A Complete, Walk Away DNA Purification Solution from 10 mL Whole Blood using Omega Bio-tek's Reagents on the Hamilton Microlab® STAR™

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Introduction

With advances in genomics research, personalized medicine and sequencing-based technologies, there is a necessity for purification of high-quality genomic DNA from large volumes of blood. The rapidly growing landscape of biorepositories that store large amounts of DNA from an enormous number of biospecimens further fuels this need to find high-throughput solutions for reliable purification of DNA. The information derived from the purified DNA is crucial to health science research and facilitates drug discovery, biomarker discovery, clinical implementation projects etc. For the success of these analyses and to arrive at relevant information, DNA extraction is the most critical step and must meet the criteria of extraction speed, yield and quality, as well as reproducibility avoiding cross-contamination. There are several kits and automated workflows that allow high-throughput processing of blood samples with volumes in the range of 100-250 µL, but not many options exist for volumes as high as 10 mL. To address this challenge, Omega Bio-tek has developed a complete, automated solution on Hamilton's Microlab® STAR™ to extract DNA from large volumes of fresh or frozen blood in a high-throughut fashion with minimal manual intervention. The most attractive feature of this workflow is its scalability -- it can work with volumes as little as 250 µL to 10 mL with the same hardware configuration and no additional expensive accessories on the Hamilton platform. In this application note, we present the automated solution along with verification studies to demonstrate the performance of the automated system. The performance of the automated system will be evaluated on three parameters -- yield, purity and integrity.

Materials & Methods

DNA from twelve 10 mL whole human blood samples was extracted using Omega Bio-tek's Mag-Bind® Blood DNA HV Kit (M3292) on Hamilton's Microlab® STAR™. The tubes containing the whole blood were inserted into the sample carriers on the Hamilton deck (Figure 1). The automated protocol begins with the initial blood sample being split into two 5 mL aliquots and dispensed into identical wells of two 24-well deep well blocks (HJ-Bioanalytik GmbH, Germany) which can accomodate a volume of 25 mL/well. The Hamilton STAR™ was programmed to perform various liquid handling and magnetic bead-based tasks as demanded by the Mag-Bind® Blood DNA HV protocol for the extraction of genomic DNA. The special 24-well deep block with a 25 mL volume allowance accomodated the large volumes of lysis and binding buffers as needed by the input sample amount. Post lysis and binding steps, the lysate was transferred into a standard 24-well deep well plate. Clickbio's XBase 24 magnet compatible with the 24-well deep well plate was mounted on the deck and used for the various magnetization steps. The lyse-bind-wash steps were all performed in the same standard 24-well deep well plate. After the wash steps, the beads were subjected to a quick nuclease-free water wash to remove the residual ethanol. DNA was eluted in 2 mL of 10 mM Tris-HCl (pH 8.5). The extraction workflow is fully automated starting with whole blood to final eluted product with minimal user intervention.

The extracted DNA from 12 samples was quantified using Thermo Scientific's NanoDrop™ 2000c system. Absorbance measurements were made at the wavelengths of 230 nm, 260

Hamilton Microlab® STAR™ Deck Layout

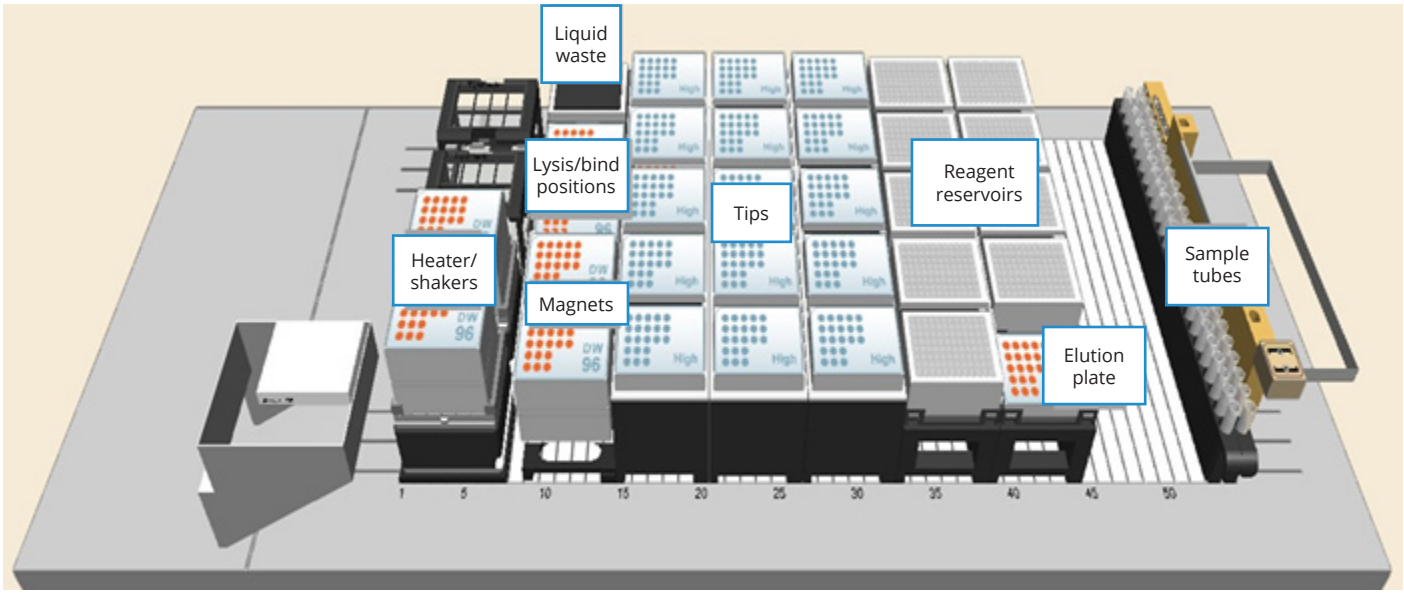

Figure 1. Deck layout on Hamilton Microlab® STAR™.

nm and 280 nm to assess the quality of the purified DNA and to probe if there was any contaminating RNA/protein or any salt carryover. DNA isolated was also analyzed on Agilent's TapeStation® 2200 by running a genomic DNA tape. This was performed to shed light on not only the size of the genomic DNA extracted but also on its integrity. TapeStation® 2200 analysis software evaluates the DNA integrity and it was displayed as the DNA Integrity Number (DIN).

The suitability of the extracted DNA for downstream applications was examined by performing real-time PCR using human-specific primers on undiluted, 10-fold and 100-fold dilutions of the purified DNA. Briefly, a qPCR reaction was set up to a total volume of 20 µL using Agilent's Brilliant III 2X SYBR® as the master mix and 2 µL of purified DNA at appropriate dilution as template with suitable primers following a standard amplification protocol on the ABI 7900.

DNA Yield & Quality

Table 1. DNA was extracted from 10 mL of whole blood samples and was eluted in 2 mL volume. The DNA yield was determined using Thermo Scientific's NanoDrop™ 2000c and Promega's QuantiFluor® dsDNA system. The absorbance at different wavelengths was determined by NanoDrop™ spectrophotometer.

Sample ID	Yield (µg) by NanoDrop™	A_{260}/A_{280}
1	256.2	1.84
2	260.4	1.84
3	257	1.84
4	264.4	1.84
5	249.2	1.85
6	257.4	1.84
7	247.8	1.83
8	250	1.82
9	232	1.82
10	241.2	1.82
11	251.8	1.84
12	254.4	1.84

Results & Discussion

The DNA yields from the human blood samples determined using the NanoDrop™ spectrophotometer was as shown in Table 1. The yields were consistently over 250 µg and in the range of 200-400 µg from normal 10 mL samples as reported by Krueger et al. (2011) using Promega's ReliaPrep Large Volume HT gDNA Isolation System. The absorbance ratio, A260/A280 was consistently between 1.82-1.85 indicating pure DNA free of contaminating RNA and proteins. The A260/A230 ratios were all greater than 2.0 (not shown) implying low contamination carryover. Both the ratios point to 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 of the genomic DNA that was extracted as well as its integrity. DNA Integrity Number (DIN) was also automatically calculated by the TapeStation® 2200 analysis software. It typically ranges from 0 to 10 and determined based on the fragmentation of genomic DNA sample. The sample with a DIN of 10 is of highest integrity and is considered intact. Figure 2 shows the TapeStation® analysis performed on the extracted DNA from the whole blood samples. The purified DNA across the 12 samples migrated as well as a well-defined band above the largest ladder peak (48,500 bp) and the software analyzed it to be over > 60 kb. The DIN values ranged between 8.6-9.2 and suggest highly intact DNA with no degradation.

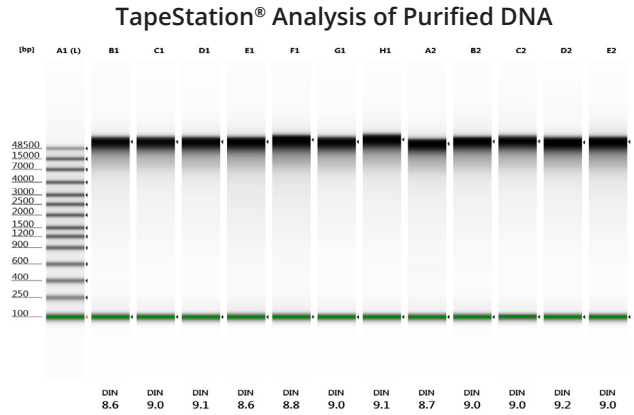

Figure 2. TapeStation® analysis of genomic DNA purified from 10 mL of whole blood using Omega Bio-tek's Mag-Bind® Blood & Tissue DNA HV Kit (M6329) automated on Hamilton's Microlab® STAR™.

Real-time PCR was performed on a representative sample set of 6 using human-specific primers. The average C_t of undiluted, 10-fold and 100-fold are as listed in Figure 3. There was no detectable fluorescence in the no template control wells. The C_t values across all the dilutions indicate positive amplification. Typically, C_t of the samples whose concentration differs by a factor of 10 are ~3.3 cycles apart. The average ΔC_t value between 10-fold and undiluted and between 100-fold and 10-fold was ~2.4 and ~3.3 respectively. The average ΔC_t value between 10-fold and undiluted suggests inhibition which could very well be because of the high concentration of the template DNA in the qPCR reaction. Template inhibition is a well-known problem in

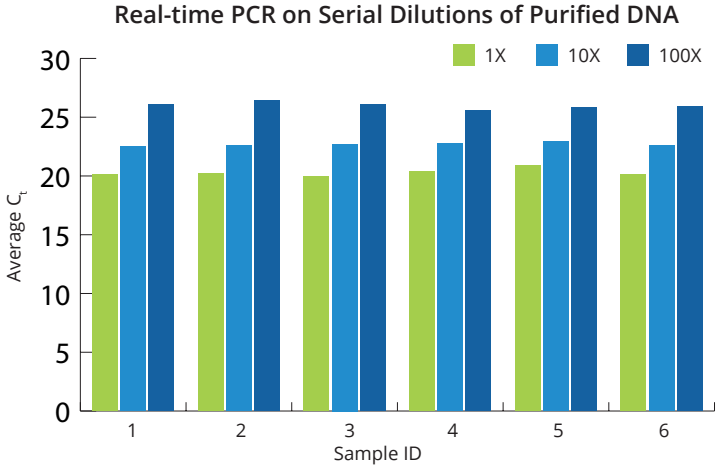

Figure 3. Average C_t values obtained using human-specific primers on 1X, 10X, and 100X dilution of purified DNA used as the template. Sample IDs 1-6 are represented here.

qPCR and is inhibitory. As expected, this problem disappeared at higher dilution with ΔC_t between 100-fold and 10-fold being ~3.3 indicating good PCR efficiency.

Conclusions

The combination of Omega Bio-tek's Mag-Bind® Blood DNA HV Kit integrated onto Hamilton's Microlab® STAR™ offers a fully automated, high-throughput purification solution of gDNA from large volume samples. Using this workflow, twenty-four 10 mL blood samples can be processed in 2.5 hours. This system is scalable (250 µL-10 mL) and by dialing down the initial sample volume, the throughput can be increased. For a 5 mL initial sample volume, the system is capable of processing 48 samples in the same 2.5 hours, essentially doubling the throughput. The same solution can be translated to other large volume sample types such as from 4 mL saliva and other body fluids. The robust performance of this automated workflow ensures high molecular weight DNA extraction, making it attractive for third-generation sequencing technologies such as those produced by Pacific Biosciences and Oxford Nanopore, that require longer single molecule DNA fragments. The purified DNA was of significantly high quality, suitable for demanding downstream applications and biorepository needs.

Ordering Information

Description	Product No.	Preps
Mag-Bind® Blood DNA HV Kit	M3292-00	1 x 24
	M3292-01	4 x 24

References

Krueger, S., Kennedy, J., Lee, S., Helt, C., Bonk, A., Cowan, C. & Vincent, E. (2011). Automated Isolation of Genomic DNA from Large Volumes of Whole Blood. Journal of Biomolecular Techniques: JBT, 22(Suppl), S30.

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