Semi-Automated High Yield cfDNA Extraction Using nRich^{DX} Revolution Sample Prep System on Eppendorf epMotion for Liquid Biopsy Development

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INTRODUCTION

Liquid biopsies have been on the rise due to their minimally invasive procedure and broad clinical applications. There are many biomarkers available in liquid biopsies, among them, cell-free DNA (cfDNA) has become one of the most useful due to its specificity in early cancer detection. However, the isolation of cfDNA from plasma and other biological fluids are extremely challenging due to their low concentration. cfDNA extraction kits often struggle to obtain sufficient cfDNA yield while maintaining precision due to their small volume input. The nRich^{DX} Revolution cfDNA system is intended to extract cfDNA and ctDNA from biological fluids using a semi-automated workflow. The Revolution semi-automated workflow precisely extracts nucleic acids from large volumes of biological fluids (1-20mL). This workflow does not utilize yield-lowering sample pooling and increases cfDNA yield for downstream applications such as qPCR, ddPCR, and NGS.

MATERIALS & METHODS

cfDNA extractions were performed using the nRich^{DX} Revolution Sample Prep System on Eppendorf epMotion. Apheresis-derived human plasma was stored in K2EDTA and was processed in preparation for cfDNA extraction. Plasma samples ranging from 1-20mL were extracted following the Revolution Semi-Automated Workflow Instructions for Use (IFU). The cfDNA yield was determined by fluorescence on the Qubit^{TM} 4 Fluorometer. The percent recovery of cfDNA was determined by a KRAS G12V mutation detection assay on the QuantStudio 3 Real-Time PCR System. The quality of extracted cfDNA was determined using Agilent TapeStation High Sensitivity D1000 ScreenTape.

RESULTS

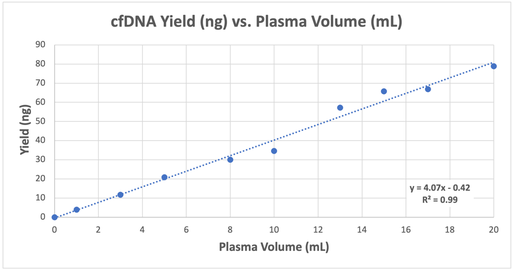


Figure 1. The total cfDNA yield (ng) was proportional across samples of 1-20mL plasma volume with a strong correlation coefficient ($R^2 \geq 0.99$) using the semi-automated workflow.

Plasma Volume (mL)	cfDNA Yield (ng/mL)	Standard Deviation	%CV
1	3.9	0.3	7%
3	3.9	0.3	7%
5	4.2	0.1	3%
8	3.8	0.1	4%
10	3.5	0.1	2%
13	4.4	0.1	3%
15	4.4	0.1	2%
17	3.9	0.0	1%
20	3.9	0.0	1%

Figure 2. For precision analysis, the percent coefficient of variation ranged from 1% to 7%. The mean cfDNA yield (ng/mL) for all sample volumes were all within ±20% of each other.

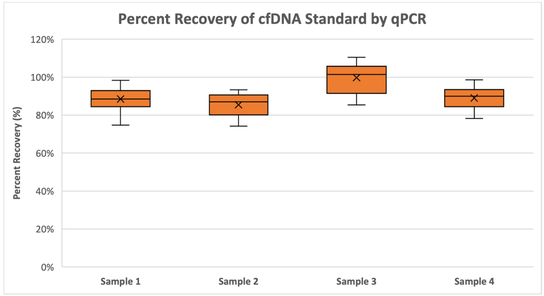
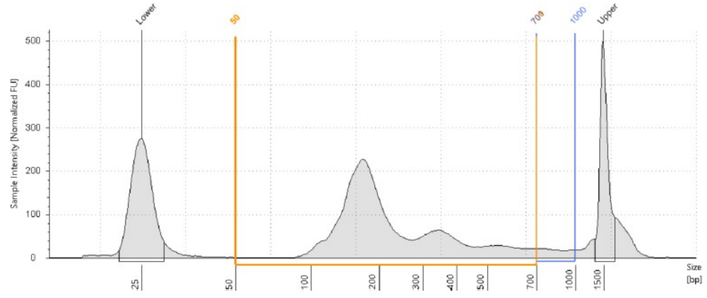


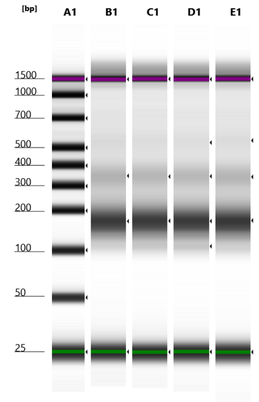
Figure 3. The yield of cfDNA recovered through a KRAS G12V mutation detection assay was determined to be between 70% and 110% as determined by Real-Time qPCR. Each sample is the result of 12 technical replicates.



Base Pair Range (bp)	Concentration (pg/µL)	Percent of Total DNA (%)
50 - 700 (cfDNA)	809.0	95%
701 - 1000 (gDNA)	38.4	5%

Figure 4. The TapeStation electropherogram traced fragment peaks at ~180bp (monomer), ~360bp (dimer), and ~540bp (trimer) corresponding to the expected tracings of the cfDNA standard. cfDNA as quantified between 50bp and 700bp was ≥90% of the total extracted DNA while genomic DNA as quantified greater than 700bp was <10%.

Figure 5. Gel electrophoresis indicated the isolation of the monomer, dimer, and trimer of cfDNA.



CONCLUSION

The Revolution cfDNA Isolation Kit with semi-automated workflow on the Eppendorf ep*Motion* 5073 instrument successfully extracts cfDNA and ctDNA with consistently high yields without yield-lowering sample pooling. This workflow can obtain high cfDNA yield with minimal genomic DNA. The workflow's high throughput allowed a single technician to process 48 samples over the course of a standard workday with high precision.