Evaluation of cfDNA Mutation Recovery from Widest Sample Volume Range using Magnetic Bead-Based Extraction

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INTRODUCTION

Sensitive and accurate detection of low allele frequency circulating tumor DNA (ctDNA) variants is essential for early cancer detection, treatment monitoring, and minimal residual disease (MRD) assessment. One of the key challenges in these workflows is achieving high cfDNA extraction efficiency, especially when working with low-input volumes or low-abundance targets. Efficient cfDNA recovery is critical for maximizing downstream sensitivity and maintaining variant detection fidelity.

MATERIALS & METHODS

Nucleosome-sized ctDNA (n-ctDNA, SensID) was generated from tumor/normal-matched breast cancer cell lines using a proprietary fragmentation method. The tumor cell line carries the TP53 p.R175H mutation and was blended into the matched normal cell line to produce VAFs of 0.5% and 1.0%. These n-ctDNA blends (SensID), exhibiting patient-like fragment profiles, were spiked into DNA-free synthetic plasma (Plasma 1X, SensID) to simulate liquid biopsy samples. cfDNA extractions were performed at three input volumes 1 mL, 5 mL, and 20 mL using the nRichDX Revolution cfDNA Max 20 Kit (n=3 per condition). Total cfDNA yield was quantified using Qubit, and mutation detection was assessed by digital PCR (dPCR). Key metrics included yield, VAF, replicate consistency, and cfDNA purity via TapeStation.

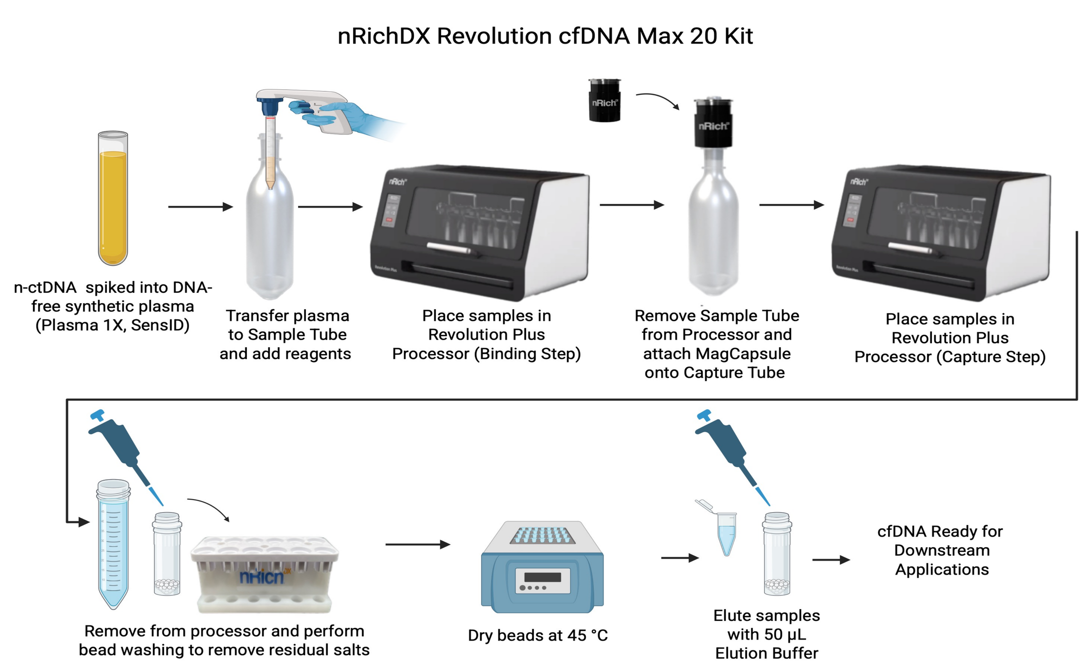


Figure 1. nRichDX Revolution cfDNA Max 20 Kit workflow.

RESULTS

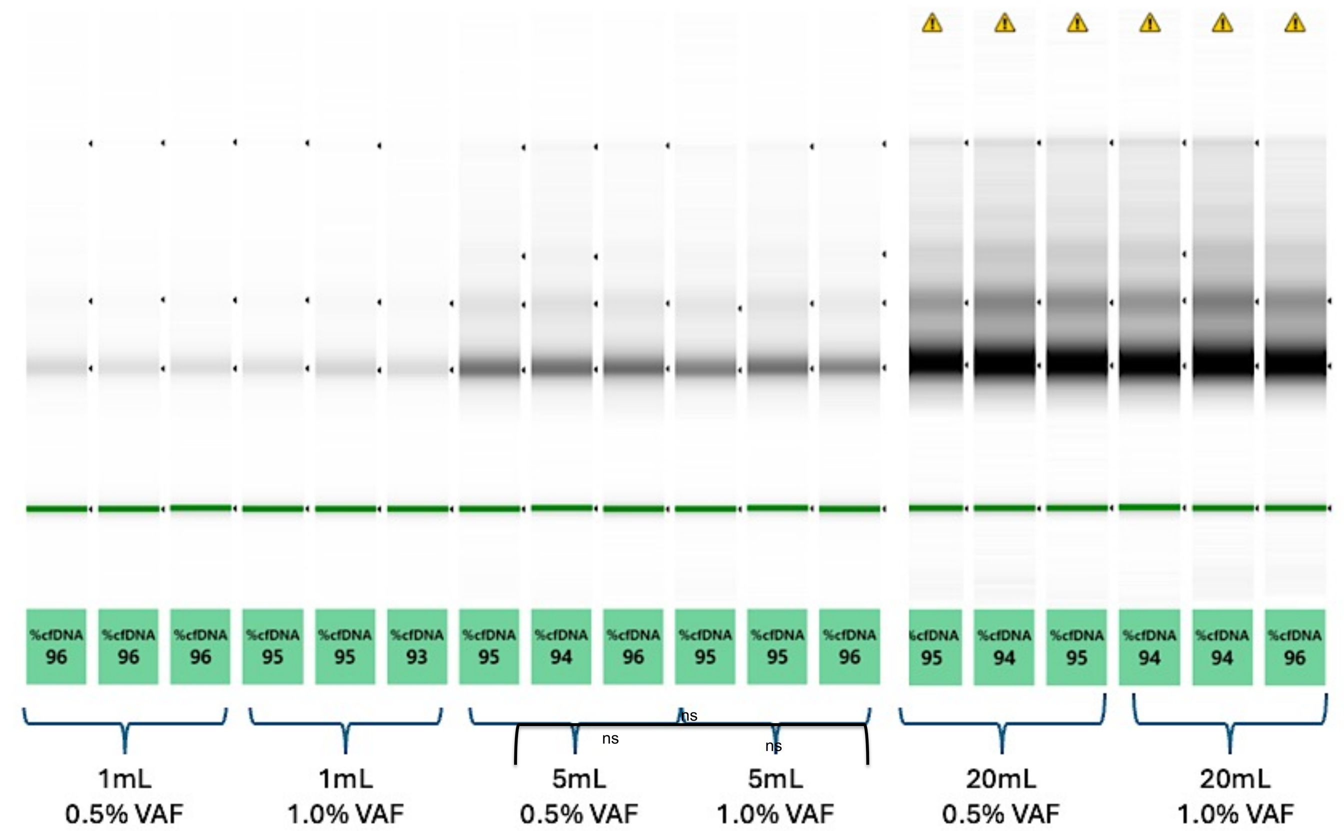


Figure 2. Agilent cfDNA ScreenTape gel. Synthetic plasma cfDNA spiked with nucleosome-sized ctDNA across volume inputs and %VAF. Analysis calculates %cfDNA recovery based on the sizing and concentration of measured total DNA in a sample. All extracted samples yielded highly effective cfDNA recovery >90%.

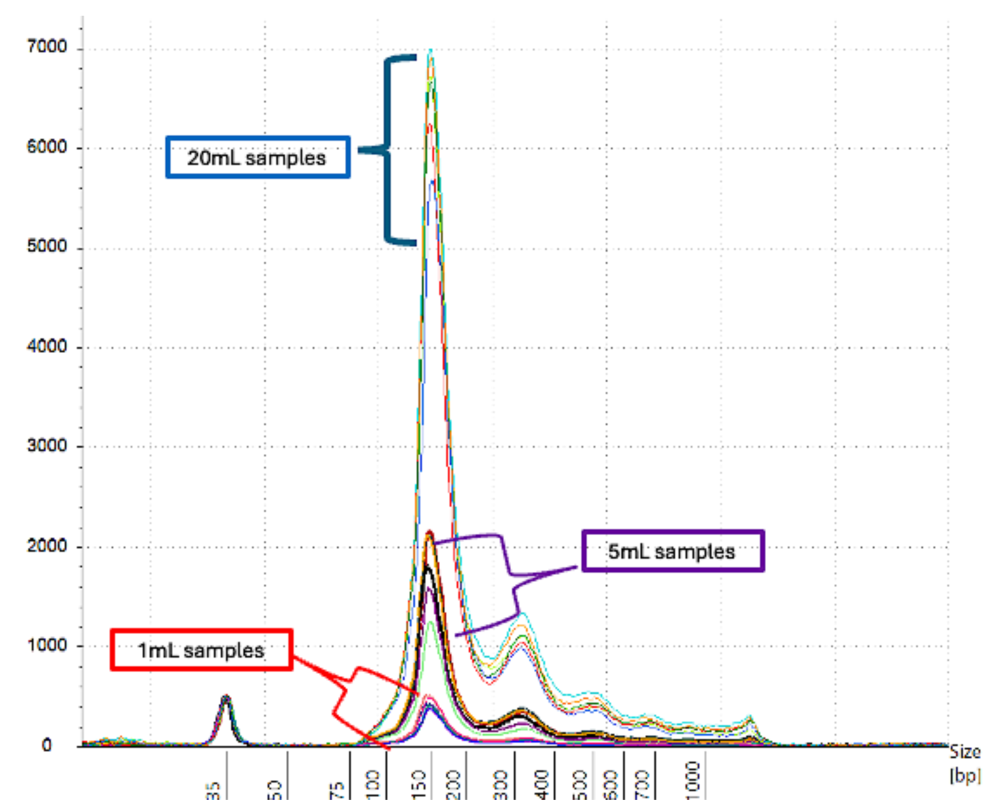
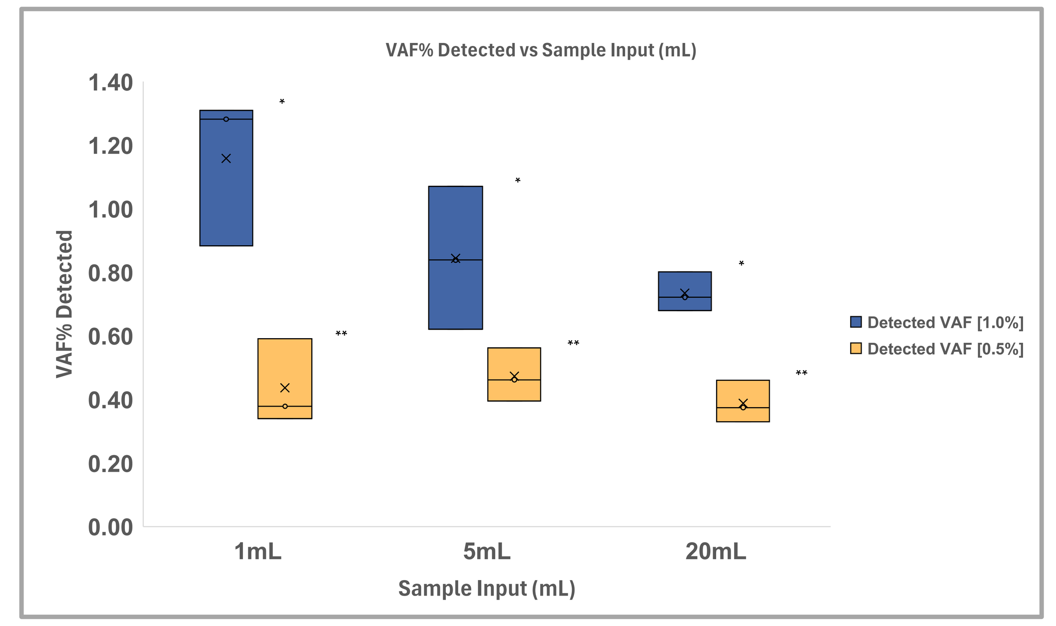


Figure 3. Agilent cfDNA ScreenTape electropherograms. Synthetic plasma spiked with nucleosome-sized ctDNA across volume inputs and %VAF. Representative image of electropherogram tracings of extracted cfDNA from synthetic plasma samples ranging from 1 mL - 20 mL. Profiles visualize increased mono-,di-, and tri-nucleosomal peaks as sample volume increases as well as minimal presence of genomic DNA (>700bp).

A)



B)

Input volume for Extraction (mL)	VAF [%]	Mean Value Detected VAF [%]	Mean Value VAF of original cfDNA Pool [%]	Recovery VAF [%]	**Mean Recovery VAF [%]**
1	0.50%	0.44	0.48	91%	**90%**
5		0.47		98%	
20		0.39		81%	
1	1.00%	1.16	1.04	111%	**88%**
5		0.84		81%	
20		0.73		71%	

Figure 4. VAF detection and recovery.

(A) Box-and-whisker plots of %VAF in 1.0% and 0.5% VAF samples across input volumes 1, 5, and 20 mL. Asterisks (*, **) denote $P > 0.05$ within each VAF group (two-tailed paired t-test).

(B) Mean %VAF used to calculate percent recovery from each extraction. Increasing input volume reduces replicate variability and improves consistency.

CONCLUSION

This study demonstrates that accurate recovery of low-frequency mutations can be achieved across a broad range of input volumes. Extractions across input volumes and VAF had consistently high cfDNA yield, accurate VAF detection, and maintained high cfDNA integrity. These results highlight the importance of efficient cfDNA extraction for applications requiring high analytical sensitivity, such as early cancer detection and MRD monitoring. Consistent results in synthetic plasma show that this magnetic bead–based extraction suits low-yield applications. Experiments used n-ctDNA spiked into DNA-free synthetic plasma (SensID), providing a standardized, workflow control that enabled reproducible performance assessment. The nRichDX Revolution cfDNA Max 20 Kit delivers high-efficiency, scalable cfDNA recovery from a wide range of inputs to support rare-variant detection.