Comparison of DNA reference material for pre-analytics: Nucleosomal plasma-spike-ins reveal true recovery efficiencies of circulating cell-free DNA extractions

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Background & objectives

The rising interest in circulating cell-free DNA (ccfDNA)-based diagnostics has led to a surge in the number of available ccfDNA extraction kits and methodologies in recent years. Current ccfDNA extraction protocols involve multiple steps, including protease digestion, lysis, binding, washing, and elution, and the efficiency of each step can affect the final yield of ccfDNA. The benchmark for evaluating extraction protocols is typically the recovery efficiency of wildtype (WT) and mutant (MUT) DNA fragments. However, clinical samples, which would provide the most realistic recovery results, may not always be accessible for benchmarking, leading to an urgent need for alternative reference materials. A widely used approach are pooled plasma samples from healthy individuals (WT control) spiked with synthetic oligonucleotides or sonicated genomic DNA (MUT control). Recently, mononucleosomes extracted from cell culture have emerged as a more physiologically relevant MUT reference material for evaluating ccfDNA extraction protocols.

In this work, we demonstrate how mononucleosomes can serve as a more realistic material for evaluating critical steps of an extraction protocol, such as protease digestion and lysis time, compared to histone-free oligonucleotides.

Methods

We evaluated the ccfDNA recovery efficiency using QIAamp Circulating Nucleic Acid Kit (QIAGEN) with varying protease and lysis times. We compared healthy plasma samples spiked with synthetic 131 base-pair sized oligonucleotides (IDT) comprising the *KRAS G13D* mutation as well as mononucleosomes from colorectal cancer cell line HCT116 carrying the same mutation.

Results

Our findings indicate that the recovery efficiency of synthetic oligonucleotides remained unaffected with decreasing protease digestion and lysis times in spiked plasma samples. Conversely, the recovery efficiency of mononucleosomes decreased as the protease digestion and lysis times were reduced, from 100% at 30 minutes to 94.8% at 5 minutes and 80.2% at 1 minute incubation, most likely due to insufficient histone unwrapping.

Conclusion

This work provides further evidence that mononucleosomes provide a more appropriate reference material as MUT spike-in for ccfDNA extraction control than histone-free oligonucleotides. This has important implications for future research aimed at benchmarking and optimizing ccfDNA extraction methods, and further argues against the use of synthetic oligonucleotides as reference material in clinical implementation of liquid biopsy-based diagnostics.