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Next-generation sequencing analysis of high-quality and high-quantity cell-free circulating DNA prepared from droplet volumes of patient plasma.

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Abstract Disclosures

Background: It is in agreement and the growing belief that “liquid biopsy” can provide a global longitudinal picture of cancer progression beyond the heterogeneity presented in the primary tumor. Cell-free DNA (cfDNA) circulating in the bloodstream could become the unprecedented non-invasive, standard-of-care molecular marker for the treatment and management of cancer. However, cfDNA is highly fragmented and presented at very low concentrations; therefore, its isolation is challenging because of the requirement of large volume input, costs, and labor-intensity. A proprietary cfDNA enrichment process with novel characteristic of ultra-low input (as low as 20 uL of plasma) and high output (> 300 ng/mL of cfDNA) has been developed and compared to the industry standard. **Methods:** The performance of our method was evaluated side-by-side with two leading extraction methods based on throughput, sample volume, yield, and cfDNA amplifiablity. The enriched products were further subjected to NGS on the Ion Torrent PGM to detect mutations. **Results:** Compared to the leading cfDNA extraction methods using different chemistries and workflows, our enrichment approach allows high-yield isolation of cfDNA directly from a small volume of unprocessed plasma, leading to a significant increase in isolation efficiency and > 20-fold recovery. The cfDNA prepared by our proprietary method displayed a high-degree genomic representation as demonstrated by SNP Cytochip array. Further NGS studies on cfDNA from 10 colorectal cancer plasma and 2 spiked samples demonstrated the superiority of our protocol in generating more usable, on-target, high-quality ≥ Q20 sequencing reads, and detecting more mutations over Qiagen kit. **Conclusions:** A seamless sample-preparation process is critical when analyzing low-quantity cfDNA in high-noise background. Our cfDNA enrichment process is the first non-invasive method that allows accurate NGS genomic analyses directly from droplet volumes of plasma. This protocol can be applied to a broad range of clinical genetic tests with the advantages of minimal sample volume, maximal yield, streamlined workflow with reduced costs and turnaround time.