A new scalable and automatable method for the extraction of cfDNA

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Introduction

Liquid biopsies represent a promising area of cancer testing as taking blood is less invasive than tumor biopsies. The cell free DNA (cfDNA) present in the blood includes DNA derived from cancer cells and cancer biomarkers can be detected in the extracted cfDNA.

Whole blood also contains genomic DNA, and can be removed by centrifugation, resulting in plasma. cfDNA is present in very small amounts in blood or plasma, and thus larger amounts of plasma are required for many applications. Larger extractions are more challenging to automate, as they require additional pipetting steps.

Here we present a novel cfDNA extraction kit and show its compatibility with extractions from 200 µl – 5 mL. We discuss the optimization of the method and demonstrate automation on a KingFisher Duo. This workflow can also be automated on a Biomek i7 Automated Workstation. We demonstrate that this kit can be used for NGS and produce results comparable to other commercial kits.

Scalable chemistry with inputs from 200 µL to 5 mL

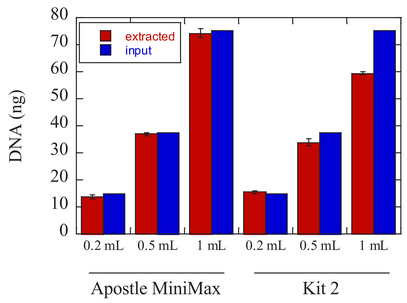

Figure 1. DNA isolation from varying plasma amounts. Nucleosomes were spiked into plasma to ensure enough DNA was present for QC. The Apostle MiniMax kit isolated nearly all the input DNA, while Kit 2 isolated less from larger plasma volumes.

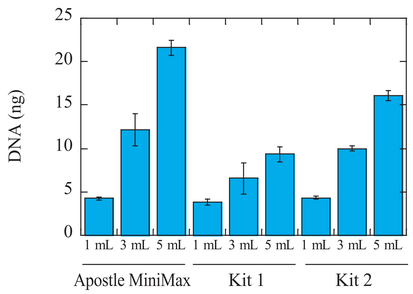

Figure 2. Isolation of DNA from 1 – 5 mL of EDTA plasma. Yields were highest with the Apostle MiniMax kit for 3-5 mL, and yields were similar across all kits at 1 mL.

Apostle MiniMax Workflow

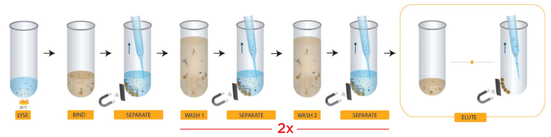

Figure 3. Apostle MiniMax Workflow. The Apostle MiniMax kit involves a lysis step, then the addition of magnetic beads to bind the DNA. Once the DNA is bound, it is washed with various wash buffers and finally eluted from the beads.

Removal of PCR inhibitors and gDNA

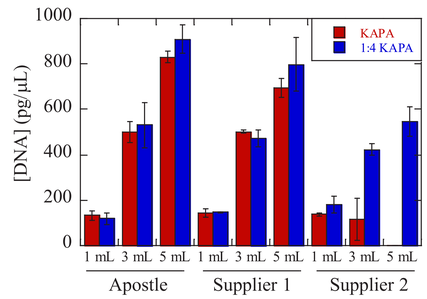

Figure 4. Comparison of PCR inhibition. The p41 primers and the KAPA hgDNA Quantification and QC kit was used to estimate [DNA]. Undiluted samples were compared to samples diluted 1:4 to measure the effect of PCR inhibitors. If PCR inhibitors are present, the estimated concentration will be higher in more dilute samples. Similar concentrations estimated from the 1:1 and the 1:4 dilution KAPA is a sign of low inhibition. As such, Apostle MiniMax and Kit 1 have low inhibition and significant PCR inhibition is seen in Kit 2.

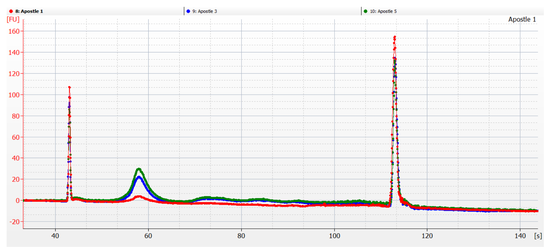

Figure 5. Increasing input amounts yield increasing amounts of cfDNA. Bioanalyzer traces show that the increase in DNA yield is due to increasing amounts of a small DNA peak. No contaminating genomic DNA was seen. The high peaks at the beginning and end of the trace are high and low markers.

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Protocol Optimization

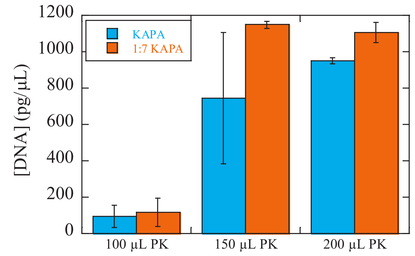


Figure 6. Optimization of Proteinase K. Changes in Proteinase K concentration have significant effects on final [DNA]. Increasing the amount of Proteinase K to 150 µL results in significantly more yield in EDTA plasma tubes.

Automation on KingFisher Duo

The extraction was automated on the KingFisher Duo instrument after the addition of the binding/ nanoparticle solution. The automated portion of the extraction was 37 min.

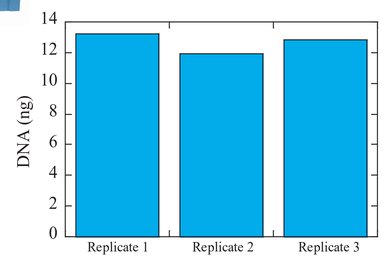
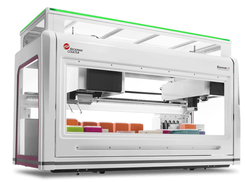


Figure 7. cfDNA was extracted from 1 mL of plasma in concentrations consistent with manual extractions. Little variability was seen between the replicates (standard deviation of 1.0).

Automation on the Biomek i7 Automated Workstation



The Apostle MiniMax kit is compatible with automation on Beckman Coulter's Biomek i7 Automated Workstation instrument with integrated KingFisher Presto. While the method can be automated on the Biomek i7 Automated Workstation alone, the run time is prohibitively long (4.5 hr). With the KingFisher integration, run time is expected to be 2.5 hr.

Sample Name	Percent Aligned Reads	Read Enrichment	Uniformity of Coverage (Pct > 0.2*mean)	Target Coverage at 1X	Target Coverage at 20X
EDTA donor 1 Apostle MiniMax	99.80%	70.10%	98.40%	100.00%	99.90%
EDTA donor 2 Apostle MiniMax	99.80%	70.80%	97.90%	99.90%	99.80%
EDTA donor 1 Kit 1	99.70%	67.90%	97.80%	100.00%	99.90%
EDTA donor 2 Kit 1	99.80%	65.10%	95.70%	99.90%	99.70%
cfDNA Tube 1 donor 1 Apostle MiniMax	99.80%	72.10%	98.60%	100.00%	99.90%
cfDNA Tube 1 donor 2 Apostle MiniMax	99.80%	71.90%	98.50%	100.00%	99.90%
cfDNA Tube 1 donor 1 Kit 1	99.80%	71.10%	97.50%	100.00%	99.80%
cfDNA Tube 1 donor 2 Kit 1	99.80%	70.20%	95.90%	100.00%	99.90%
cfDNA Tube 2 donor 1 Apostle MiniMax	99.80%	68.10%	97.90%	100.00%	99.90%
cfDNA Tube 2 donor 2 Apostle MiniMax	99.80%	69.60%	98.40%	100.00%	99.90%
cfDNA Tube 2 donor 1 Kit 1	99.80%	68.40%	96.70%	100.00%	99.90%
cfDNA Tube 2 donor 2 Kit 1	99.70%	67.20%	96.30%	100.00%	99.90%

Table 1. Quality Control of NGS run. Libraries were prepared from 25 ng DNA with the Accel-NGS 2S Hyb DNA Library prep kit for NGS and a target capture library was prepared from that library using the IDT xGen Pan-cancer panel. Libraries were pooled and run on an Illumina NextSeq. Data was analyzed via BWA enrichment. Human genome UCSC hg19 was used as the reference genome. Quality control metrics from all runs are good and comparable between extraction methods.

Detection of Cancer Mutations

Sample Name	Indels	Indel Het/ Hom Ratio	SNVs	SNV Het/ Hom Ratio	SNV Ts/ Tv Ratio
EDTA donor 1 Apostle MiniMax	108	4.4	374	1.7	2.3
EDTA donor 2 Apostle MiniMax	127	4.5	453	1.9	2.8
EDTA donor 1 Kit 1	110	4.8	373	1.7	2.3
EDTA donor 2 Kit 1	126	4.3	452	1.9	2.7
cfDNA Tube 1 donor 1 Apostle MiniMax	108	3.3	398	1.7	2.6
cfDNA Tube 1 donor 2 Apostle MiniMax	99	3.1	403	1.7	2.2
cfDNA Tube 1 donor 1 Kit 1	108	3.3	401	1.7	2.5
cfDNA Tube 1 donor 2 Kit 1	99	3.3	402	1.7	2.3
cfDNA Tube 2 donor 1 Apostle MiniMax	115	4.8	410	2.1	2.2
cfDNA Tube 2 donor 2 Apostle MiniMax	112	4.3	434	2.5	2.6
cfDNA Tube 2 donor 1 Kit 1	116	4.8	414	2.1	2.2
cfDNA Tube 2 donor 2 Kit 1	113	4.4	427	2.5	2.6

Table 2. Mutation Detection with Different Extraction Methods. The detection of indels and SNVs was similar with both extraction methods.

Sample Name	Indels	Indel Het/ Hom Ratio	SNVs	SNV Het/ Hom Ratio	SNV Ts/ Tv Ratio
cfDNA Tube 1 donor 2 Apostle MiniMax Run A	99	3.1	403	1.7	2.2
cfDNA Tube 1 donor 2 Apostle MiniMax Run B	101	3.4	399	1.7	2.3
cfDNA Tube 1 donor 2 Kit 1 Run A	99	3.3	402	1.7	2.3
cfDNA Tube 1 donor 2 Kit 1 Run B	104	3.5	402	1.7	2.3
cfDNA Tube 2 donor 2 Apostle MiniMax Run A	112	4.3	434	2.5	2.6
cfDNA Tube 2 donor 2 Apostle MiniMax Run B	112	4.1	432	2.5	2.7
cfDNA Tube 2 donor 2 Kit 1 Run A	113	4.4	427	2.5	2.6
cfDNA Tube 2 donor 2 Kit 1 Run B	114	4.4	431	2.5	2.7

Table 3. Run Variation. The duplicate libraries were sequenced to determine the amount of intra-run variation. As you can see, the variation between runs A and B of the same library have equal or greater variation than the runs observed for the different extraction methods, implying that the two methods sequence equaling well with NGS.

Conclusions

- DNA can be extracted from 200 µL to 5 mL of plasma
- The Apostle MiniMax kit removes the PCR inhibitors present in plasma
- Genomic contamination is not present in the extracted cfDNA
- Extraction of 1 mL plasma can be automated on a KingFisher instrument with yields similar to manual extraction
- Similar numbers of mutations were found in cancer plasma with the Apostle MiniMax kit and another commercial kit.

The Apostle MiniMax kit is a versatile new cfDNA kit that can extract from a wide range of sample amounts and be run either manually or on a variety of automation systems.