Method Comparison of Commercially-Available cfDNA Extraction Products for Minimum Residual Disease Next Generation Sequencing

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Background

For liquid biopsy applications, high recovery of total cfDNA is essential for detection of the ctDNA component within an individual's total cfDNA. Due to requisite low limits of detection, accurate tumor-informed minimum residual disease (MRD) by next generation sequencing (NGS) testing is highly dependent upon maximum recovery of cfDNA from a cancer patient's separated plasma. In the following study, 4 different commercially-available cfDNA extraction methods were compared to determine the most suitable method for MRD NGS.

Methods

8 tubes of whole blood were collected for 20 healthy individuals during a single draw using the Qiagen PAXgene blood ccfDNA collection device. Samples were transported to Strata Oncology overnight and processed the following day. Plasma was isolated from cellular material by high-speed centrifugation. All separated plasma was stored at -80°C until cfDNA extraction was performed.

Plasma was extracted using 4 different commercially available cfDNA extraction methodologies: Qiagen QIAamp Circulating Nucleic Acid Kit, Promega Maxwell RSC ccfDNA Plasma Kit, Thermo Fisher MagMAX Cell-Free DNA (cfDNA) Isolation Kit, and nRichDX Revolution cfDNA Max 20 Kit. 8mL plasma was utilized for each cfDNA extraction. Purified cfDNA was evaluated for quality and quantity using the Agilent High Sensitivity D1000 ScreenTape Assay and the Agilent cfDNA ScreenTape Assay for the Agilent TapeStation 4200. During quality evaluation, cfDNA quality and quantity was assessed from 50-700bp, and gDNA was assumed at >700bp. Datasets for each extraction method were compared by a nonparametric analysis.

Results

Upon visual examination of the Agilent TapeStation electropherogram data, the fragmentation profiles of healthy individuals are remarkably consistent. However, fragmentation profiles differ with cancer patients, and cfDNA fragmentation contains crucial information. The Thermo, Promega, and nRichDX extraction methods all display strong preservation of cfDNA integrity cfDNA, given the consistent isolation of di-nucleosomal cfDNA fragments. In addition, all three bead-based methods showed lower levels of gDNA contamination as compared to Qiagen QIAamp Circulating Nucleic Acid Kit.

While Thermo Fisher MagMAX Cell-Free DNA (cfDNA) Isolation Kit yielded a higher percent cfDNA, and both Thermo Fisher MagMAX and Promega Maxwell RSC ccfDNA Plasma Kit yielded similar average cfDNA size in bp when compared to nRichDX Revolution cfDNA Max 20 Kit, the nRichDX Revolution consistently provided both higher cfDNA concentration by ~2x as well as the highest overall yield in comparison to the other 3 methods.

Figure 1. Agilent High Sensitivity D1000 ScreenTape Electropherogram Comparison (Orientation Indicated at 500 RFU)

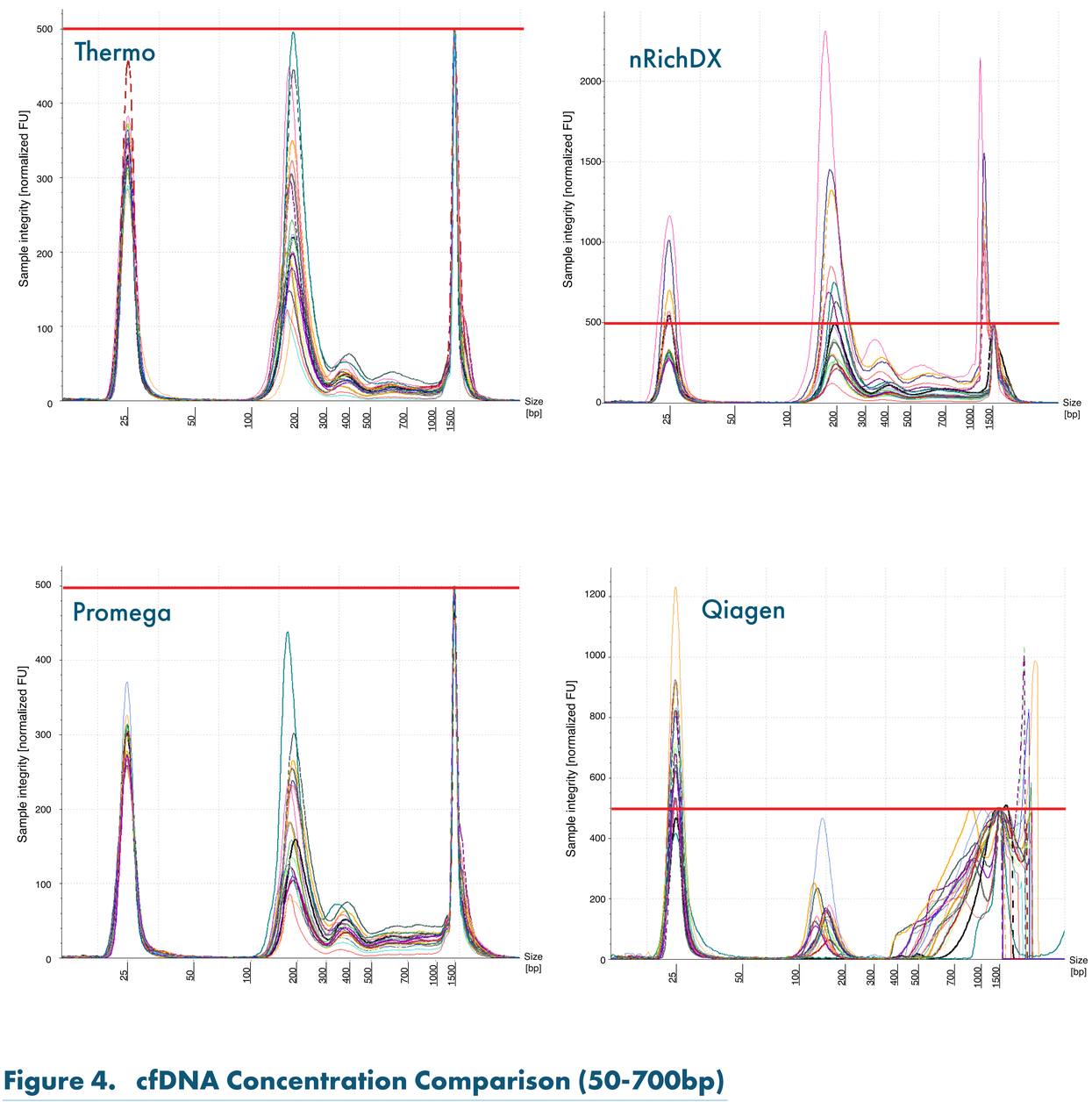
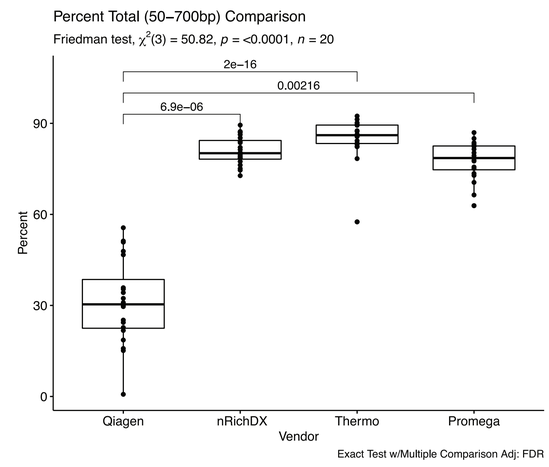


Figure 2. Percent cfDNA Comparison



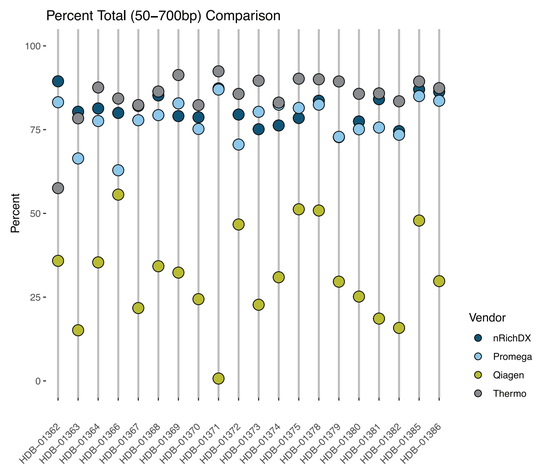
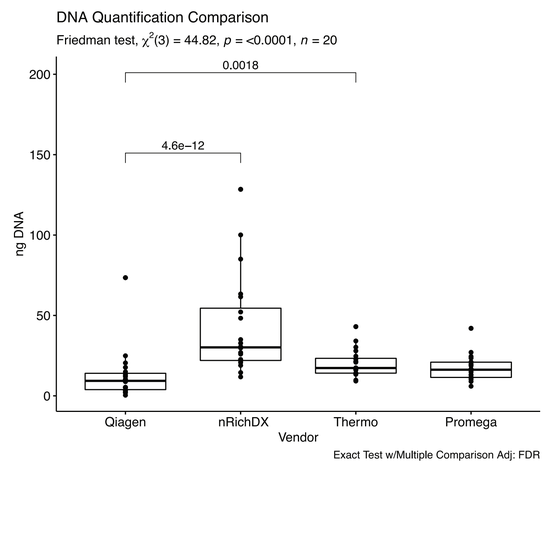


Figure 3. cfDNA Quantification Comparison



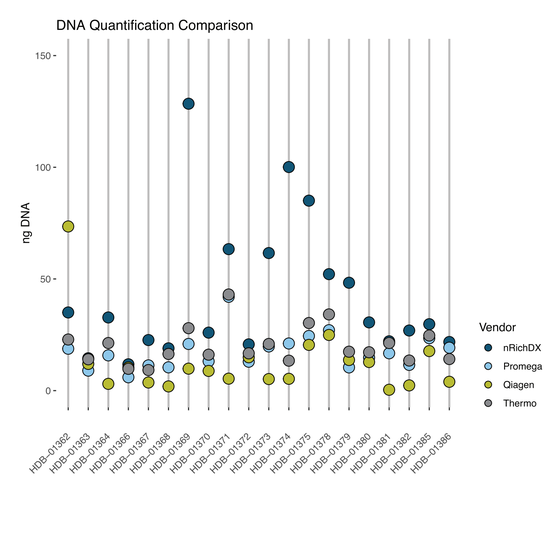
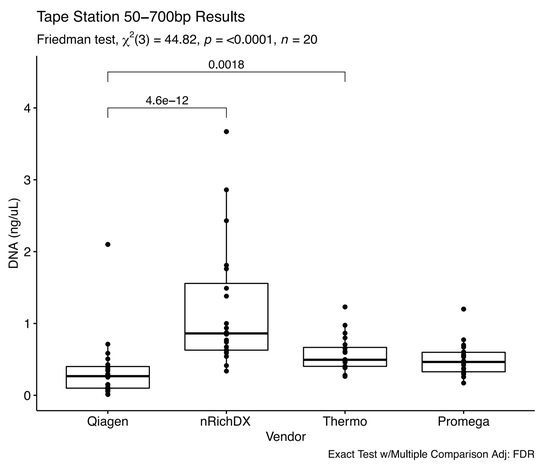
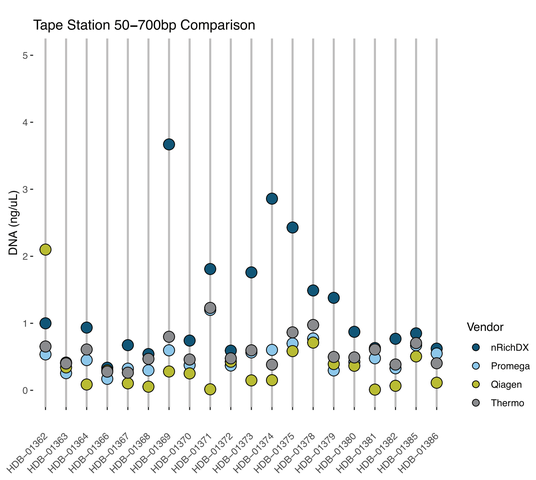


Figure 4. cfDNA Concentration Comparison (50-700bp)





Conclusions

The nRichDX Revolution cfDNA Max 20 Kit with the nRichDX Revolution Sample Prep System yields higher quality cfDNA with less evidence of gDNA contamination when compared to other commercially available cfDNA extraction methodologies. The consistent performance of nRichDX Revolution makes this cfDNA extraction method a strong candidate for applications such as minimum residual disease testing where yield directly impacts limit of detection (LoD).

Additional Information

Questions? Contact Travis Reeder at travis.reeder@strataoncology.com