Poster Number 5441

Agilent

Analytical Performance of Magnetic Bead-Based cfDNA Extraction with a Low-Input Targeted DNA Enrichment

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INTRODUCTION

Analytical alignment between cell-free DNA (cfDNA) extraction chemistry and downstream targeted DNA assays is essential for reliable molecular counting, especially at low plasma input. This study evaluated whether two magnetic bead-based cfDNA extraction methods exhibit differences in analytical performance within a low-input targeted DNA enrichment workflow.

MATERIALS & METHODS

Experiment I: Sixteen 3 mL plasma aliquots per donor were extracted in parallel using two bead-based kits—the Revolution cfDNA Max 20 Kit and MagMAX—producing paired eluates from identical input samples. QC utilized analysis via Agilent TapeStation cfDNA ScreenTape assay for nucleosomal-profile assessment. **Experiment II:** Plasma matrices containing a 0.2% allele-frequency cfDNA spike-in were extracted in duplicate from 1mL inputs using the same extraction kits. The primary metrics were the library-eligible cfDNA concentration and molecule recovery following the Agilent Avida targeted DNA enrichment workflow using the Avida DNA Expanded Cancer Panel, covering 105 cancer-associated genes. Secondary endpoints included VAF concordance, on-target fraction, background noise, and library complexity.

RESULTS

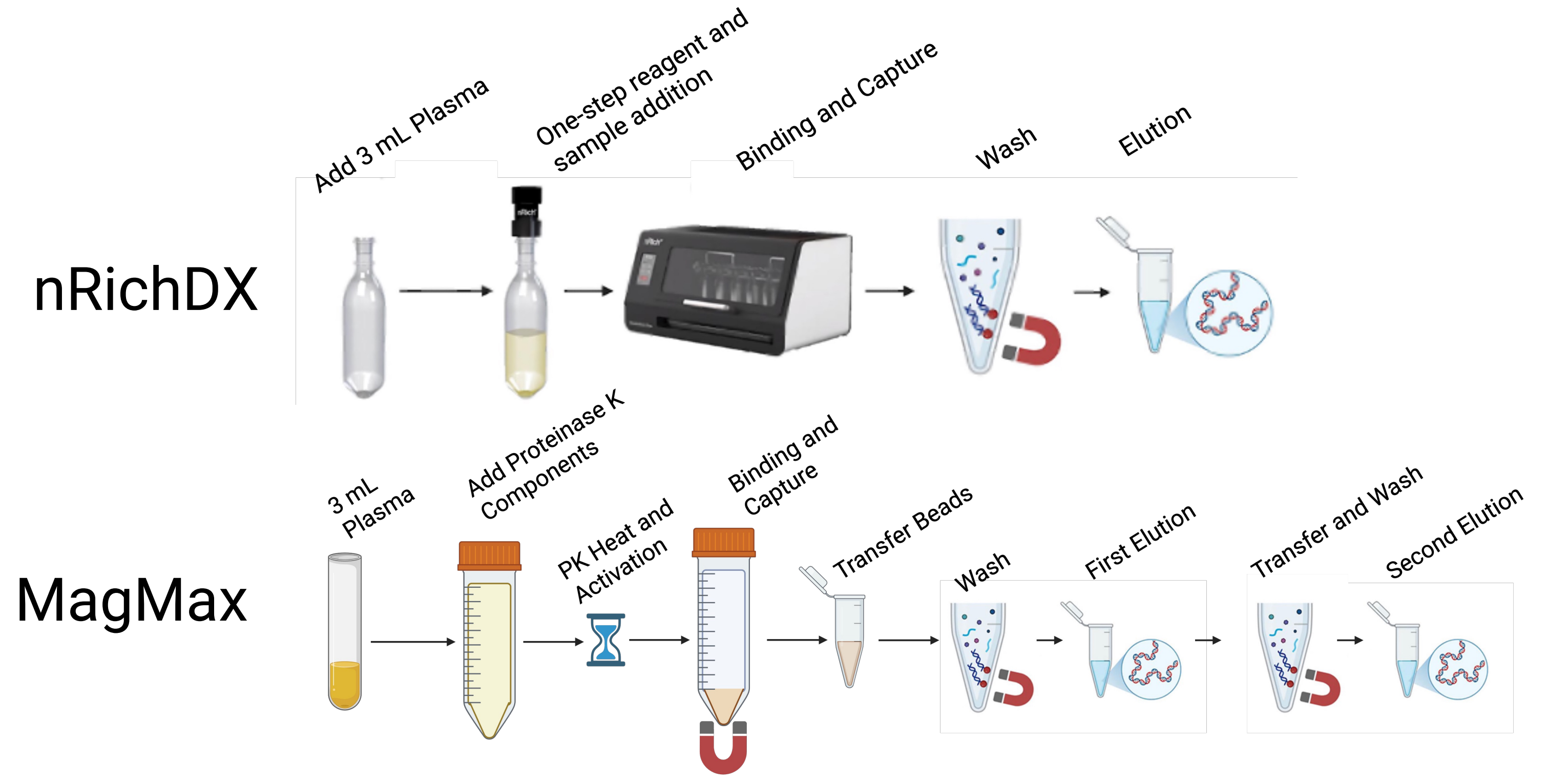

Figure 1. Comparison of cfDNA extraction workflows. The nRichDX platform enables a streamlined high-volume workflow with minimal hands-on time and no sample transfer steps, whereas the MagMax workflow involves multiple manual handling and transfer steps, increasing workflow complexity.

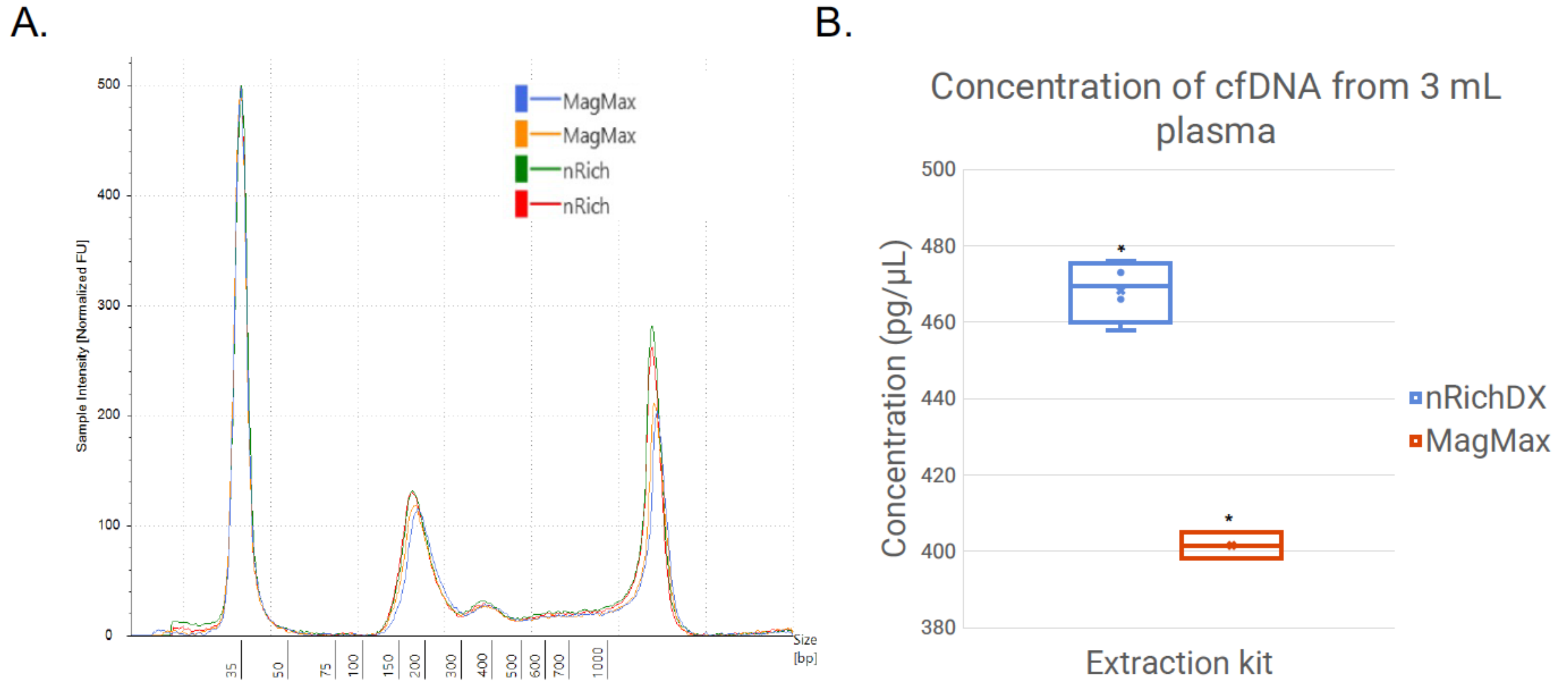

Figure 2. Total yield (ng) vs plasma volume. Total cfDNA yield as a function of plasma input volume. (A) Agilent cfDNA ScreenTape analysis confirmed successful recovery of library-eligible cfDNA fragments in the 50–700 bp size range. (B) Qubit fluorometric quantification was performed on plasma samples extracted using the nRichDX and MagMAX workflows. Box-and-whisker plot analysis showed significantly higher cfDNA yield with nRichDX relative to MagMAX (two-tailed paired t-test, $P < 0.05$), indicating improved recovery of cfDNA suitable for downstream library preparation.

A.

cfDNA extraction method	Uniformity measure across regions in the panel			
	pct0.2x_region_wise	Mean of replicates	pct0.5x_region_wise	Mean of replicates
MagMax	99.6	99.6	92.2	92.8
	99.6		93.3	
nRichDX	99.9	99.9	95.6	95.8
	99.9		96.1	

cfDNA extraction method	Uniformity measure across base in the panel			
	pct0.2x_base_wise	Mean of replicates	pct0.5x_base_wise	Mean of replicates
MagMax	98.5	98.6	88.9	89.4
	98.6		89.8	
nRichDx	99.0	99.0	92.0	92.1
	99.0		92.1	

B.

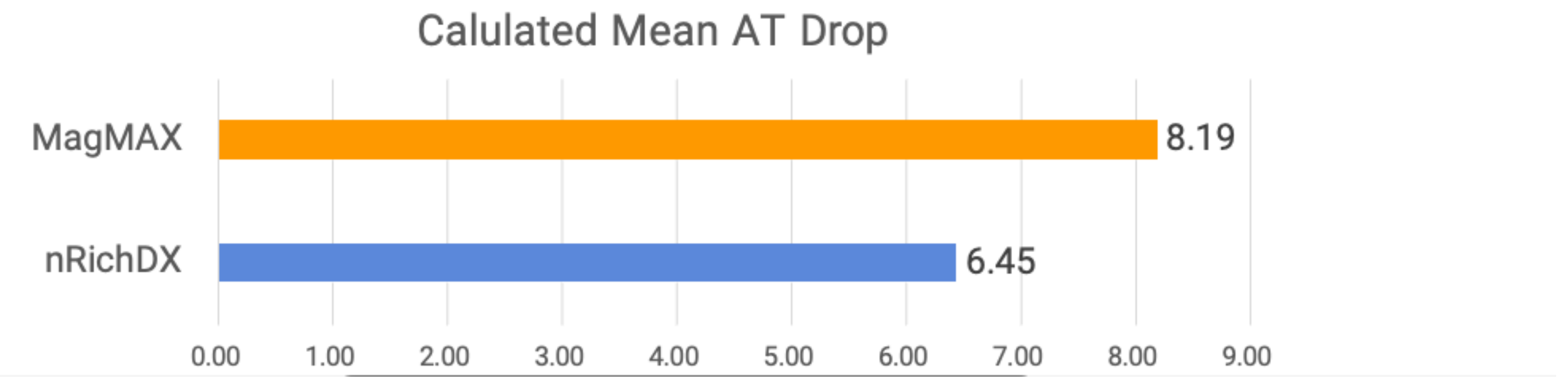

Figure 3. Improved sequencing uniformity from contrived spike-in plasma. (A) Target region and base capture analysis showed that samples extracted with nRichDX achieved high library capture at both 0.2× and 0.5× mean coverage thresholds. (B) Capture bias analysis, based on detection of high adenine-thymine (AT) content in low-coverage regions, indicated that nRichDX extractions produced libraries with greater sequencing uniformity and reduced coverage bias.

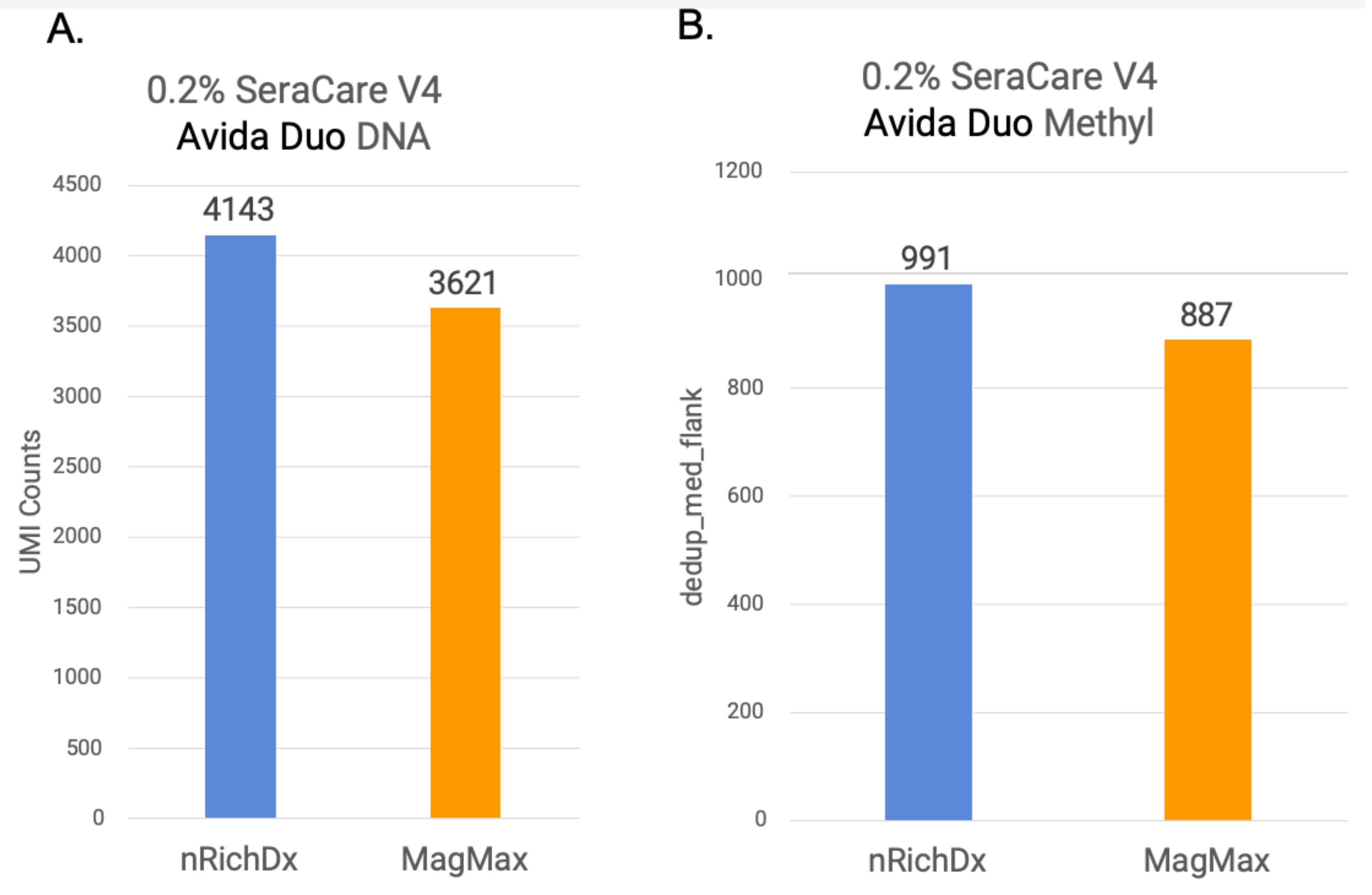

Figure 4. nRichDX Improved Molecular Recovery. (A) Avida Duo DNA capture from libraries of plasma samples spiked with Seraseq ctDNA V4 standard at 0.2% allele-frequency show nRichDX extractions produced greater molecule recovery at an input of 1 mL plasma. (B) The Avida Duo Methyl analysis also showed nRichDX as the better performing kit for molecule recovery. Both assays show nRichDX as the robust extraction method for low-input targeted DNA analysis.

CONCLUSION

Both magnetic bead-based methods generated cfDNA suitable for low- input targeted sequencing; however, the nRichDX Revolution cfDNA Max 20 Kit consistently showed superior pre-analytical performance. nRichDX improved recovery of library-eligible cfDNA, increased mutation and molecule detection metrics, and produced libraries with better sequencing uniformity and reduced bias compared with the TFS MagMax cfDNA Isolation Kit. Together, these findings support the Revolution cfDNA Max 20 Kit as a superior extraction workflow for low-input liquid biopsy applications requiring sensitive and reproducible downstream sequencing performance under the tested conditions.