HAMILTON

Automated Circulating Cell-Free DNA Extraction from Large Sample Volumes

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Introduction

Circulating cell-free DNA (cfDNA) has a small fragment size (distribution peak of approximately 170 bp), and is typically found circulating in plasma, serum, or other body fluids. This type of DNA is clinically important for the non-invasive detection, diagnosis, and monitoring of disease. In fact, it holds tremendous potential in the screening of tumors, cancer, and some fetal genetic abnormalities. Despite its promise, however, cfDNA is present in relatively low quantities throughout the body. Therefore, large sample volumes are often required in order to obtain enough genetic material for downstream analysis. Moreover, some isolation methods, such as those that employ silica spin columns, generally require large buffer volumes. This limitation, when combined with already high sample volumes, create conditions that are often unsuitable for automation. If an isolation protocol does allow for automated manipulation of greater volumes, then sample throughput is oftentimes limited. Protocols based on manual workflows are likewise limited in sample throughput and they invite the risk of performance variability.

Here, we report an automated method to isolate cfDNA using a magnetic particle-based assay workflow (see Workflow at end) in a high throughput format while accommodating volume requirements, and compare this method to a manual method. The automated method combines the Omega Bio-tek Mag-Bind® cfDNA Kit on a Hamilton Microlab® STAR™ automated liquid handling workstation. The Microlab STAR contains all necessary peripheral devices needed for the assay (see Deck Layout at end) and was previously pre-programmed and optimized for use with the Mag-Bind cfDNA Kit. This system is capable of processing up to 48 samples at once in as little as 2.5 hours. It also accepts samples in volumes ranging from 1-8 mL. The system yields cfDNA in elution volumes that are suitable for a variety of downstream applications such as qPCR and next-generation sequencing. We show that the automated method offers excellent performance with minimal genomic DNA contamination, with results equivalent to those obtained when performing the isolation protocol manually.

Benefits-Based Highlights

- Automate circulating cell-free DNA sample extractions in volumes ranging from 1 to 8 mL.
- Process up to 48 samples in approximately 2.5 hours.
- Eliminate time-consuming and repetitive manual pipetting.
- Reduce or eliminate risks of human error and variability to ensure consistency and quality of downstream results.

Materials and Methods

Plasma Samples

Plasma samples were obtained commercially from Innovative Research, Novi, MI (P/N IPLAK3E500ML and IPLAK3E1000ML).

Manual cfDNA Isolation

Circulating cell-free DNA was isolated from four 4 mL plasma samples using Omega Bio-tek's Mag-Bind cfDNA Kit (P/N M3298) following the manufacturer's standard manual protocol. Briefly, 60 µL proteinase K and 270 µL DS buffer included in the kit were added to 15 mL conical tubes, each containing 4 mL plasma sample. Sample tubes were vortexed briefly at maximum speed, and then incubated at 60 °C for 30 minutes with mixing by inversion every 10 minutes. Samples were then incubated at room temperature for an additional 10 minutes. JSB buffer, in a volume of 4 mL, was added to each tube, and the samples were vortexed briefly at maximum speed. Upon complete mixing of the tube contents, 20 µL of Mag-Bind particles were added, and the samples were incubated for 10 minutes at room temperature with continuous slow mixing on a rocking platform to allow binding of the magnetic particles to the DNA.

At the end of the incubation period, tubes were placed on a magnetic separation device until the Mag-Bind® particles completely cleared from solution and were held to the side of the tubes. Cleared supernatant was aspirated and discarded from the sample tubes, after which 1 mL GT7 buffer was added to each. Tubes were vortexed for 5 minutes at maximum speed to completely resuspend the particles.

The suspensions were then each transferred to separate 1.5 mL microtubes that were placed on the magnetic separation device until the particles completely cleared from the solution and were held to the side of the tubes. After the solution was cleared of the particles, supernatant was aspirated and discarded, the tubes were removed from the magnetic separation device, and 1 mL GT7 buffer was added to each tube.

The tubes were vortexed for 5 minutes, placed on the magnetic separation device as previously described to affix the magnetic particles, and supernatant was aspirated and discarded.

After removing the tubes containing particles from the magnetic separation device, 1 mL prepared SPW buffer was added to each, and the tubes were vortexed at approximately 3,000 RPM for 5 minutes to resuspend the particles. The tubes were again placed on the magnetic separation device to affix the magnetic particles, and the cleared supernatant was aspirated and discarded. The tubes were removed from the magnetic separation device, and the washing process was repeated starting with the addition of 1 mL prepared SPW buffer.

Once the second SPW buffer wash step was completed, the tubes were incubated on the magnetic separation stand at room temperature for 25 minutes to dry the Mag-Bind particles, after which 100 µL Elution Buffer was added to each tube. The tubes were then vortexed at approximately 3,000 RPM for 5 minutes to ensure thorough particle suspension.

Finally, the tubes were returned to the magnetic separation device and incubated at room temperature until all particles were retained on the tube sides, then the cleared supernatants containing purified cfDNA were aspirated and transferred to new 1.5 mL microtubes.

Automated cfDNA Isolation

The Mag-Bind cfDNA Kit was also used in the automated workflow in order to compare it with the manual method. The Microlab STAR integrated all necessary tools, including the XBASE24 24-position magnetic device (P/N XBASE24, Clickbio, Reno, NV) and was programmed and optimized for the Mag-Bind cfDNA Kit workflow. Here, four tubes each containing 8 mL of plasma were inserted into sample carriers on the Microlab STAR deck, after which 8 mL of each plasma sample were automatically aspirated from the original

tubes and dispensed into separate wells in a 24-well deep well microplate (P/N 750 250, HJ-Bioanalytik GmbH, Erkelenz, Germany). Each well in the microplate had a 25 mL capacity limit. All lysis and binding steps were performed automatically by the liquid handler, and lysates from the microplate wells were automatically transferred to separate wells of a standard 24-well deep well plate (Axygen/Corning, Corning, NY; P/N P-DW-10ML-24-C) for subsequent steps.

The wash and elute steps were all performed in the same standard 24-well deep well plate. Magnetic particles were incubated on the magnetic device at room temperature for 25 minutes to dry, and 100 µL Elution Buffer was added to each well to subsequently elute the purified cfDNA.

Pure cfDNA Sample Analysis

Manually and automatically purified cfDNA samples were electrophoretically analyzed on the TapeStation® 2200 (Agilent Technologies, Santa Clara, CA). Fragment size profiling was used for cfDNA quantification.

Results and Discussion

cfDNA Purity Assessment

Results from plasma samples processed using the Mag-Bind cfDNA Kit in either a manual or automated workflow and analyzed via electrophoresis on the TapeStation 2200 were compared. Results indicate that both approaches captured cfDNA with little to no genomic DNA contamination (Figure 1).

cfDNA Yield Assessment

The TapeStation 2200 Analysis Software's regional analysis functionality was used to determine cfDNA concentration within the expected 100-300 base pair region without interference from any present genomic DNA. The peak heights and separation on the electropherogram corresponding to cfDNA fragment and gDNA sizes can shed light on the relative proportions of each, and can help to draw conclusions about cfDNA extraction efficiency.

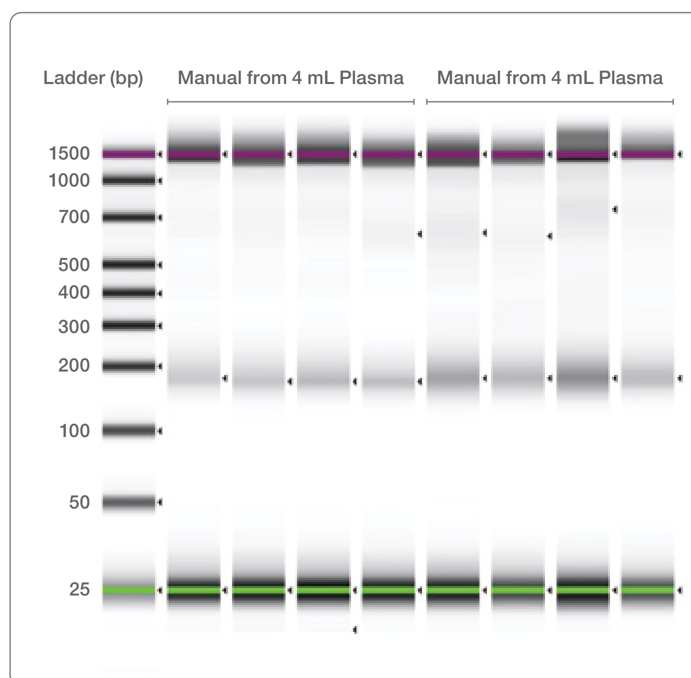


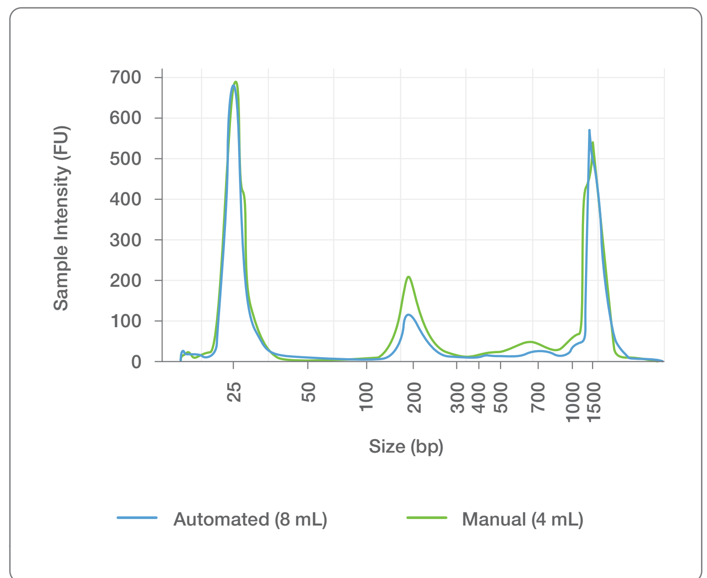
Figure 1. TapeStation analysis of cfDNA purified from 4 mL (manual protocol) or 8 mL (automated protocol) of un¬spiked plasma samples using Omega Bio-tek's Mag-Bind cfDNA Kit.

Table 1: The TapeStation 2200 Analysis Software's Regional Analysis Functionality at 100-300 bp was Used to Arrive at the cfDNA Concentration Excluding Any Potential Genomic DNA.

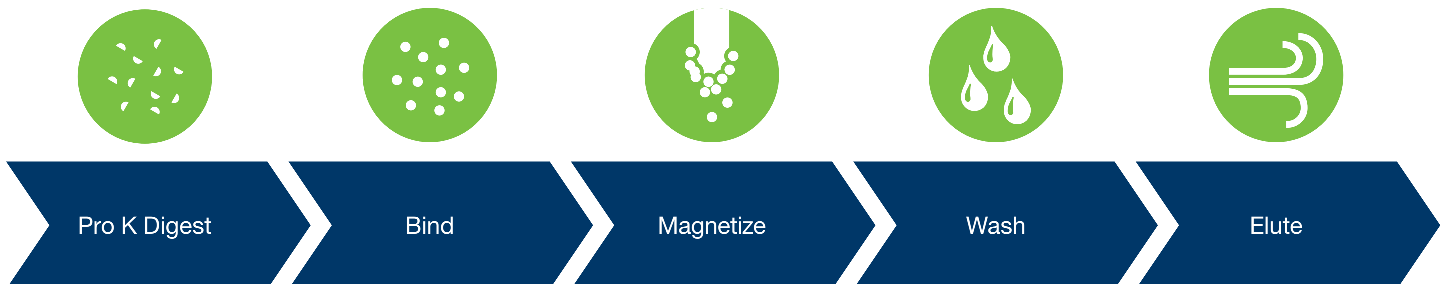
Isolation Approach	Sample ID	Average cfDNA Peak Size (bp)	100-300 bp Region Concentration
Manual	1	196	87.7
	2	192	88.1
	3	192	94.3
	4	186	88.8
Automated	1	192	143
	2	194	148
	3	193	281
	4	195	175

Average cfDNA peak sizes and 100-300 bp region concentrations are listed in Table 1. The manual and automated methods yielded comparable average cfDNA peak sizes (approximately 192 bp and 194 bp, respectively). At the same time, the average cfDNA yield of the automated method, using 8 mL of starting sample volume, was roughly twice that of the manual method, using 4 mL of starting sample volume (approximately 187 pg/µL and 90 pg/µL, respectively).

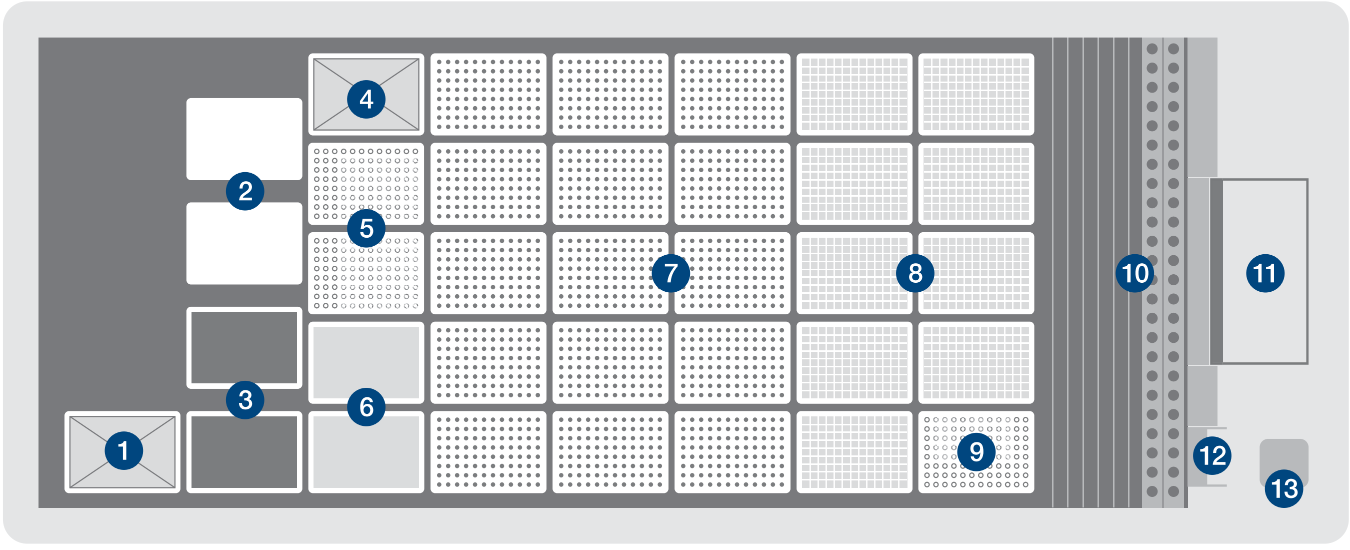
These findings are reinforced in a representative electropherogram overlay of the purified cfDNA (Figure 2). Here, the automated method sample intensity was two-fold higher compared to the manual method sample intensity (approximately 200 FU and 100 FU, respectively). This confirms efficacy of the automated approach, and also indicates that the automatic method produced high quality, pure cfDNA that is suitable for a variety of challenging downstream applications.



Automated Mag-Bind cfDNA Workflow



Microlab STAR deck layout for the Mag-Bind cfDNA assay



1. 96 Multi-Probe Head Waste
2. Expansion Positions
3. Heater Shaker
4. Liquid Waste
5. 96-Well PCR Plates
6. Plate Magnets
7. 300 µL Conductive Tips
8. 300 mL ANSI/SLAS Reagent Troughs
9. 96-Well PCR Plates
10. Microtubes
11. Channel Waste
12. CO-RE® Grippers
13. Autoload

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