Broad-Range cfDNA Capture Beyond Nucleosome-Sized Fragments Enhances Liquid Biopsy Fragmentomic Resolution

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ABSTRACT

Background: Liquid biopsy is increasingly used in oncology for early detection, disease monitoring, and minimal residual disease (MRD) assessment. These applications require comprehensive recovery of circulating cell-free DNA (cfDNA), particularly in low-input and low–tumor burden samples. Conventional silica- or bead-based cfDNA purification methods and double-stranded DNA library preparation workflows are optimized for nucleosome-sized fragments and may underrepresent ultra-short and single-stranded cfDNA species. Such bias may limit analytical sensitivity and fragmentomic resolution.

Results: Plasma samples from healthy donors and patients with lung cancer were processed using a magnetic bead-based cfDNA purification workflow designed to improve retention of cfDNA across a broad fragment size range. Recovery efficiency, fragment size distribution, and cfDNA yield were compared with commercially available cfDNA extraction kits.

To assess the strandedness of recovered cfDNA, aliquots from the same healthy donor were treated with or without a single-strand–specific nuclease prior to library preparation. Purified cfDNA was prepared using a single-stranded DNA–compatible library preparation method to enable capture of both double- and single-stranded cfDNA molecules. Fragment size distributions were analyzed from mapped sequencing reads. Additional fragmentomic features, including short-to-long fragment ratios, were evaluated to assess discrimination between cancer and healthy plasma samples as well as differences among purification methods.

Conclusions: A magnetic bead-based broad-range cfDNA purification method combined with single-stranded DNA library preparation enables recovery of ultra-short and single-stranded cfDNA fragments that are typically underrepresented in standard liquid biopsy workflows. Inclusion of these fragments enhances fragmentomic resolution and improves discrimination between lung cancer and healthy plasma samples. This clinically adaptable approach may improve analytical sensitivity for oncology applications, including early detection and MRD assessment, and warrants further validation in larger cohorts.

A NOVEL WORKFLOW FOR CFDNA ANALYSIS

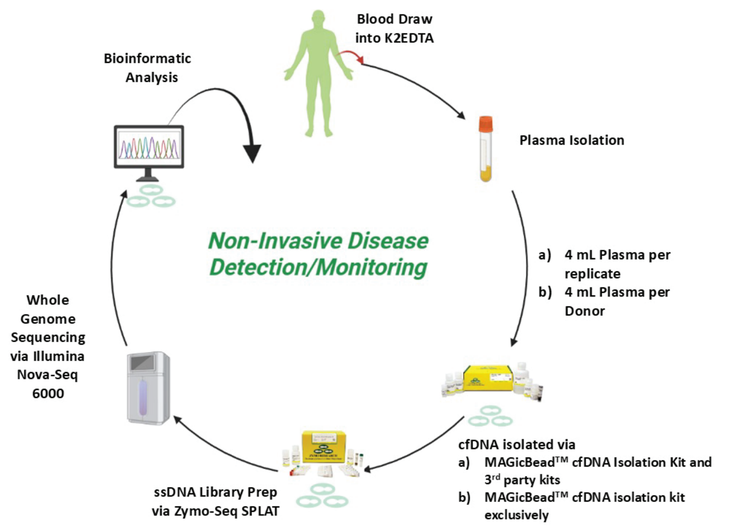


Figure 1. A novel workflow for ultra-short cfDNA fragmentomics and multiomic profiling in liquid biopsies. We developed an integrated workflow to enable comprehensive capture and analysis of cell-free DNA by combining a novel nucleic acid purification method with an ssDNA-compatible library preparation approach. This strategy enhances recovery of both short and degraded cfDNA fragments, supporting more complete fragmentomic profiling. Plasma samples were used as a model system to demonstrate workflow performance.

RESULTS

MAGicBead™ Technology Enables Comprehensive cfDNA Recovery

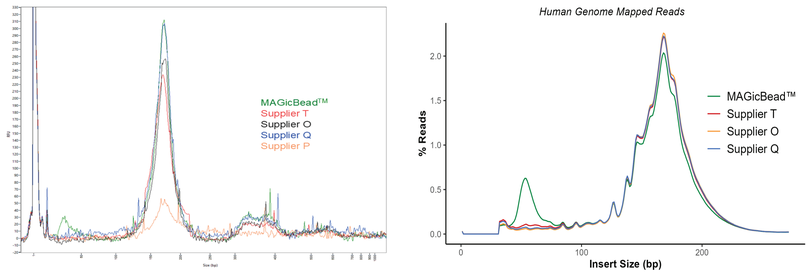


Figure 2. a) Single-donor whole blood was collected in K2EDTA blood collection tubes. Whole blood was spun at 1,000 x g for 10 minutes at room temperature to isolate plasma. cfDNA was extracted from 1 mL of plasma using various commercially available magnetic bead-based cfDNA Isolation Kits, including the **MAGicBead™ cfDNA Isolation Kit**. Extracted cfDNA was run on the Fragment Analyzer 5200 (Agilent). **b)** cfDNA extracted from 4 mL of plasma using various commercially available magnetic bead-based cfDNA Isolation Kits was library prepared using the **Zymo-Seq™ SPLAT DNA Library Kit (Zymo Research, Cat No. D5464)**. Percent reads from WGS at 30x coverage reveal MAGicBeadTM chemistry's ability to isolate ultra-short cfDNA fragments. For each insert length, the plot value for each condition was generated by averaging the proportion at each insert length across both replicates.

Differential Recovery of Cell-Free DNA Fragment Sizes Across Commercial Purification Technologies

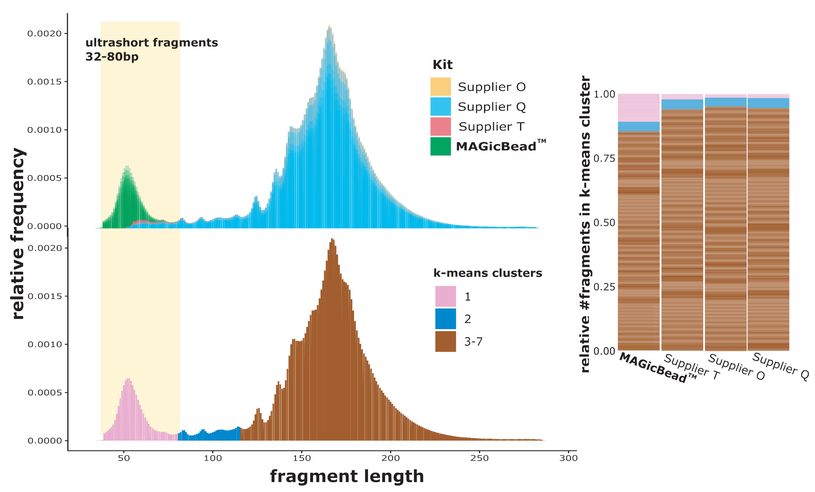


Figure 3. cfDNA fragment size distributions were compared between the MAGicBead™ cfDNA Isolation Kit and competing vendors (n=2 per kit). Samples extracted with the MAGicBead™ cfDNA Isolation Kit were enriched for sub-nucleosomal, ultra-short fragments. K-means clustering was conducted on fragment lengths to avoid a heuristic cutoff to define ultra-short fragments. Clustering identified 7 groups; cluster 1 consisted of ultra-short fragments of sizes 32-80 bp.

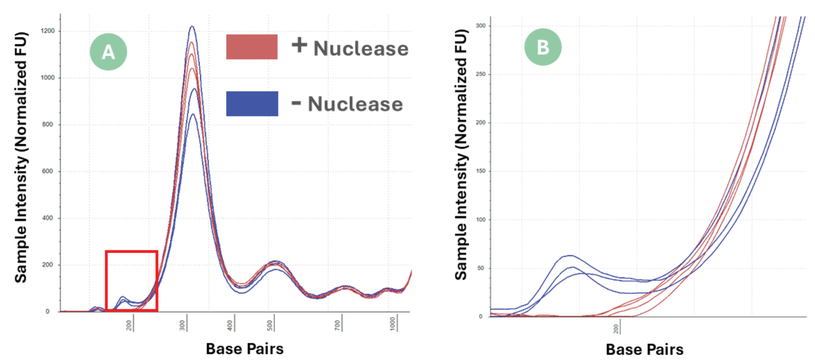


Figure 4: Ultra-short cfDNA fragments characterized as natively singe-stranded, Screentape quantification of final libraries (A) To test our ultra-short ssDNA hypothesis, six cfDNA replicates were isolated from pooled plasma. All replicates were treated with an optimized treatment protocol with three replicates containing 5 units of single-strand-specific nuclease. **(B)** Post-treatment, library prep revealed that the ultra-short cfDNA fragments were absent (red trace), supporting their single-stranded origin.

Fragmentomics Analysis of Cancer Plasma Samples Processed Using MAGicBead™ Technology and SPLAT Library Preparation

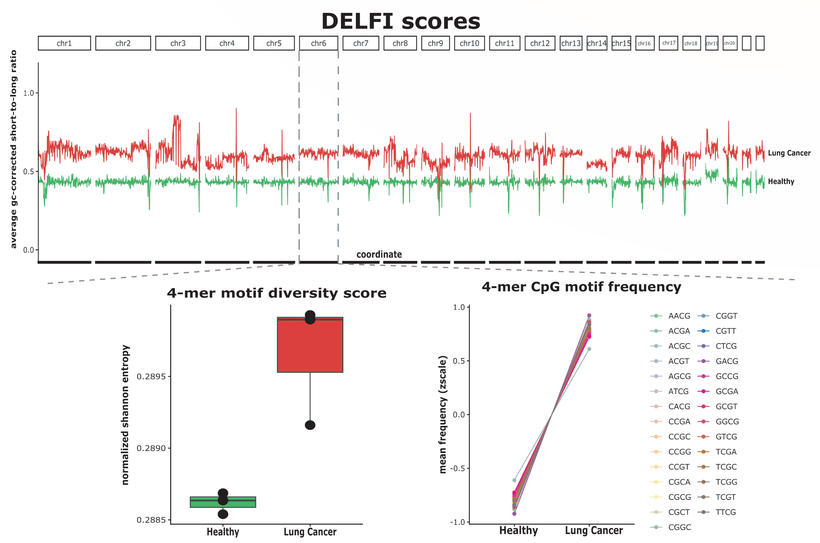


Figure 5. cfDNA was isolated from plasma from lung cancer donors (n = 3) and healthy donors (n = 3) using the MAGicBead™ cfDNA Isolation Kit. Donor samples were independently processed through cfDNA extraction, library preparation, NGS, and fragmentomic analysis, with technical duplicates generated for each donor. The ratio of short to long fragments was quantified in 1 Mb bins as described by Cristiano et al. Lung cancer samples showed a higher short-to-long fragment ratio than healthy controls, consistent with a previously reported DELFI-like fragmentation landscape. End-motif analysis on chromosome 6 also revealed higher 4-mer motif entropy and increased frequencies of CpG-containing motifs in lung cancer samples.

CONCLUSIONS

- **The MAGicBead™ cfDNA Isolation Kit, in combination with SPLAT library preparation, enables efficient recovery of ultra-short cfDNA fragments (<100 bp)** that are underrepresented or lost in conventional extraction and library workflows.
- **This workflow preserves the full cfDNA fragment size spectrum,** including sub-nucleosomal and ultra-short populations, enabling high-resolution fragmentomic analysis.
- **Enhanced recovery of ultra-short fragments reveals previously underexplored cfDNA features,** including distinct fragmentation patterns and regulatory region–associated signals.
- **These results highlight the impact of extraction chemistry and library compatibility on fragmentomic signal detection,** with implications for improving analytical sensitivity in cfDNA-based cancer diagnostics and biomarker discovery.

REFERENCES

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