Detection of Somatic Variants in Breast Cancer from Large Volumes of Plasma Using the nRich^{DX} Revolution System™

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INTRODUCTION

Breast cancer is the most common cancer in women in the United States; about 1 in 8 women will have breast cancer during their lifetime. Detecting breast cancer early can significantly increase the survival rate. Liquid biopsy can potentially revolutionize the methods of identifying breast cancers early. However, the circulating tumor DNA (ctDNA) needed for diagnosis comprises <1% of cfDNA during the early stages of cancer. cfDNA isolation kits with higher yields are scarce due to lower extraction efficiency and limiting sample capacity (≤5mL). The nRich^{DX} Revolution cfDNA isolation method can significantly increase cfDNA yield through larger sample capacity in a single extraction (up to 20mL) and higher recovery rates. Higher analyte yield translates to accurate molecular diagnostic results at low allele frequencies.

MATERIALS & METHODS

Blood was collected in K2EDTA tubes and was processed following the nRich^{DX} centrifugation protocol in preparation for the cfDNA extraction. 5mL and 20mL plasma samples were extracted using the nRich^{DX} Revolution cfDNA Isolation Kit. All samples were spiked with a cfDNA standard containing the PIK3CA E545K mutation, a known breast cancer mutation, at a concentration of 10ng/mL. Samples were eluted in 50µL. The cfDNA yield was determined by fluorescence on the Qubit™ 4 Fluorometer. Recovery of the breast cancer mutation was assessed using Ct values obtained through a PIK3CA E545K mutation detection assay on the QuantStudio 3 Real-Time PCR System. The quality of extracted cfDNA was determined using Agilent High Sensitivity cfDNA ScreenTape.

RESULTS

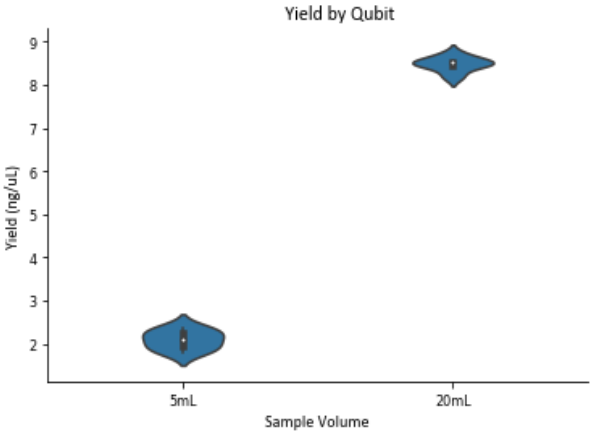


Figure 1. Yield quantified by Qubit (ng/µL) of 5mL and 20mL samples display high precision and proportion across the two sample volumes.

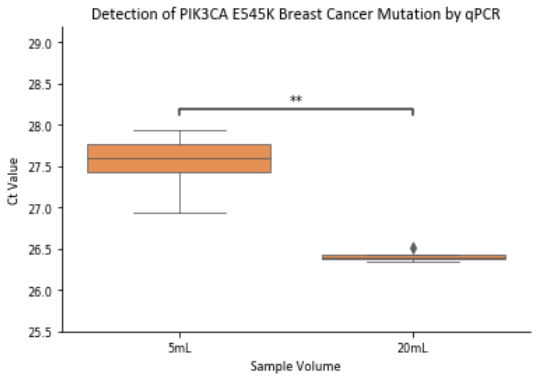
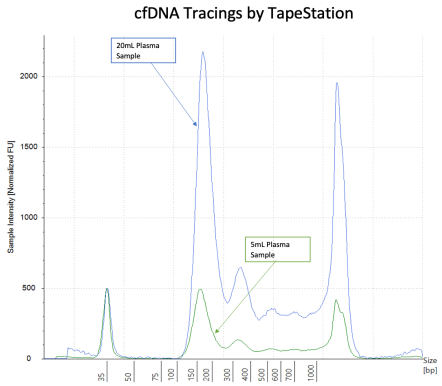


Figure 2. Ct values using a PIK3CA E545K mutation detection assay determined a significant difference (p-value <0.05) between the 5mL and 20mL sample volumes. Indicating that extracting from larger sample volumes leads to increased analyte sensitivity.



Sample Volume (mL)	Base Pair Range (bp)	Concentration (pg/µL)	cfDNA Percentage (%)
5	50-700 (cfDNA)	964	92%
	701-1000 (gDNA)	87.5	
20	50-700 (cfDNA)	4250	91%
	701-1000 (gDNA)	413	

Figure 3. TapeStation electropherogram tracings show the 20mL sample volume demonstrating ~4X times cfDNA concentrations (50-700 bp) compared to the 5mL sample volume. cfDNA extraction at both sample volumes shows minimal genomic DNA (gDNA) contamination (≤9%).

CONCLUSION

The extracted cfDNA yield quantified by the area under 50-700bp of TapeStation tracings showed ~90% extraction efficiency. Qubit and TapeStation results illustrated that yield from the 20mL sample volume is ~4X greater than the 5mL sample volume. TapeStation tracings also demonstrated a high quality of cfDNA with very low genomic DNA contamination (≤9%). qPCR confirmed the detection of the PIK3CA E545K breast cancer mutation while maintaining high precision. In previous studies, we showed the capability of the Revolution System to detect cfDNA from lung cancer; in the current study, we demonstrated the detection of cfDNA from breast cancer. The nRich^{DX} Revolution system allows for scalability up to 20mL with no transfer steps and no eluant pooling. The combination of larger samples and higher recovery rates can lead to better somatic mutation detection and early detection of different cancer types.