Automated Cell-Free DNA Purification from 4 mL Plasma with the Dynamic Devices Lynx®

Purify cell-free DNA from 4 mL plasma with Omega Bio-tek's Mag-Bind® cfDNA Kit (M3298) on the Dynamic Devices Lynx® robotic liquid handler.

Kit

Mag-Bind® cfDNA Kit (M3298)

Sample Type

Plasma collected in Streck Cell-Free DNA BCT

Sample Amount

4 mL

Analysis

qPCR, TapeStation®

Materials

Mag-Bind® cfDNA Kit (M3298)
Dynamic Devices Lynx® LM1200 Robotic Liquid Handler

- 24ST Pipetting Tool
- 96 MagRod Tool
- 4x iMagZ Magnet Elevators
- Passive Waste Block
- Source Tube Holders

Alpaqua FLX HV 24 Magnet (Alpaqua, Cat.# A000640)

For a complete list of system components and plastic consumables on Dynamic Devices Lynx®, please contact us at automation@omegabiotek.com

Methods

Automated method developed by Omega Bio-tek for the Dynamic Devices Lynx®
96 samples extracted in ~3 hours

innovations in nucleic acid isolation

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Reagent Setup & Deck Layout

Reagent Container	Reagent	Volume per Sample	Volume Required for 96 Samples†
E-Z Select® Robotic Reservoir, 600 mL	JSB Binding Buffer + Mag-Bind® Particles CH‡	4 mL + 20 µL	420 mL + 2.1 mL
4 Column Trough (position 3)	Proteinase K	60 µL	6.5 mL
4 Column Trough (position 4)	DS Buffer	270 µL	34 mL
E-Z Select® Robotic Reservoir, 600 mL	GT7 Wash Buffer	2 mL	125 mL
E-Z Select® Robotic Reservoir, 600 mL	SPW Wash Buffer	2 mL	125 mL
300 mL Reservoir	Elution Buffer	50-100 µL	60 mL

†Indicated volumes include required dead volume for reagent troughs.
‡To be prepared as a master mix.



Position	Hardware/Consumable
A1	24 Tip 5 mL Pipette Core
A3	Empty 5 mL tip rack
A4, A5, A6, B3, B4, B5, B6	DDX 24 5 mL Tips Filtered (Dynamic Devices, Cat.# DDX-24-5000F-1)
C3, C4, C5, C6	Sample Input Tubes
D1	Passive Waste Block with No-Bottom Microplate
D3, D4, D5, D6	24 MagRod Well Plate (Dynamic Devices, Cat.# MKS-24-DWP-1)
E2	Proteinase K + DS Buffer
E3	JSB Binding Buffer + Mag-Bind® Particles CH
E4	GT7 Wash Buffer
E5	SPW Wash Buffer
E6	Elution Buffer
G1	96 MagRod Tool
G2, G3, G4, G5	96 MagRod Well Plate (Dynamic Devices, Cat.# MKS-96-DWP-1)
G6	96 Microplate

Figure 1. Illustration of the deck layout for the Dynamic Devices Lynx® LM1200 configured for cfDNA extraction from 4 mL plasma.

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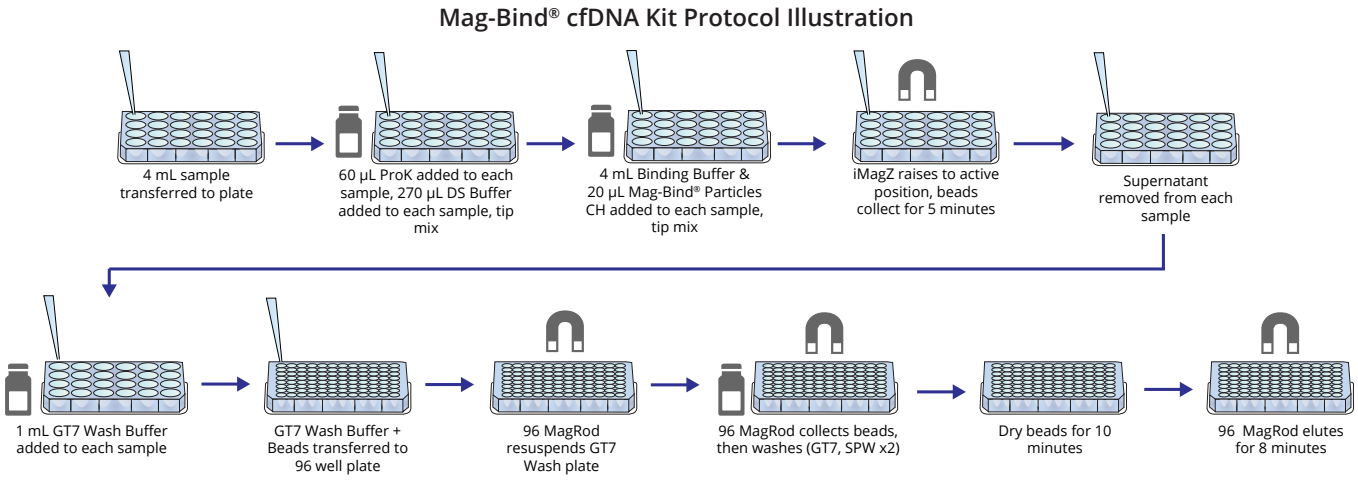


Figure 2. Workflow illustration of cfDNA extraction using the Mag-Bind® cfDNA Kit (M3298) automated on the Dynamic Devices Lynx® LM1200.

Methods

The workflow and protocol described above (**Figures 1 & 2**) were used to purify cell-free DNA from 4 mL of plasma using the Dynamic Devices Lynx® LM1200. Purified cfDNA was quantified via TapeStation® analysis and subjected to qPCR. Results were compared to a control which followed the same protocol manually. In addition, cfDNA was purified from 4 mL plasma (n=4) across four plates to evaluate reproducibility across multiple plates. This purified cfDNA was also quantified via TapeStation® analysis.

Results

cfDNA was purified from 4 mL plasma samples and quantified via Agilent's TapeStation® 4150 (**Figure 3**). As expected, yields and integrity between the manual and automated protocols were comparable.

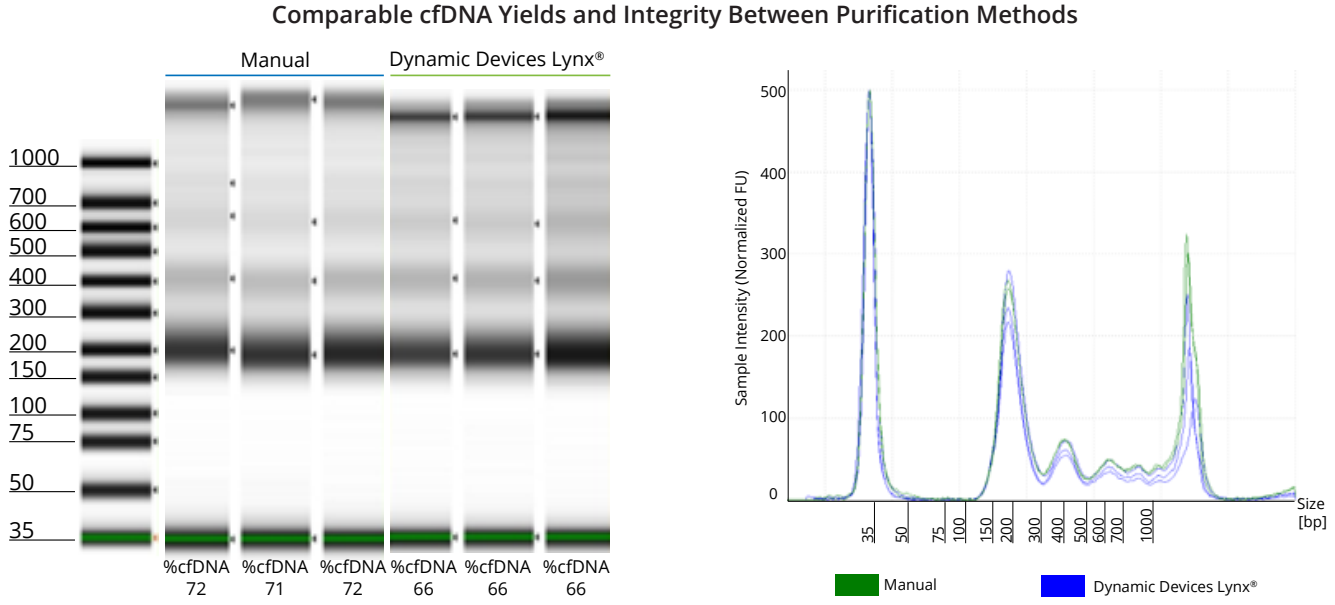


Figure 3. TapeStation® analysis illustrates comparable cfDNA yields and integrity between automated and manual purification methods.

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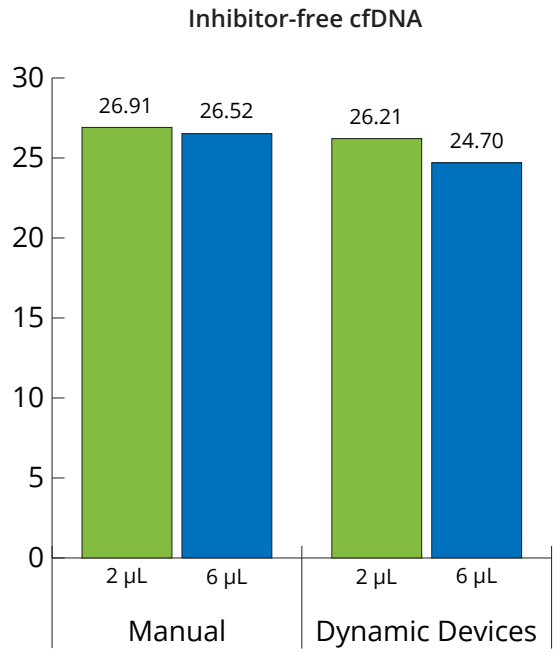


Figure 4. Ct values obtained from qPCR analysis of 2µL and 6 µL amounts of purified cfDNA. The ΔCt of the manual protocol was 0.39, and the ΔCt of the Dynamic Devices protocol was 1.51.

qPCR analysis was also performed on purified cfDNA using 2 µL and 6 µL input amounts of template DNA to evaluate the presence of PCR inhibitors. The average ΔCt between the input amounts of manually purified cfDNA was below the expected 1.5 value, indicating the presence of inhibitors, whereas the average ΔCt between inputs of automated samples aligned with the expected value of 1.5, indicating the samples were pure and contained minimal traces of inhibitors (**Figure 4**).

Subsequently, cfDNA was purified from 4 mL plasma samples (n=4) using the same protocol, but the samples were extracted across four plates to evaluate consistency. cfDNA was again quantified via TapeStation® and showed consistent, reproducible results across all four plates tested (**Table 1**). Integrity analysis via TapeStation also reveals comparable dark bands of cfDNA in the targeted region between 50 - 700 bp for each sample on all four plates (**Figure 5**).

Table 1. Yields of cfDNA across four plates purified using the Dynamic Devices Lynx®

Sample ID	% cfDNA	50-700 bp Nucleic Acid Concentration (pg/µL)	Yield (ng)	Average Yield (ng)
Plate 1	58	354	24.78	25.41
	59	411	28.77	
	59	353	24.71	
	58	334	23.38	
Plate 2	59	306	21.42	22.35
	59	297	20.79	
	59	349	24.43	
	56	325	22.75	
Plate 3	56	275	19.25	23.84
	58	407	28.49	
	59	382	26.74	
	54	298	20.86	
Plate 4	60	424	29.68	28.70
	58	466	32.62	
	59	418	29.26	
	59	332	23.24	

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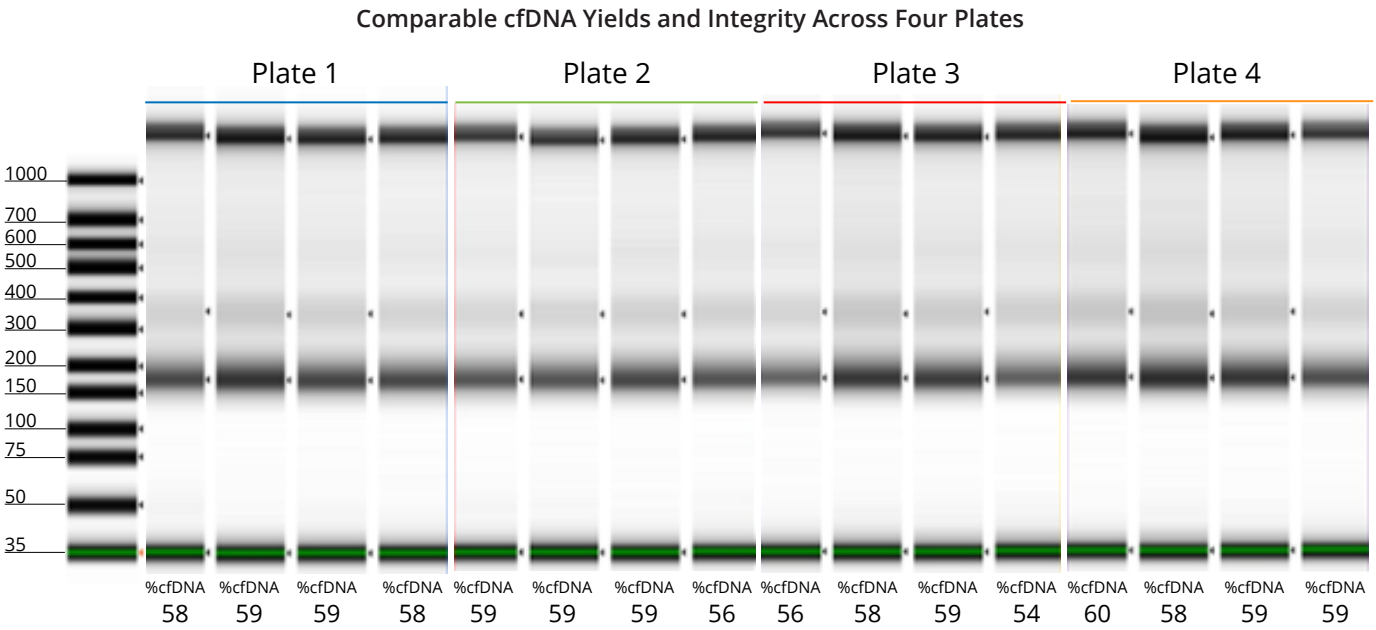


Figure 5. TapeStation® analysis illustrates comparable cfDNA yields and integrity between automated and manual purification methods.

Conclusions

Omega Bio-tek and Dynamic Devices have collaborated to create a robust method for automating cfDNA purification from plasma samples up to 4 mL. This workflow eliminates upfront manual processing and the need for sample splitting across multiple plates, providing a true walk-away solution. cfDNA purified using this automated workflow is shown to be equivalent or better in terms of yield and purity compared to cfDNA purified from manual techniques.

Product Information

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

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