Assessment of Cell-free DNA (cfDNA) Extraction using the nRich^{DX} Revolution Sample Prep System from Plasma, Urine, and Pancreatic Cyst Fluid

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INTRODUCTION

As tumor cells undergo apoptosis, cfDNA is generated. The need for an efficient, standardized cfDNA extraction method to isolate cfDNA and a fraction of cfDNA tumor-specific genetic alterations, or circulating tumor DNA (ctDNA), is essential to optimize biomarker analysis in oncology. Here, the performance of the Revolution Sample Prep System cfDNA isolation kit was evaluated using different clinical specimens.

METHODS

Proof of principle concept workflow, sample processing, and cfDNA extraction method

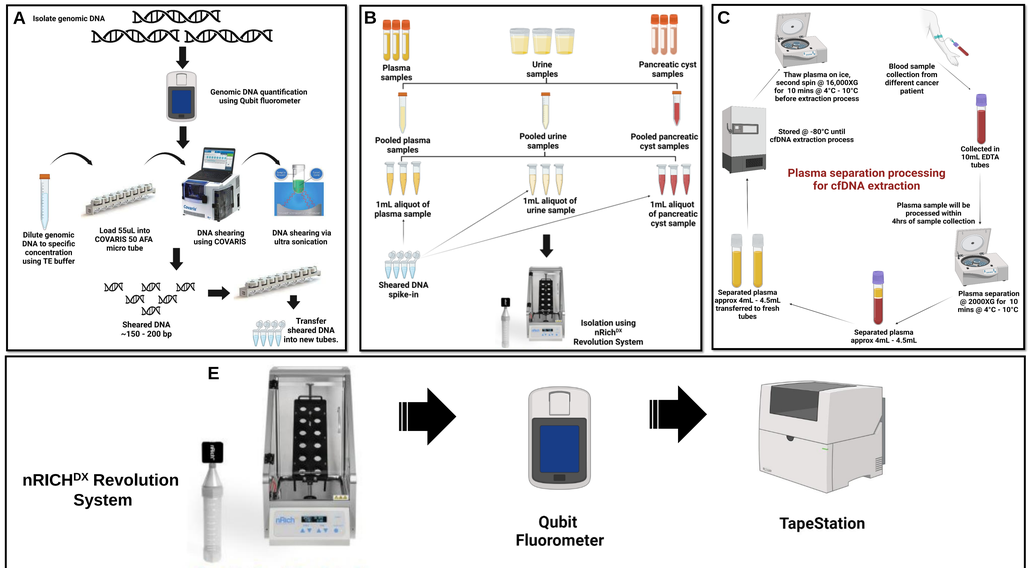

Figure 1: cfDNA extraction workflow. *(A) Isolation & quantification of genomic DNA (gDNA) using pooled blood samples. The isolated gDNA (5µg) samples were sheared equivalent to cfDNA base pair (~150 – 200bp) using COVARIS for the spike-in study. (B) The sheared DNA samples were quantified and spiked at 30ng/mL concentration into 1mL of pooled plasma, urine, or pancreatic cyst fluid samples respectively. The samples were processed for cfDNA extraction using the nRich^{DX} Revolution System. (C) cfDNA extraction validation workflow from plasma. (D) All the samples were extracted using the nRich^{DX} Revolution System and quantified using a Qubit fluorimeter and TapeStation for quality control.*

RESULTS

1. Genomic DNA spike-in proof of principle study using plasma, urine, and pancreatic cyst fluid samples

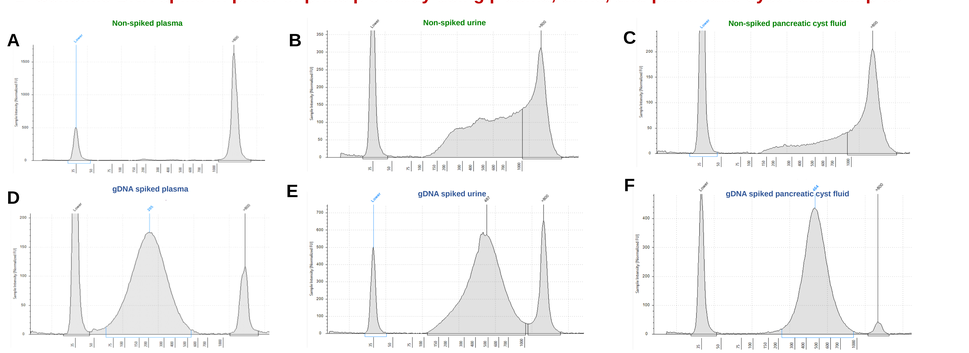

Figure 2: Evaluation of plasma, urine and pancreatic cyst fluid samples using nRich^{DX} Revolution System for the extraction of cfDNA. *(A), (B), (C) TapeStation electropherogram from plasma, urine and pancreatic cyst fluid samples (non spiked). (D), (E), (F) gDNA spiked into plasma, urine and pancreatic cyst fluid samples displaying fragment peaks at 295bp, 487bp and 464bp respectively after cfDNA extraction using nRich^{DX} and cfDNA ScreenTape quantification.*

2. Linearity and limit of detection using nRich^{DX} cfDNA reference material.

3. Extraction specificity controls for quality control assessment.

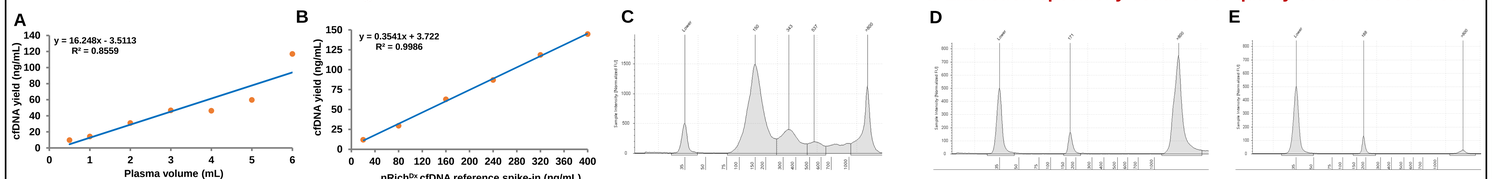

Figure 3: Evaluation of linearity, detection limit, and quality control. *(A) The total nRich^{DX} cfDNA reference material spike-in yield (ng/mL) is proportional across samples of 0.5 to 6mL DNA free plasma volume with a good correlation coefficient R^2≥0.85. (B) LoD with 20 - 400µl nRich^{DX} cfDNA reference material spike-in displayed strong correlation coefficient R^2≥0.99. (C), (D) TapeStation electropherogram for linearity and limit of detection. (E) TapeStation electropherogram displaying fragment peaks at ~ 150 -180bp (monomer), ~350bp (dimer), and ~540bp (trimer) >800bp (gDNA) for extraction specificity quality control material.*

4. Extraction of cfDNA using clinical samples.

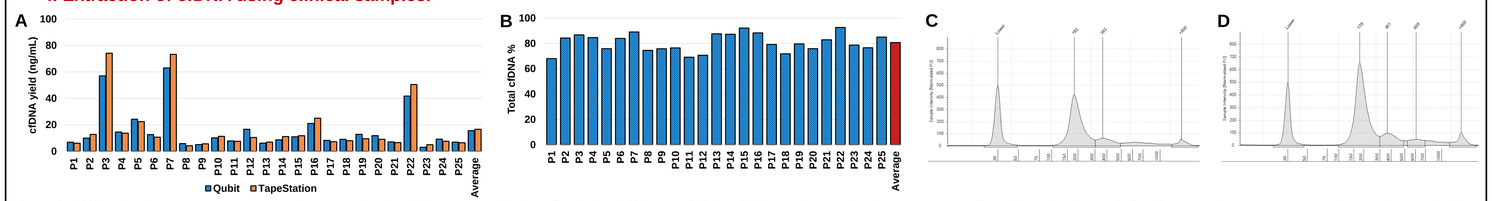

Figure 4: cfDNA extractions from clinical samples of cancer patients. *(A) Comparison of Qubit and TapeStation cfDNA yield. (B) Total cfDNA percentage in patient samples based on Tapestation data. (C) and (D) TapeStation electropherogram displaying fragment peaks at ~180bp (monomer), ~375bp (dimer), and ~600bp (trimer) >800bp (gDNA) from two different clinical samples.*

5. Precision and reproducibility.

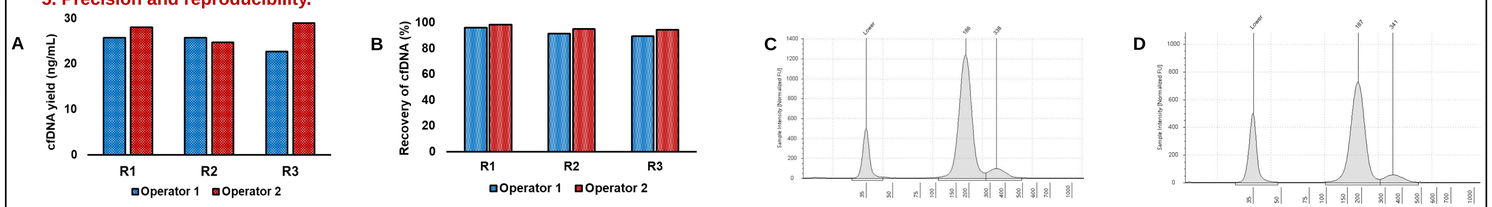

Figure 5: Data representing precision and reproducibility. *(A) Comparison of cfDNA yield between two operators. B) Inter-operator comparison for the cfDNA recovery. (C) and (D) TapeStation electropherograms from two different operators using the same sample.*

6. Evaluation of sample stability

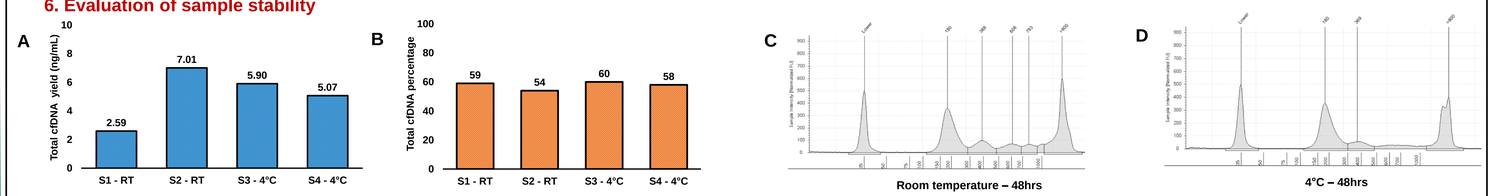

Figure 6: Assessment of sample stability. *(A) Comparison of cfDNA yield from blood samples stored at room temperature (RT) versus 4°C for >48hrs. (B) Amount of total cfDNA present in the samples stored at room temperature (RT) versus 4°C for >48hrs. (C) TapeStation electropherogram from the extracted samples (RT and 4°C) displaying fragment peaks at ~180bp (monomer), ~370-380bp (dimer), ~600bp (trimer) with significant traces of gDNA (>800bp).*

CONCLUSIONS

- The proof of study using a sheared gDNA spike-in to the plasma, urine and pancreatic cyst fluid showed a effective cfDNA extraction and yield from different bodily fluids.
- Linearity and LoD data showed a good correlation and cfDNA extraction from the patient samples displayed an average yield of 16ng/ml cfDNA and presence of 80% total cfDNA. Intra-operator data showed a good correlation between cfDNA yield and its recovery.
- Sample stability demonstrated variability in cfDNA yield at room temperature compared to 4°C. Based on the TapeStation data the average total cfDNA presence was around 57% present in these samples (RT and 4°C).
- This study demonstrates the feasibility of cfDNA extraction from different bodily fluids (plasma, urine and pancreatic cyst fluid) using the nRich^{DX} Revolution System.
- The development of reliable and non-invasive tests using different bodily fluids holds great promise for early detection and improved outcomes in different cancer types.

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