagents on Tecan Fluent® 780 Workstation

Kiranmai Durvasula[1], Evan Wolfson[1], Julie Baggs[1], Travis Butts[1]
[1]Omega Bio-tek, Inc, Norcross GA 30071

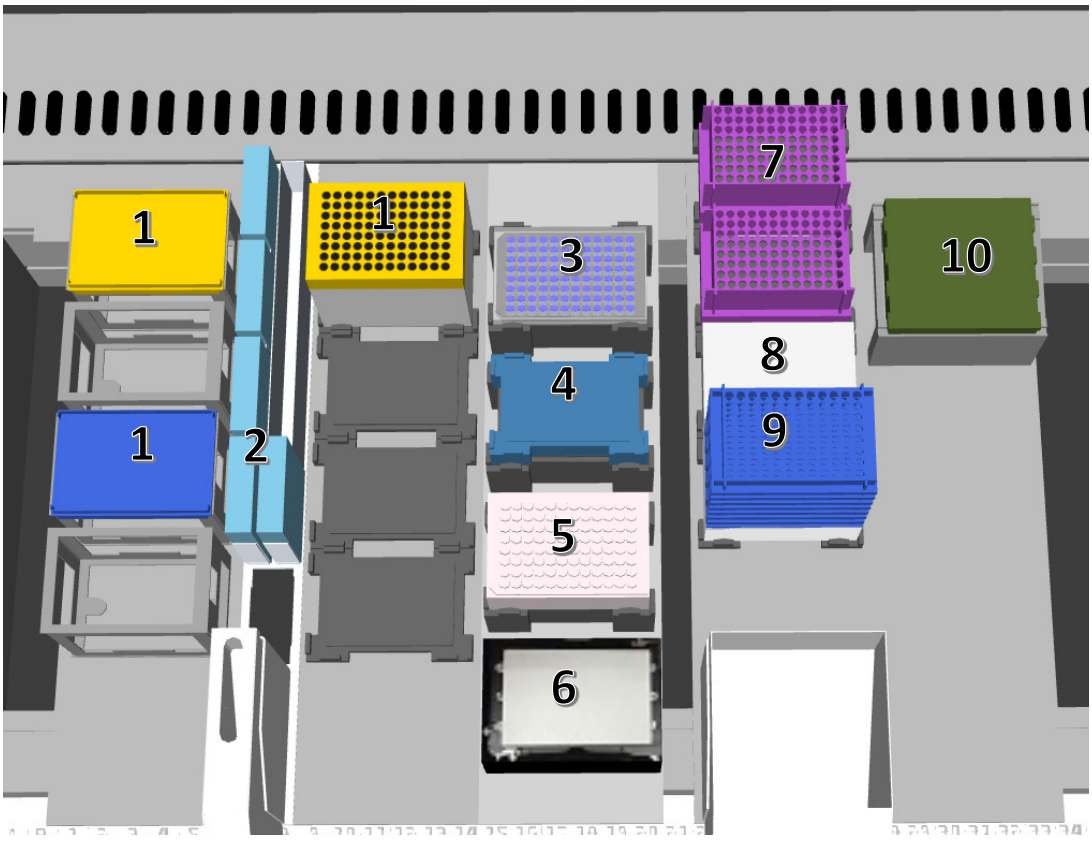
Introduction

Blood is the most common biospecimen used to obtain genomic DNA for use in many genomic-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 using their kit, Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399) on Tecan Fluent 780 workstation to extract DNA from 250 µL blood in a high throughput fashion with minimal manual intervention. In this application note, we present the automated solution along with validation studies to demonstrate the performance of the system. The performance of the automated system was evaluated based on how closely it represents the manual approach in terms of yield, purity, and integrity of DNA extracted. Our results indicate that this automated workflow is capable of extracting high-quality, high molecular weight DNA from ninety-six 250 µL whole blood samples in less than 75 minutes.

Materials and Methods

Eight 250 µL aliquots from the same lot of human whole blood were transferred to a 96-well deep well plate and moved to the Tecan Fluent 780 workstation for purification of DNA. The Tecan instrument was programmed to perform various liquid

Tecan Fluent 780 Example Deck Layout



1. 1000 µL & 200 µL DiTi Storage (Hanging or SBS)
2. Reagent Troughs
3. Standard 96-Well Elution Plate
4. Alpaqua Magnum FLX Plate Magnet
5. NUNC 96 Deep-Well Sample Plate
6. BioShake 3000-T elm Heater/ Shaker
7. 500 µL MCA96 Filtered DiTi
8. 300 mL Trough for Liquid Waste
9. 200 µL 8-stack Nested MCA96 DiTi
10. EVA Adapter

Figure 1. Tecan Fluent 780 deck layout for extraction of 96 blood samples with an input volume of 250 µL each.

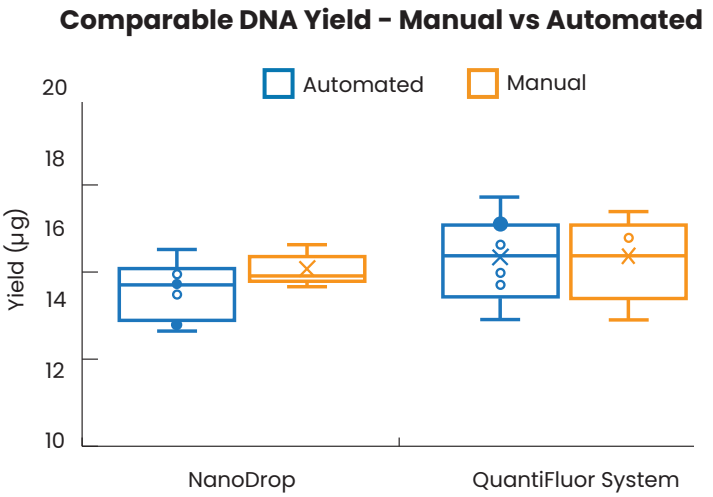


Figure 2. DNA was extracted from 250 µL of whole blood samples and was eluted in 100 µL volume. The DNA yield was determined using Thermo Scientific's NanoDrop 2000c and Promega's QuantiFluor dsDNA system. The average DNA yield from manual and automated extractions were found to be comparable and not significantly different *(Tukey's post-hoc analysis; p>0.05*).

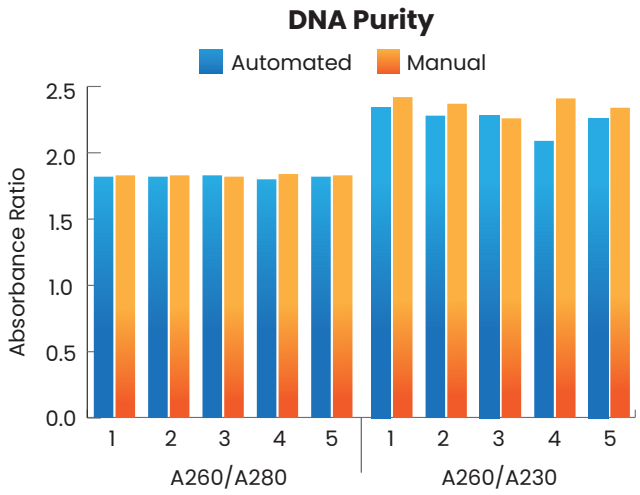


Figure 3. The purity of DNA isolated using manual and automated protocols was analyzed through spectrophotometry focusing on A260/A280 and A260/A230 ratios.

handling and magnetic bead-based tasks as demanded by the Mag-Bind Blood & Tissue DNA HDQ 96 protocol for the extraction of genomic DNA. DNA was eluted in 100 µL of 10 mM Tris-HCl (pH 8.5). All consumables and carriers were placed onto the Tecan deck configured as shown in Figure 1. The extraction workflow was fully automated starting with the sample aliquot in the 96-well deep well plate to final eluted product. Manual extraction from the same lot of human blood was performed in parallel and compared to validate the automated purification methodology and instrument set-up.

The purified DNA was quantified using Thermo Scientific's NanoDrop™ 2000c system and absorbance measurements were made at the wavelengths of 230 nm, 260 nm, and 280 nm to assess the quality of the purified DNA and to probe if there was any contaminating RNA/protein or salt carryover. Promega's QuantiFluor® dsDNA system was also employed to enable specific quantification of double-stranded DNA in the eluate without any interference from single-stranded DNA (ssDNA) and RNA. The size and integrity of the isolated genomic DNA was analyzed on Agilent's TapeStation® 2200 with a genomic DNA tape. The suitability of the extracted DNA for downstream applications were examined by performing real-time PCR using human-specific primers on 10-fold and 100-fold dilutions of the purified DNA. Agilent's Brilliant III 2X SYBR® mix was used as the master mix following a standard amplification protocol on Agilent AriaMx.

Results and Discussion

The DNA yields from the whole blood samples determined using the NanoDrop 2000c system as well as Promega's QuantiFluor System are as shown in Figure 2. The average DNA yield from manual extraction was found to be comparable and not significantly different (*Tukey's post-hoc analysis; p>0.05*) to the average DNA yield obtained following automated protocol. These results validate the instrument set-up and automated purification protocol. DNA purity and quality were analyzed looking at the A260/A280 and A260/A230 ratios obtained post spectrophotometric analysis (Figure 3). For both manual and automated protocols, the absorbance ratio of A260/A280 was consistently between 1.80-1.84 indicating pure DNA free of contaminating RNA and proteins (Figure 3; data corresponding to the first 5 samples shown). The A260/A230 ratios were all greater than 2.0 (Figure 3) following either of the protocols implying low contamination carryover. Both the ratios indicate high-quality DNA which is typically considered suitable for a variety of downstream applications. The purified DNA was also analyzed on TapeStation to derive information about the size and integrity of the genomic DNA extracted. DNA Integrity Number (DIN) was determined by the TapeStation 2200 analysis software and typically DNA with a DIN of 10 is considered intact and of the highest integrity. Figure 4 shows the TapeStation analysis performed on DNA extracted from the first three samples using automated and manual protocols. The purified DNA following automated or manual protocol is of high molecular weight and migrated as a well-defined band above the largest ladder peak (48,500 bp) with the software analyzing it to be > 60 kb. The DIN values following the automated protocol are 8.3, 8.5, 8.5 and following the manual protocol are 7.8, 7.9, 8.2 for the first three samples respectively (Figure 4). Overall, the DIN values are all >7.7 that suggests a highly intact DNA of superior quality regardless of the extraction methodology.

Real-time PCR was performed on a representative set of first four samples following manual extraction and automated extraction on Tecan Fluent 780 platform using human-specific primers. The average Ct value of the purified DNA diluted 10-fold and 100-fold are as shown in Figure 5. The Ct values across all the dilutions indicate positive amplification and were comparable irrespective of the extraction methodology adopted. The average ΔCt value between 100-fold and 10-fold for the manual and automated protocols were 3.14 ± 0.19 and 3.80 ± 0.35 respectively. Typically, Ct of the samples whose

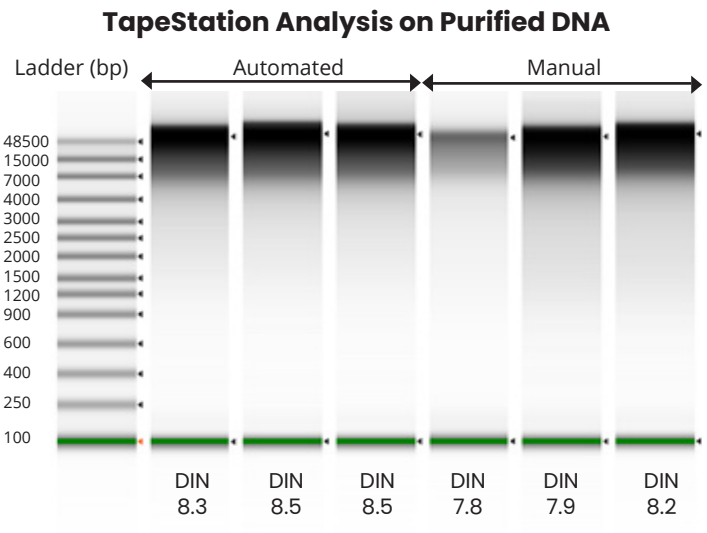


Figure 4. TapeStation analysis performed on the DNA extracted from 250 µL of blood following protocol automated on Tecan Fluent 780 workstation and protocol performed manually.

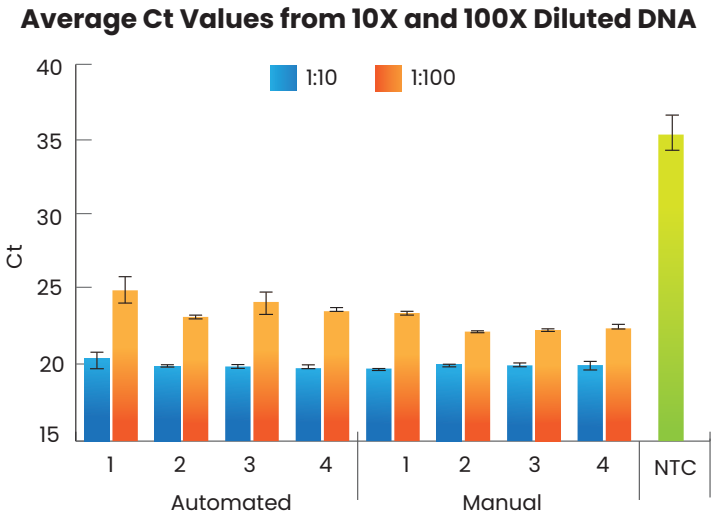


Figure 5. Average Ct values obtained amplifying the purified DNA following the automated and manual protocols using Omega Bio-tek's kit.

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 irrespective of the extraction approach.

Conclusions

Omega Bio-tek's Mag-Bind Blood & Tissue DNA HDQ 96 Kit (M6399) integrated onto Tecan Fluent 780 workstation offers an automated, high throughput purification solution for gDNA purification from whole blood samples. The automated approach matches the quality parameters of the DNA extracted following manual extraction and using this workflow, ninety-six 250 µL blood samples can be processed in less than 75 minutes. This deck configuration using this workflow has the capability to run 4 plates but can easily be adapted and scaled throughput wise to different Fluent configurations according to liquid handling arm availability and size. The high molecular weight and quality of the purified DNA substantiate its use in various downstream applications and make it particularly attractive for next-generation sequencing technologies including those by Pacific Biosciences and Oxford Nanopore that require long single-molecule DNA fragments.

Product Information

Product No.	Description
M6399-00	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (1 x 96 Preps)
M6399-01	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (4 x 96 Preps)

400 Pinnacle Way,
Suite 450
Norcross, GA 30071
www.omegabiotek.com
info@omegabiotek.com
(800) 832-8896