



Automating cfDNA extraction using the Apostle MiniMax[®] High Efficiency Kit on a Biomek i-Series workstation

Introduction

The Apostle MiniMax[®] High Efficiency cfDNA Isolation Kit isolates cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) from plasma collected from blood collection tubes containing EDTA and other blood collection tube types as well as from serum and urine. The kit produces high quality extracted cfDNA that can be used in downstream genomic assays, such as PCR amplification and next-generation sequencing. Proteins in cell-free plasma are digested and cfDNA is captured using Apostle's proprietary magnetic nanoparticles. Contaminants are removed from the samples through several simple washes, leaving high-quality cfDNA samples that are ready for elution. In this application overview, we demonstrate the automated performance of the Apostle MiniMax[®] High Efficiency cfDNA Isolation Kit on the Biomek i7 Hybrid Genomics Workstation.

When compared to manual operations, the Apostle MiniMax[®] High Efficiency cfDNA Isolation Kit automated on Biomek platforms provides:

- Reduced hands-on time and increased throughput
- Option to run the method end-to-end with only setup and tear-down touch points
- Reduction in pipetting errors
- Standardized workflow for improved results
- Quick implementation with demonstrated methods
- Knowledgeable support for reagents, automation and methods all from a single vendor

Spotlight

Biomek i7 Hybrid Genomics Workstations

System features provide the highest throughput and flexibility of all Biomek Genomics workstations for maximized efficiency and reliability to increase user confidence and walk-away time.

- 300 μ L or 1200 μ L Multichannel head with 1-300 μ L and 1-1200 μ L pipetting capability
- Span-8 with 1-1000 μ L pipetting capability
- Enhanced Selective Tip pipetting to transfer custom array of samples
- 2 independent 360[°] rotating gripper with offset fingers
- 45 positions
- Orbital Shakers, Peltiers and Tip washing for controlling sample processing
- Optional enclosure



Figure 1: Biomek i7 Hybrid Genomics Workstation with optional enclosure on a Biomek Cart. The deck has a maximum capacity of 45 positions to maximize efficiency and increase walk-away time.



Figure 2: Apostle MiniMax® High Efficiency cfDNA Isolation kit protocol.

Automated Method

We automated the Apostle MiniMax® High Efficiency cfDNA Isolation kit protocol on the Biomek i7 workstation, incorporating on-deck lysis. Due to the high temperature required for lysis, we used the Inheco incubator, a device that can be integrated onto the deck of the workstation. In general, the automated protocol enables cfDNA extraction from up to 96 plasma and serum samples up to 4 mL, in multiples of 24, in approximately 5 hours (**Table 1**).

The handling of plasma and plasma waste requires special precautions and waste disposal procedures. Therefore, the automated apostle MiniMax® method isolates plasma waste using a waste drain, facilitating proper disposal of biohazardous waste.

Major Process Description	Automated/ Hands-on Time	
	24 Samples	96 Samples
Sample Extraction - 4 mL input		
Prepare Reagents/Set up Inst	30 min	30 min
Method Run Time	2 hr, 32 min	4 hr, 36 min
Total	3 hr, 2 min	5 hr, 6 min
Major Process Description	Automated/ Hands-on Time	
	24 Samples	96 Samples
Sample Extraction - 2 mL input		
Prepare Reagents/Set up Inst	30 min	30 min
Method Run Time	2 hr, 24 min	4 hr, 11 min
Total	2 hr, 54 min	4 hr, 41 min

* Timing does not include reagent and sample thawing or plasma/serum collection or preparation.

Table 1: Estimated run times for Apostle MiniMax® cfDNA automated method on the Biomek i7 workstation.

Demonstrated Method Interface (DMI)

Three modules used to set up the Apostle MiniMax[®] protocol provide the user with full instructions to set up the method and reduce setup errors.

1. Biomek Method Launcher (BML)

BML is a secure interface for selecting methods without affecting method integrity and manual control (**Figure 3**).

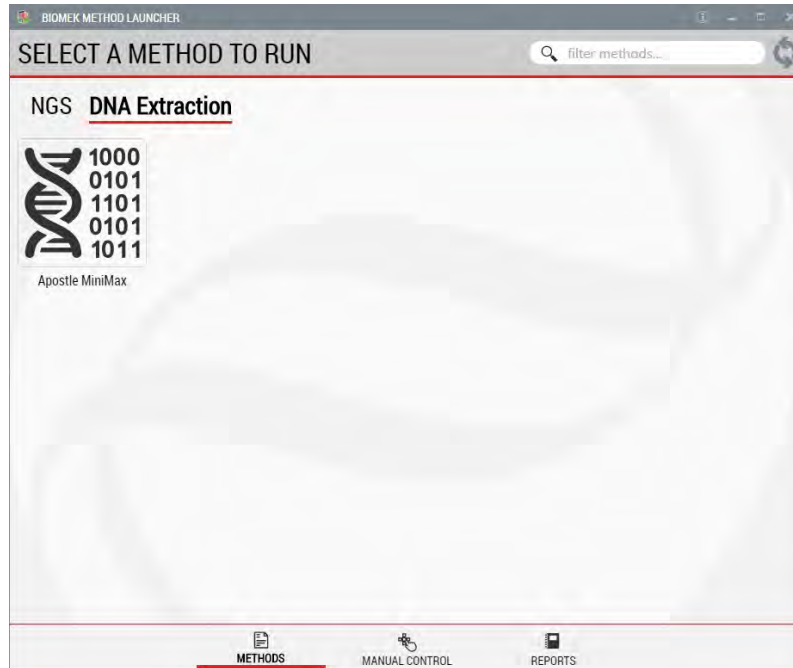


Figure 3: Biomek Method Launcher provides an easy interface to start the method.

2. Method Options Selector (MOS)

MOS enables the selection of run-time options to maximize the flexibility in the set up of the method execution (**Figure 4**). For the Apostle MiniMax[®] method, the automated protocol allows for up to 96 samples in the volume range of 1-4 mL for processing.

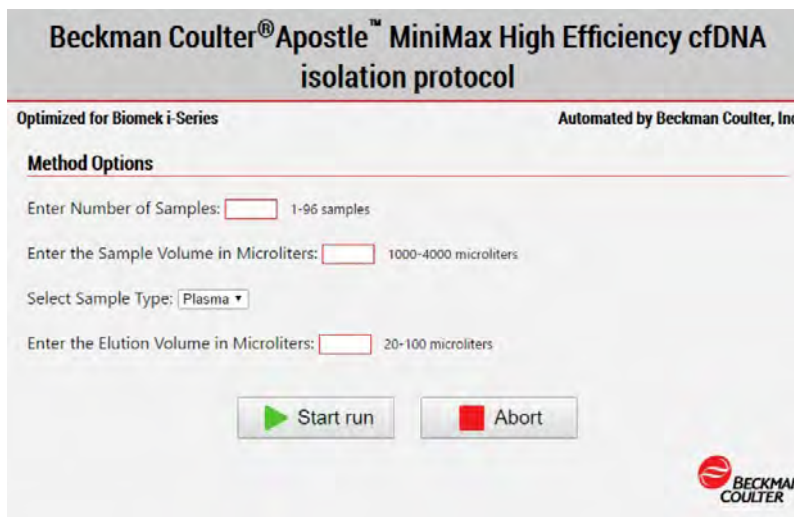


Figure 4: Biomek Method Options Selector indicates sample number and processing options.

3. Guided Labware Setup (GLS)

GLS is generated based on user-selected options in the MOS and provides the user-specific text and step-by-step graphical setup instructions with reagent calculations (**Figure 5**).

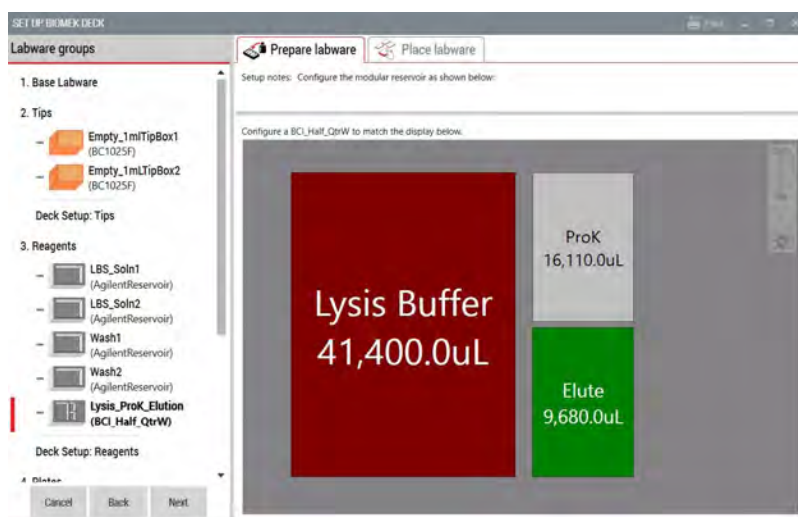


Figure 5: Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup.

Experimental Design

Blood plasma from seven donors was collected in blood collection tubes, four coated with EDTA and three donors collected in other blood collection tubes. Plasma was isolated from whole blood and frozen at -80°C until date of extraction. Plasma samples were thawed at room temperature and three technical replicates for each donor were used for a total of 21 samples of 4 mL aliquots. cfDNA was then extracted using the Apostle MiniMax[®] High Efficiency cfDNA Isolation automated method both manually and implemented on the Biomek i7 Hybrid platform. All extracted cfDNA samples were quantified using the Quant-iT[™] PicoGreen[™] dsDNA Assay Kit (Thermo Fisher Scientific) and analyzed using the 2100 Bioanalyzer with the High Sensitivity DNA kit (Agilent Technologies) and qPCR with the KAPA Human Genomics DNA Quantification and QC Kit and 41 bp primer (KAPA Biosystems) performed in duplicate.

Results

The quantity of extracted cfDNA from the automated protocol was comparable to those of the manual protocol (**Figure 6**). To further characterize the cfDNA samples, extracts were also analyzed using a Bioanalyzer with a High Sensitivity DNA kit. Those traces confirmed expected cfDNA peaks at 170bp, 340bp, and 510bp in both manual and automated samples (**Figure 7**).

Both manually and automated extraction of cfDNA were amplified in the range of Ct 25 (**Figure 8**). Our results show that potential inhibitors have been removed and that samples are suitable for downstream applications such as PCR, next-generation sequencing library preparation, and genotyping.

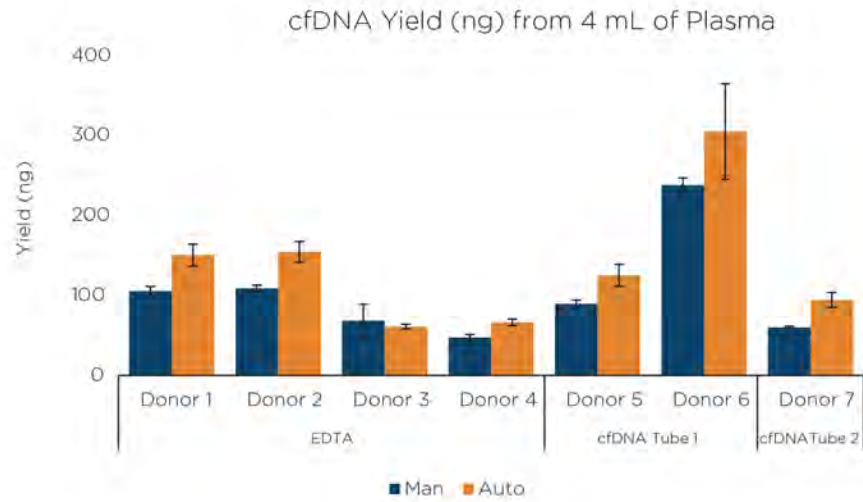


Figure 6: Average DNA yield of manual and automated replicate samples and blood tube types as indicated by Quant-iT™ PicoGreen™.

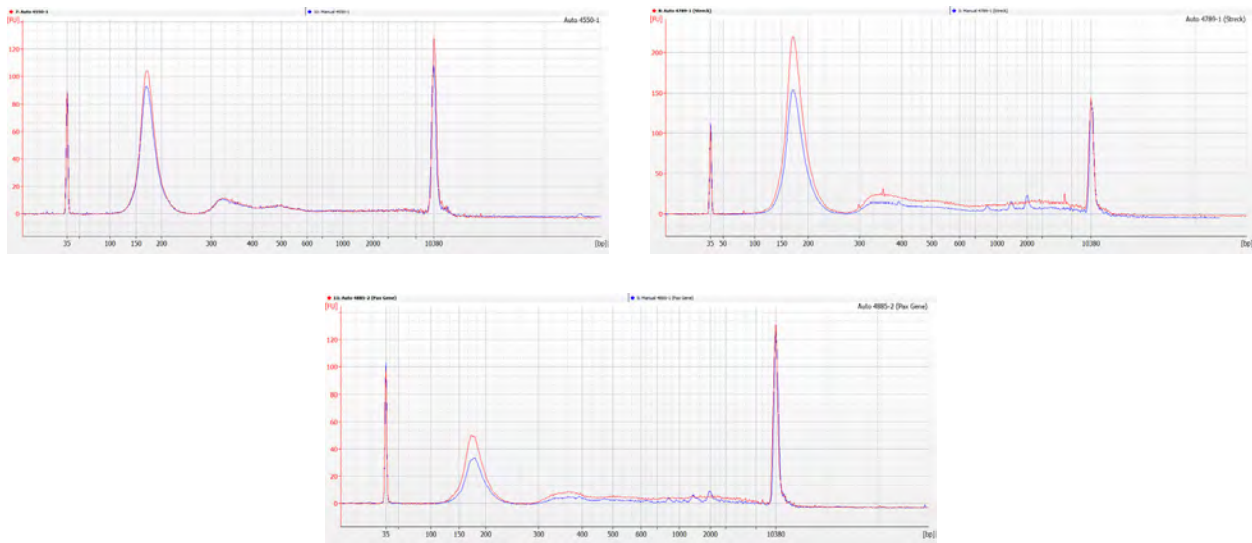


Figure 7: Bioanalyzer overlaid traces of representative cfDNA manually extracted samples (blue lines) and extracted on a Biomek i7 workstation (red lines) from EDTA blood collection tubes (A) and two other blood collection tubes (B & C).

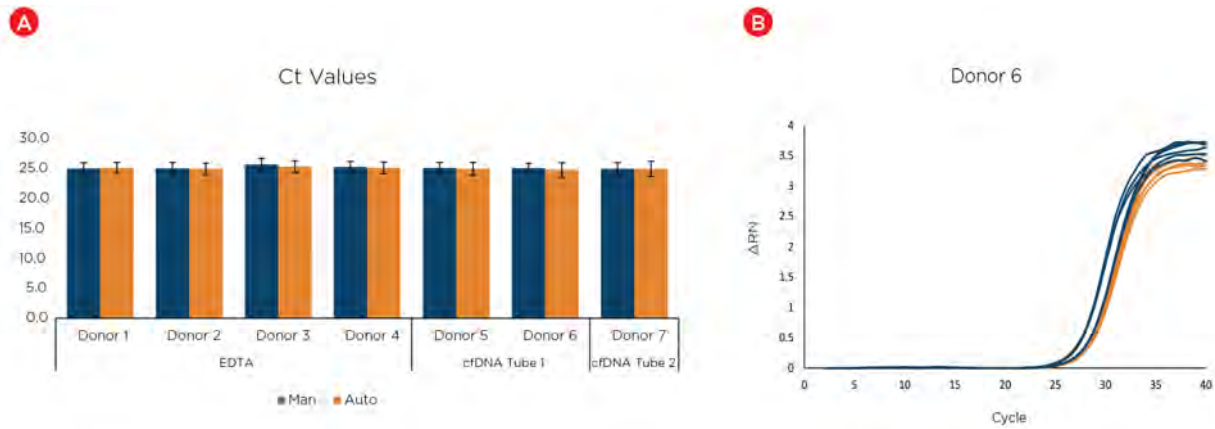


Figure 8: qPCR of cfDNA samples from KAPA Human Genomic Quantification and QC kit with 41 bp primer. (A) The average Ct values of replicate samples of cfDNA extracted manually and automated on the Biomek i7 workstation. (B) A representative curve of cfDNA amplification extracted by a manual user (orange lines) and a Biomek i7 workstation (blue lines).

Summary

We have demonstrated automation of the Apostle MiniMax® High Efficiency cfDNA Isolation kit on the Biomek i7 Hybrid Genomics Workstation results in high-quality cfDNA and yields comparable to manual extraction from blood plasma. Furthermore, samples are free from inhibitors and ready to use in downstream genomic applications. The automated method allows for flexible input/output volumes and sample numbers. The method was implemented using Biomek Method Launcher and provides a convenient interface to run the method.

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Biomek Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

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