



Comparative analysis of cell-free DNA extraction efficiency from plasma

Introduction

With recent advancements in nucleic acid-based molecular technologies, applications for precision medicine in clinical research have grown exponentially over the last few years. In particular, analysis of cell-free DNA (cfDNA) for cancer detection, prognosis, and monitoring has significantly improved patients' clinical management and outcomes. The primary challenge with cfDNA-based liquid biopsy techniques is that cfDNA is typically found at very low concentrations in body fluids, with a range of 1-50 ng cfDNA from 1 mL plasma. Of the total cfDNA found in plasma, fragments originating specifically from tumors (ctDNA) can represent as low as 0.01%. Thus, efficient techniques for cfDNA extraction are essential for ctDNA detection in precision medicine.

Two of the most commonly used cfDNA extraction techniques are spin column-based and magnetic bead-based purification. To evaluate the performance of these cfDNA extraction techniques, we invited an independent principal investigator to conduct a study using the bead-based Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Beckman Coulter Life Sciences, U.S.) and the spin column-based QIAamp® circulating nucleic acid kit (Qiagen, Germany). Based on cfDNA quantification and sizing, the independent investigator found that the Apostle MiniMax cfDNA Isolation Kit significantly outperformed the QIAamp® circulating nucleic acid kit. The findings of this study highlight the importance of selecting a highly efficient cfDNA extraction method to avoid the loss of crucial ctDNA fragments and achieve optimal detection for liquid biopsy applications.

Materials and methods

Sample processing and cfDNA isolation

Blood was collected in EDTA tubes from healthy individuals and plasma was isolated by centrifugation at 2,000 x *g* for 10 min at room temperature (RT). To remove additional impurities, the isolated plasma was subsequently centrifuged at 16,000 x *g* for 10 min, and the supernatant was collected. The plasma samples were then mixed thoroughly and split into 4 aliquots of 2 mL. cfDNA was isolated from duplicate plasma samples using either the Apostle MiniMax™ High Efficiency cfDNA Isolation Kit (Beckman Coulter Life Sciences, U.S.) or the QIAamp® circulating nucleic acid kit (Qiagen, Germany) according to the manufacturers' instructions. For comparison, cfDNA extracted from both kits was eluted in 35 µL elution buffer.

Quantification of cfDNA

cfDNA was quantified using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher U.S.) and a droplet digital PCR (ddPCR) method (QX200 Droplet Digital PCR System, Bio-Rad Laboratories, U.S.) according to the manufacturers' instructions. ddPCR primer/probe sets were designed in-house at the investigator's lab to target actin gamma 1 (*ACTG1*) and DnaJ heat shock protein family (Hsp40) member C5 gamma (*DNAJC5G*) (**Table 1**). No template control (NTC) samples were used to set the threshold for positive droplets. The absolute count of positive droplets was normalized to the total amount of droplets generated for each well. ddPCR was performed in technical duplicates for each purified sample.

Oligo	Sequence
ACTG1-fwd	GTTTCTTTTCGCTGTTCCA
ACTG1-rev	GCAGGCAGAAACCAAAT
ACTG1-probe	HEX-CCCGGCATTTCCTCCCTGAAGCCTCC-BHQ1
DNAJC5G-fwd	GCGATATCACCACCAAGAGCCT
DNAJC5G-rev	GGTACACAGAGTTGCAACAAGGG
DNAJC5G-probe	FAM-ATCCTGTCCCTGGAGCTGCTGCACCCATTA-BHQ1

Table 1. Primer and probe sequences used for ddPCR quantification.

Evaluation of cfDNA fragment size

cfDNA fragment size was assessed using the Agilent High Sensitivity DNA Kit on the Agilent Bioanalyzer instrument (Agilent Technologies, U.S.) according to the manufacturer's instructions.

Results and discussion

Mean cfDNA concentrations in the eluates were determined to be 8.30 ng/μL for samples extracted with the QIAamp kit and 11.15 ng/μL for samples extracted with the Apostle kit when measured with the Qubit assay (**Figure 1A**). This illustrates a 34.3% higher yield when using the Apostle kit as compared to the QIAamp kit.

When evaluating the contents of specific genes in the eluate, the Apostle purification displayed a higher yield than QIAamp (**Figure 1B**). 8358 *DNAJC5G* positive droplets were detected in the Apostle samples compared to 7269 droplets in the QIAamp samples. Similarly, 10849 *ACTG1* droplets were detected in the Apostle-extracted samples versus 9463 droplets detected in the QIAamp-extracted samples. This data demonstrates an average of 14.8% higher yield using the Apostle kit.

Finally, when evaluating cfDNA fragments via the Agilent bioanalyzer instrument, the Apostle kit demonstrated the highest yield (**Figure 1C and D**). Mononucleosomal cfDNA concentration in the eluate of Apostle samples was estimated to be 10.64 ng/μL versus 6.26 ng/μL in the eluate of QIAamp samples. The dinucleosomal cfDNA concentration was low for both kits, with 0.13 ng/μL recorded for Apostle samples and 0.24 ng/μL for QIAamp samples. This indicates a slightly better purification of dinucleosomal cfDNA using QIAamp; however, this could also be an artifact given the low concentrations. The Apostle mononucleosomal cfDNA yield was 70.0% higher than the yield for the QIAamp samples.

A summary provided in Table 2 shows the consistently higher yield achieved by the Apostle MiniMax™ High Efficiency cfDNA Isolation Kit as compared to the QIAamp® circulating nucleic acid kit. The average 40% improvement in yield underscores the importance of extraction kit selection in cfDNA assay development and highlights the potential impact of cfDNA isolation efficiency in ctDNA detection.

Acknowledgments

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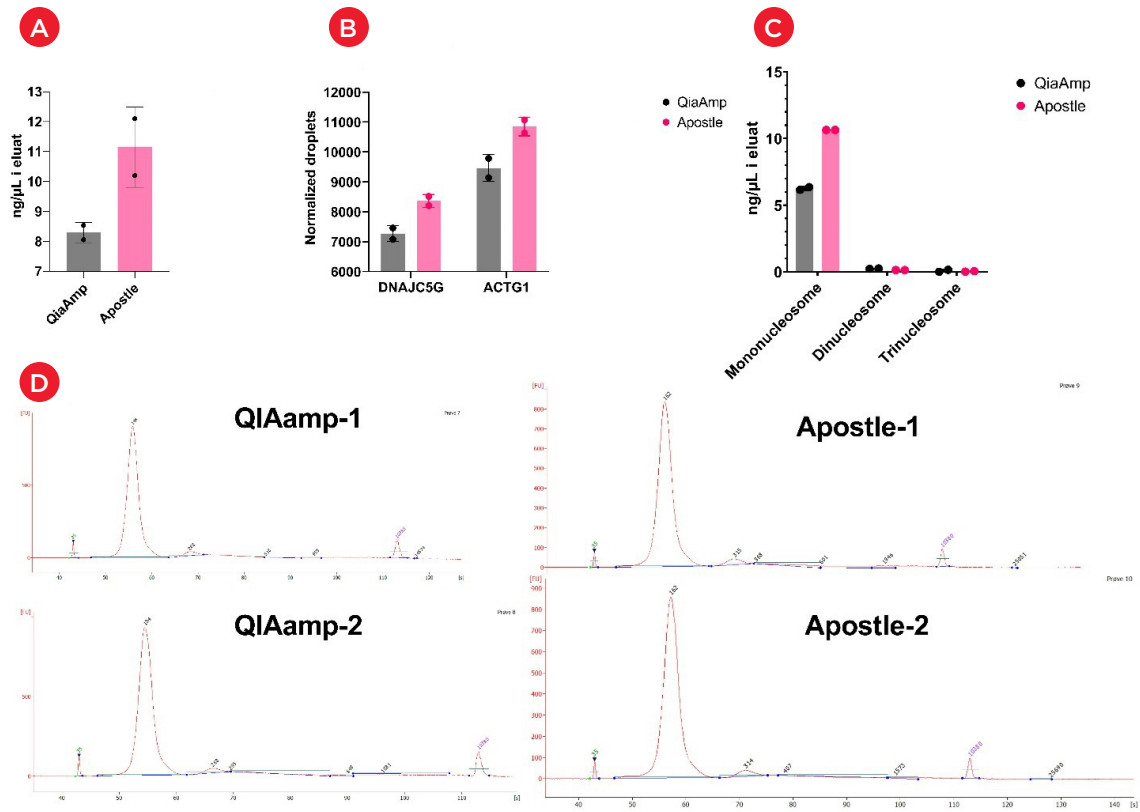


Figure 1. Evaluating Apostle MiniMax and QIAamp cfDNA purification efficiency. **A)** cfDNA concentration in final eluates. **B)** Normalized positive droplets for *DNAJC5G* and *ACTG1* in the purified cfDNA samples. **C)** Quantified contents of cfDNA corresponding to mono-, di-, and tri-nucleosomal DNA in the purified samples based on analysis with the Agilent Bioanalyzer system. **D)** Size distribution plots for purified cfDNA samples. N=2 in all plots.

Method	Percent higher yield with Apostle compared to QIAamp
Qubit	34.4%
ddPCR	14.8%
Bioanalyzer	70%
Average	40.0%

Table 2. Summarizes the quantification methods and demonstrates that the Apostle MiniMax purification kit outperformed the QIAamp purification kit for all quantification methods, resulting in an average of 40% higher yield.

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