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Life Sciences

Isolation of cell-free DNA (cfDNA) from plasma using Apostle MiniMax™ High Efficiency cfDNA Isolation kit—comparison of fully automated, semi-automated and manual workflow processing

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Summary

Cell-free DNA is present in plasma, urine, and other bodily fluids. Typically cfDNA is at low concentration and comprises double-stranded DNA fragments that are overwhelmingly short (140 to 180 base pairs). Its small size and low quantities present a challenge for sequencing and other downstream applications due to insufficient cfDNA yield. Subsequently, resulting in a need to extract from more substantial volumes of bodily fluid; yet higher input volumes can be more challenging to manage for high-throughput sample processing.

This application note compares workflows and yield for the extraction of cfDNA using Apostle MiniMax™ High Efficiency cfDNA Isolation Kit, following the manual protocol, automating the extraction using the Biomek i7 Hybrid Workstation, and semi-automating the extraction using the KingFisher™ Duo Prime Sample Purification System. These potential solutions help mitigate some of the challenges of processing large volume samples. Automating the chemistry can also reduce the risk of human error and reduce hands-on time, therefore giving the user the ability to run more samples in a day.

Materials and Methods

Samples

Plasma was collected in EDTA and two types of cfDNA blood collection tubes (BCT). All samples used in this study were stored at -80°C for greater than six months. Before extraction, the plasma samples were thawed at room temperature. The plasma samples were centrifuged on a Beckman Coulter Avanti J-26 XPI centrifuge equipped with a JA-14.50 rotor set to 9500 RPM for 10 minutes.

Manual

Extraction of cfDNA from 1, 2 and 4 mL of plasma followed the Apostle MiniMax protocol.

Fully Automated

Twenty-four 4mL samples were processed on a Biomek i7 Hybrid Workstation (Figure 1).

Figure 1. A picture of a representative deck layout of a Biomek i7 Hybrid Workstation and a photo of the workstation.

Semi-Automated

Extraction of cfDNA from 1 mL and 2 mL plasma collected in EDTA tubes was semi-automated on the KingFisher Duo Prime using Apostle MiniMax. The plasma samples were processed manually for lysis as detailed in the protocol, followed by cfDNA purification on the KingFisher Duo Prime. The parameters for KingFisher Processing are explained further in reference 1.

Results

Fully-Automated on a Biomek i7 Hybrid Workstation vs Manual Processing

The yield of cfDNA was estimated by using the Quant-iT™ dsDNA Assay (ThermoFisher). Yields between manual and fully automated were not significantly different (t=0.69 p-value=0.5). The yields are shown in Figure 2. There was variation between the samples, which is expected due to the varying biological yields in patient samples.

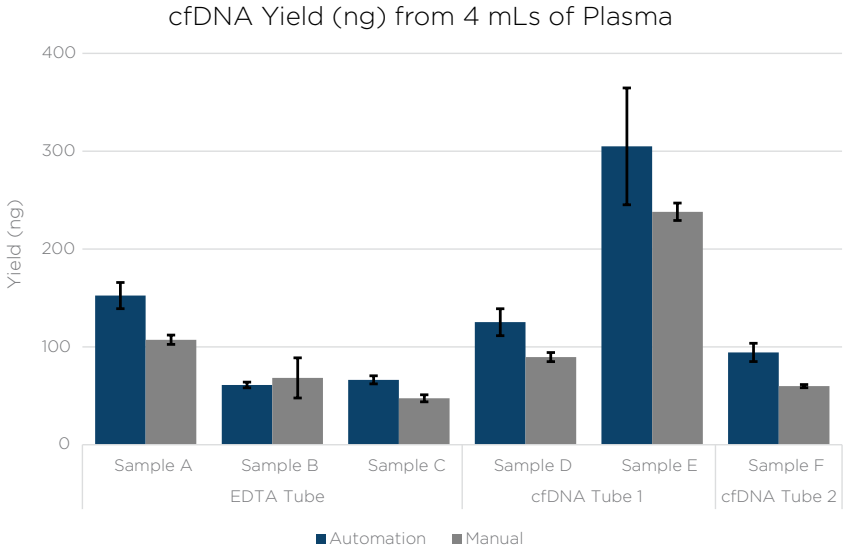

Figure 2. The cfDNA extracted by a manual processing and a Biomek i7 Hybrid Workstation processing. The error bars are representative of the standard deviation of technical replicates.

To better estimate the percent recovery of a known standard, a low mass DNA ladder was spiked into 4 mLs of plasma at a concentration of 329 ng/mL of plasma. Extraction using Apostle MiniMax on a Biomek i7 Hybrid Workstation recovered 92% of the total nucleic acid at 1211 ng/4 mL of sample. The percent coefficient of variation (CV) was estimated for this sample. Automating the extraction resulted in a 2.5% CV, indicating a low amount of variation when using the automated platform.

After the yields were analyzed, the size range of the recovered DNA was assessed on an Agilent Bioanalyzer High Sensitive DNA Chip. The extraction of a low mass DNA ladder showed that all input sizes were recovered equally with both manual processing and Biomek i7 Hybrid Workstation processing (Figure 3).

The absence of any genomic DNA was evaluated on an Agilent Bioanalyzer High Sensitive DNA Chip. Traces for all samples showed a peak near 175 bp, indicating that the majority of the DNA extracted was cfDNA (Figure 4).

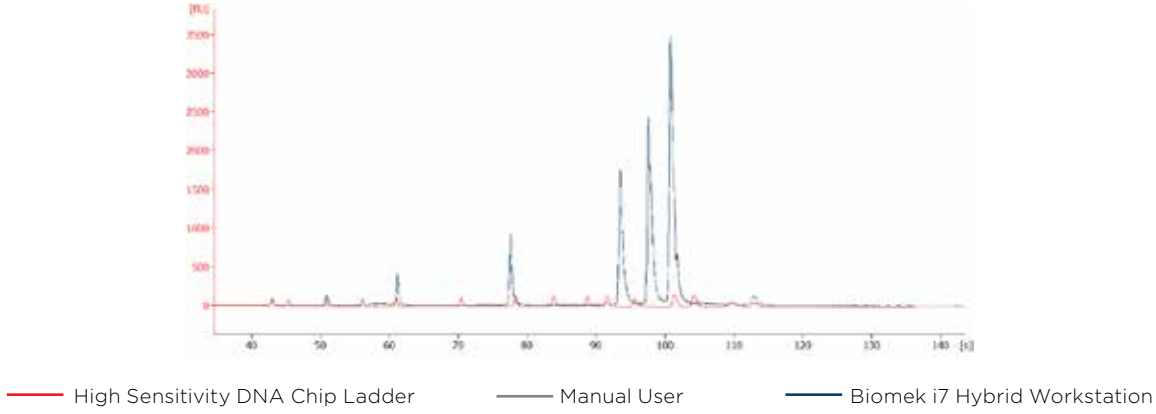

Figure 3. An overlay of the low mad DNA ladder extracted by manual processing (grey line) and a Biomek i7 Hybrid Workstation processing (blue line).

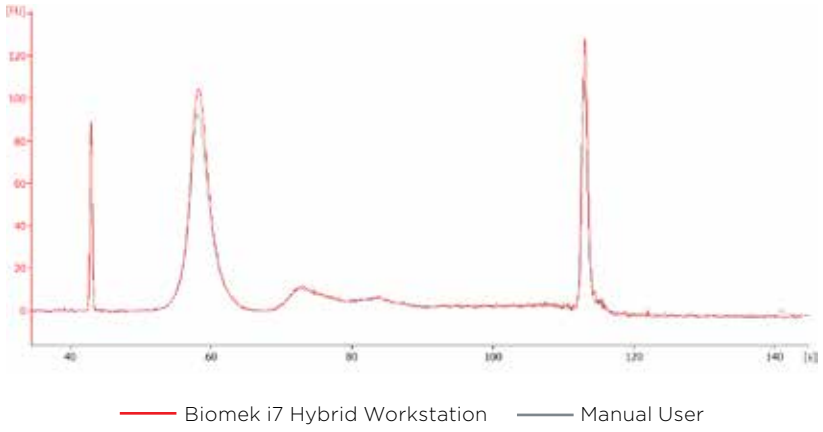

Figure 4. An overlay of cfDNA extracted from EDTA BCT tubes by a manual user (grey line) and on a Biomek i7 Hybrid Workstation (red line). The characteristic cfDNA peak can be seen at 60 s, which corresponds to 140-200 bp. The second small peak at about 75 s, which corresponds to about 350 bp, is also associated with cfDNA.

Lastly, to see whether all PCR inhibitors were removed from the samples, a qPCR using the KAPA hgDNA Quantification and QC Kit (KAPA Biosystems) was performed using only the small primer. The Ct value averaged 25 for Biomek i7 Hybrid Workstation processing and 25.2 for manual processing; a T-test showed that the Ct values were not significantly different (t=-1.27, P=0.23) (Figure 5).

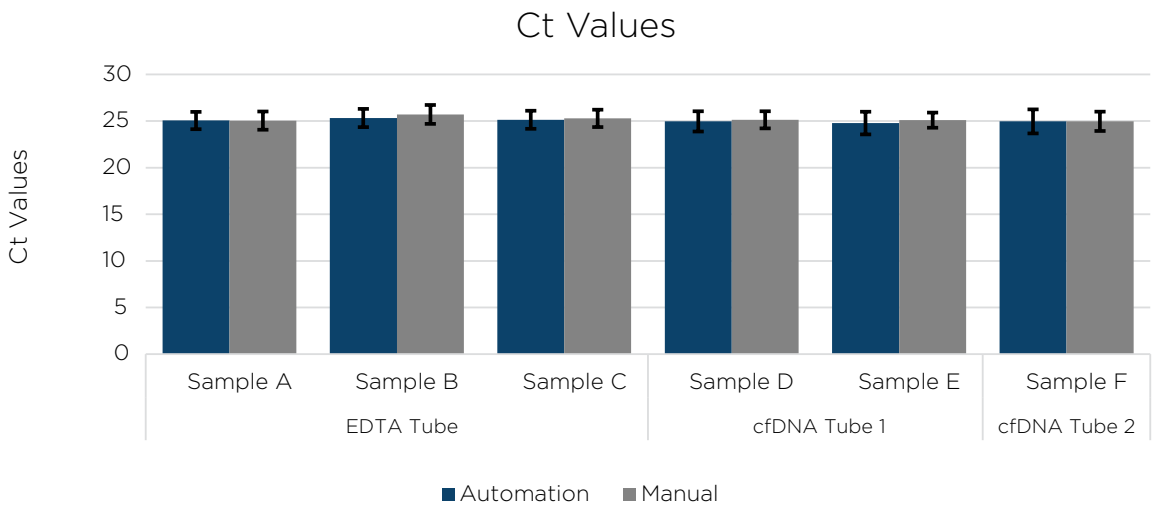

Figure 5. The average Ct values of three technical replicates for all 6 samples of cfDNA extracted by a manual user and on a Biomek i7 Hybrid Workstation.

Semi-Automated vs. Manual Processing

Manual processing of cfDNA using Apostle MiniMax was compared to a semi-automated processing of Apostle MiniMax on a KingFisher Duo Prime. The yield of DNA was estimated by using Quant-it™ dsDNA Assay Kit (Figure 6). The yield varied for the manual and automated extraction for the two different input volumes, due to experimental variation. For both of the plasma volumes, the coefficient of variation (CV) for the semi-automated processing was less than for the manual processing, indicating that there is less variability in relation to the average yield when processing on the KingFisher Duo Prime. Due to the volume limitations on the KingFisher Duo Prime, the maximum input sample volume is 2 mL. If a larger volume is desired, the sample volumes can be split between multiple wells and the elution can be combined.

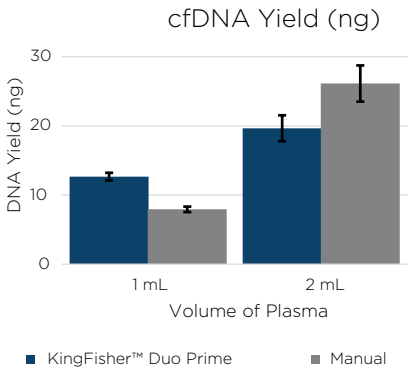

Figure 6. The cfDNA extracted using the KingFisher Duo Prime and by manual processing. The error bars are representative of the standard deviation of three technical replicates.

Volume of Plasma	KingFisher Duo Prime	Manual
1 mL	4.4%	5.0%
2 mL	9.6%	10.0%

Table 1. The coefficient of variation for the 4 different conditions.

Conclusions

Using a fully or semi-automated platform reduces hands-on-time without sacrificing cfDNA yield (Table 2). Automation can also help reduce the risk of human errors, as there are less human intervention steps (Table 2). The throughput of the Biomek i7 Hybrid Workstation is 96 x 4 mL samples per run, and the throughput of the KingFisher Duo Prime is 6 x 2 mL samples per run (Table 2). At maximum capacity in an 8 hour day, 96 samples can be processed on the Biomek i7 Hybrid Workstation and 36 samples can be processed on a KingFisher Duo Prime.

	Maximum Sample Input Volume	Human Intervention Steps	Throughput per run	Hands-on Time	Total Time
Manual	10 mL	39	24	45 minutes	75 minutes
Biomek i7 Hybrid Workstation	4 mL	1	96	30 minutes	5 hours 20 minutes
KingFisher Duo Prime	2 mL	12	6	10 minutes	75 minutes

Table 2. The throughput and time for manual, fully-automated and semi-automated cfDNA extraction.

References

1. Supplemental Protocol for Apostle MiniMax™ High Efficiency cfDNA Isolation kit for 1 and 2 mL sample on a KingFisher™ Duo Prime AAG-5748SP08.19

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