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 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 qPCR. The Apostle MiniMax kit isolated nearly all the input DNA, while kit 2 isolated less from larger plasma volumes.

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 withucci wash buffers and finally eluted from the beads.

Protocol Optimization

Figure 4. Optimization of Protease K. Changes in Protease K input amounts yield increasing amounts of cfDNA. The Apostle MiniMax kit and another commercial kit.

Removal of PCR inhibitors and gDNA

Figure 2. Comparison of PCR inhibition. The pHL primers and the KAPA htgDNA 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 P and P44 kit indicate that the concentration of the gDNA 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. Bioreactor 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 matters.

Detection of Cancer Mutations

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.

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.

Automation on 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.

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 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.

Table 1. Quality Control of NGS run. Libraries were prepared from 25 ng DNA with the Accel-NGS 25‑hd 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 Hiseq X1001. Human genomic DNA (Catalog #604507) and 1 µg of UCSC hg19 was used as the reference genome. Quality control metrics from all runs are good and comparable between extraction methods.

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

Table 3. Run Variation. The duplicate libraries were sequenced to determine the amount of intrarun variation. As you can see, the variation in average reads 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.