Automated Circulating Cell-Free DNA Extraction from 8 mL Sample Volumes

Kiranmai Durvasula[1], Jeff Roeder[1], Chris Lee[1], Travis Butts[1]
[1]Omega Bio-tek, Inc. Norcross, GA 30071

Introduction

Circulating cell-free DNA (cfDNA) holds great clinical significance for non-invasive disease detection, diagnosis and monitoring. cfDNA are usually small fragments of DNA (size distribution peaking at ~170 bp) found circulating in plasma, serum, or other bodily fluids and offers a tremendous potential as a screening method for tumors, cancer, as well as in fetal DNA studies. Circulating DNA extractions are found in low quantities and often require large sample input volumes to isolate the less abundant cfDNA to sufficient amounts needed for downstream analysis. The existing methods are often silica spin column-based, which do not lend themselves to automation. Also, these methods require large volumes of binding buffers making it unsuitable for extractions involving sample volumes as high as 8 mL. These existing workflows impose certain limitations in terms of the sample volume and number of samples that they can process. Automated solutions to process sample volumes greater than 1 mL in a high throughput fashion are far and few. To tackle this necessity, Omega Bio-tek has developed a fully automated solution to extract cfDNA from 8 mL volumes from 48 samples in 2.5 hours when integrated on Hamilton's Microlab® STAR™ platform. The system is scalable and can extract from sample volumes ranging from 1-8 mL without any hardware modification or additional expensive accessories on the Hamilton workstation. The system supports low elution volumes to purify concentration cfDNA suitable for a variety of downstream applications such as qPCR, next-generation sequencing, etc. In this application note, we elucidate the workings of the automated protocol using Omega Bio-tek's Mag-Bind® cfDNA Kit (M3298) and compare its performance to that of an existing extraction protocol from 4 mL of plasma when processed manually using the same kit.

Materials & Methods

Circulating cell-free DNA was extracted manually from four 4 mL unspiked plasma samples using Omega Bio-tek's Mag-Bind® cfDNA Kit following manufacturer's instructions. Conversely, an automated approach was adopted for extracting cfDNA from four 8 mL unspiked plasma samples on the Hamilton Microlab® STAR™ using the same Omega Bio-tek kit. For the automated protocol, the tubes containing the plasma sample were first inserted into the sample carriers on the Hamilton deck (Figure 1). The automated protocol begins with 8 mL of plasma sample being dispensed into a 24-well deep well block (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® cfDNA protocol. The special 24-well deep well 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 liquid handler transfers the lysate into a standard 24-well deep well plate for the subsequent steps. Clickbio's XBase 24 magnet compatible with the 24-well deep well plate was mounted on the deck and used for various magnetization steps. The lyse-bind-wash steps were all performed in the same standard 24-well deep well plate. For both manual and automated extraction methods, the beads were air dried for 15 minutes post-wash steps to remove the residual ethanol and the cfDNA was eluted in 100 µL of 10 mM Tris-HCl (pH 8.5). The extraction workflow is fully automated starting with the plasma sample to the final eluted cfDNA product with minimal user intervention. cfDNA purified using both the approaches were analyzed on Agilent's TapeStation® 2200 to compare the fraction of cfDNA that was extracted and to test the efficacy of the automated protocol.

Hamilton's Microlab® STAR™ Deck Layout

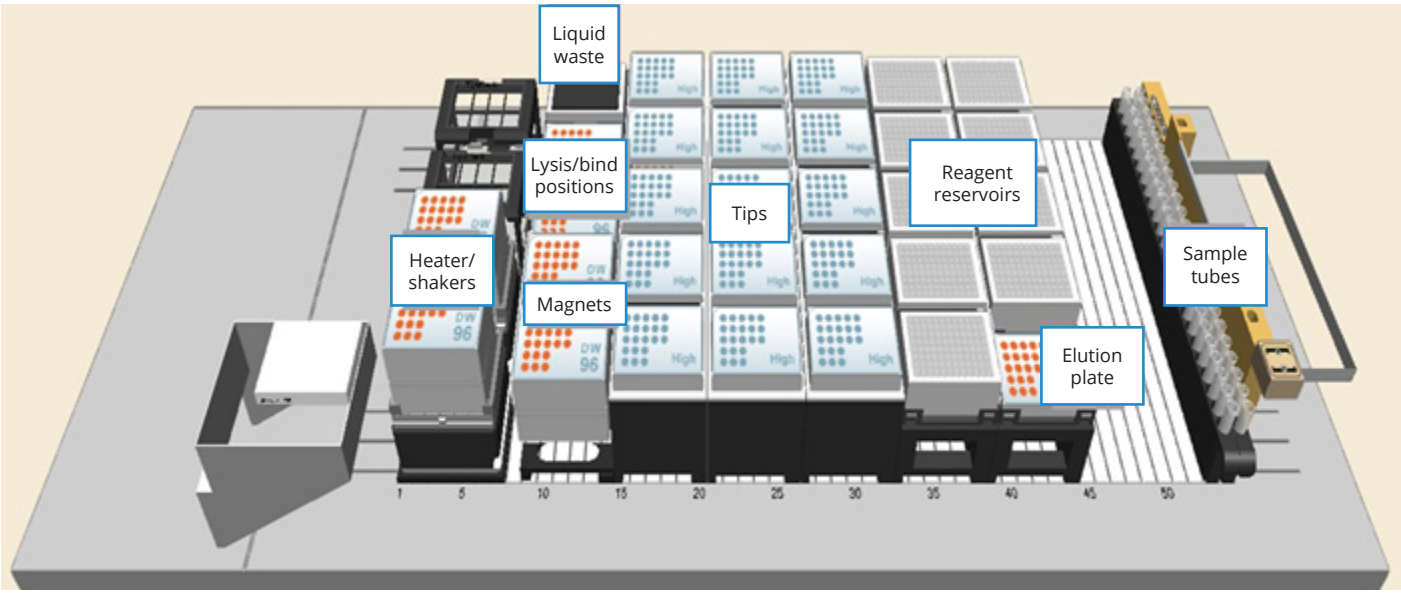

Figure 1. Deck layout on Hamilton Microlab® STAR™.

TapeStation® Analysis of Purified cfDNA from Unspiked Plasma Samples

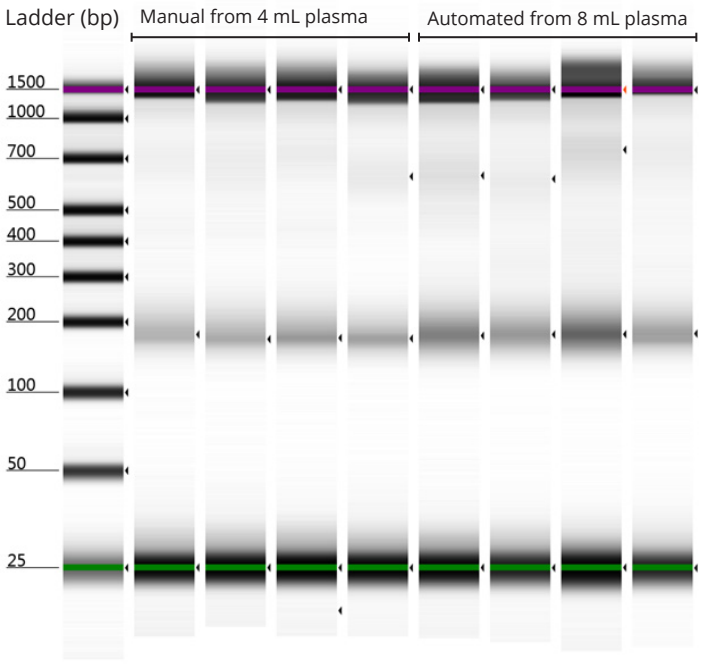

Figure 2. TapeStation® analysis of cfDNA purified from either 4 mL or 8 mL of unspiked plasma samples following manual and automated protocols using Omega Bio-tek's Mag-Bind® cfDNA Kit (M3298).

Results & Discussion

Figure 2 shows the TapeStation® analysis of cfDNA purified using manual and automated methods using the same Omega Bio-tek kit. The TapeStation® results indicate that both the approaches could capture the circulating, cell-free DNA with little to no genomic DNA contamination. To arrive at the cfDNA concentration without any interference from the genomic DNA, we utilized the regional analysis functionality of the TapeStation® 2200 Analysis Software.

Determination of cfDNA Concentration Ranging from 100-300 bp Region Using TapeStation® 2200 Analysis Software

Table 1. The cfDNA region (100-300 bp) was examined using the regional analysis functionality of the TapeStation® 2200 Analysis Software to arrive at the cfDNA concentration excluding any genomic DNA that might be present.

Extraction Approach	Sample ID	Average cfDNA Peak Size (bp)	100-300 bp Region Concentration (pg/µL)
Manual	1	196	87.7
	2	192	88.1
	3	192	94.3
	4	186	88.8
Automated	1	192	143
	2	194	148
	3	193	281
	4	195	175

Table 1 shows the DNA concentration quantified using the software within the 100-300 bp region where cfDNA was most likely present. The average cfDNA peak following manual extraction was comparable to that of automated extraction (~192 bp vs. ~194 bp) (Table 1). Also, the average cfDNA yield roughly doubled with double the sample input volume (4 mL for manual vs. 8 mL for automated). It was ~187 pg/µL using the automted method vs. ~90 pg/µL when processed manually (Table 1). A representative electropherogram overlay of the cfDNA purified using both the approaches also reinforces this finding (Figure 3). The cfDNA peak from 8 mL unspiked plasma was at a sample intensity of ~200 FU compared to ~100 FU from 4 mL unspiked plasma, indicating a two-fold increase in cfDNA yield with the automated approach. The TapeStation® analysis proves the efficacy of the automated approach and indicates that cfDNA purified was of sufficiently high quality and suitable for various demanding downstream applications.

Electropherogram Overlay of cfDNA Purified Following Manual or Automated Approaches

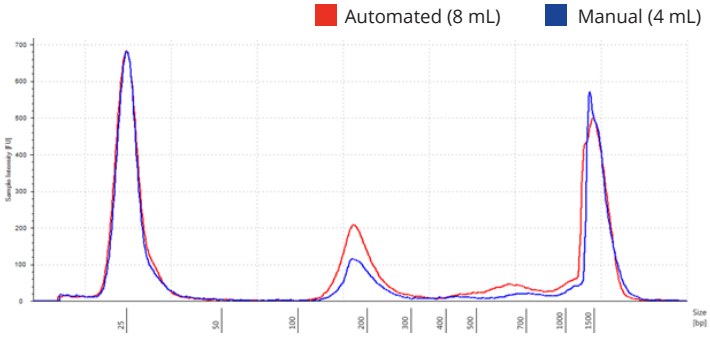

Figure 3. 4 mL and 8 mL of unspiked serum was purified following manual or automated methods using Omega Bio-tek's Mag-Bind® cfDNA Kit (M3298). Purified DNA was analyzed on Agilent's TapeStation® 2200.

Conclusions

Omega Bio-tek's automated cell-free DNA purification method provides excellent performance from 8 mL sample input volumes with minimal genomic DNA contamination. Using this fully automated workflow, forty-eight 8 mL samples can be processed in 2.5 hours using a Hamilton Microlab® STAR™ liquid handler. Omega Bio-tek's Mag-Bind® cfDNA Kit , which uses magnetic bead-based purification technology, can be automated on most open-ended liquid handling platforms.

Ordering Information

Description	Product No.	Preps
Mag-Bind® cfDNA Kit	M3298-00	5
	M3298-01	50
	M3298-02	200

400 Pinnacle Way Suite 450
Nocross, GA 30071
(800) 832-8896

www.omegabiotek.com
info@omegabiotek.com