MinION Nanopore Sequencing of cell-free DNA (cfDNA) and cell-free RNA(cfRNA) extracted from human plasma using nRich^{DX} Revolution System

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INTRODUCTION

Circulating cell-free DNA (cfDNA) found in various biofluids can harbor genetic alterations that serve as hallmarks of cancer. These analytes can be isolated with a minimally invasive procedure and can help track disease state and progression. Next Generation Sequencing (NGS) has emerged as a strong candidate for analyzing cfDNA due to its ability to detect the mutation status in genes at various loci. The Oxford Nanopore NGS technology is capable of sequencing short to ultra-long (>4Mb) DNA or RNA from as little as 100ng of nucleic acid. It possesses advantages over qPCR and fluorescence analysis where there are limitations in mutation detection capabilities, or where information regarding the DNA sequence can not be obtained.

Here we utilize Nanopore Sequencing to compare the whole genome coverage from samples extracted using the nRich^{DX} cfDNA Isolation Kit, Qiagen QIAmp Circulating Nucleic Acid Kit, and the Thermo Fisher MagMAX Cell-Free Total Nucleic Acid Isolation Kit. We compare coverage across the genome and the quality of the sequencing to observe the impacts of extraction method on sequencing results.

MATERIALS AND METHODS

Apheresis derived pooled human plasma was processed with a 10-minute spin at 16000g to isolate cell free plasma. All plasma used in this study was from the same manufacturing lot, minimizing variability in background cfDNA between samples. Once processed, cell-free plasma was stored at -20˚C until time of use in this study. For each extraction method tested, thawed human plasma samples were spiked with 100ng/sample (20ng/mL) of internal spike standard and mixed prior to use. cfDNA extraction was performed using the nRich^{DX} cfDNA Isolation Kit, Qiagen QIAmp Circulating Nucleic Acid Kit, and the Thermo Fisher MagMAX Cell-Free Total Nucleic Acid Isolation Kit following the manufacturer protocol. All samples were extracted for the 5mL sample volume for consistency and due to maximum volume constraints of the three kits: 5mL for Qiagen, 6mL for Thermo Fisher, and 20mL for nRich^{DX}. All samples were eluted in 50µL. 10µL of each extracted sample was quantified on Qubit 1X dsDNA HS Assay (Thermo Fisher) and 2µL of the sample was assayed on the TapeStation Cell-free DNA ScreenTape (Agilent). 80-100ng of each eluate was prepared for sequencing using the Ligation Kit SQK-LSK110 and sequenced on the MinION Device (Oxford Nanopore Technologies) for 24 hours. Sequencing results from each sample were analyzed through whole genome alignment (human) on the EPI2ME Agent software (Oxford Nanopore Technologies).

RESULTS

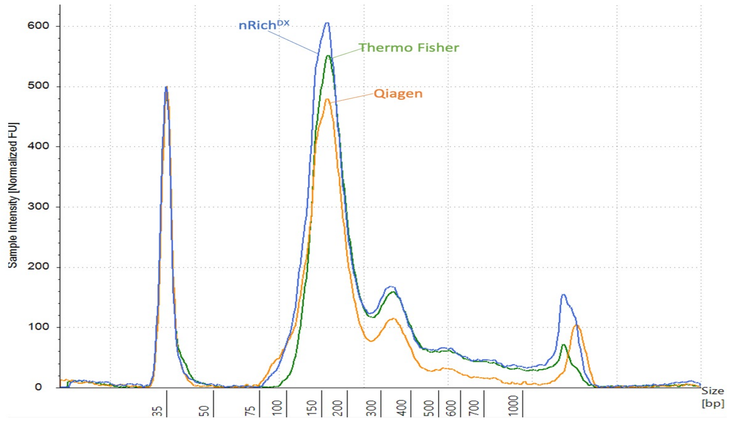

Figure 1. Agilent TapeStation Cell-free DNA ScreenTape Analysis of extracted DNA. The TapeStation electropherogram traced large fragment peaks at ~180bp (monomer), ~360bp (dimer), and ~540bp (trimer) consistent with the internal spike standard's fragment size profile. All samples show trace amounts of genomic DNA.

	Average Sequence Length (bp)	Average Quality Score	Average Alignment Accuracy (%)
nRich^{DX} Sample	507	14.71	97.0
Qiagen Sample	441	14.27	96.9
Thermo Fisher Sample	460	14.12	96.6

Figure 2. Sequencing results of eluates obtained using various commercially available cfDNA isolation methods. Sequencing Quality Scores and Average Accuracy are calculated through the Oxford Nanopore Technologies EPI2ME Agent software. All samples had a quality score >14.0 and an average accuracy above 96.0%.

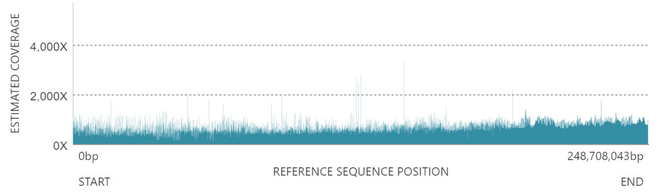

Figure 3. Whole genome alignment of sequenced nRich^{DX} sample.

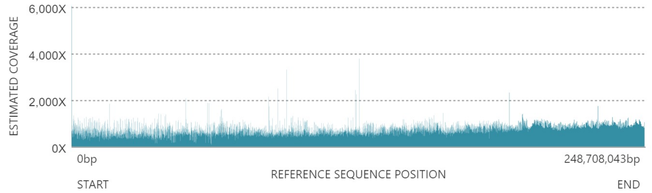

Figure 4. Whole genome alignment of sequenced Qiagen sample.

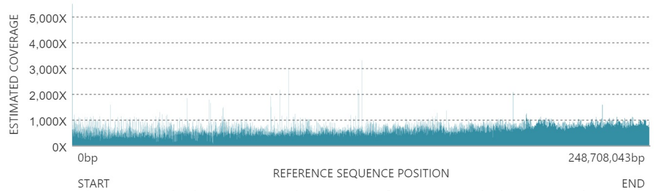

Figure 5. Whole genome alignment of sequenced Thermo Fisher sample.

CONCLUSION

Here we demonstrated the feasibility of using the Oxford Nanopore Technologies Ligation Kit SQK-LSK110 and the MinION device for the sequencing of cfDNA from human plasma samples. The MinION device was able to sequence short DNA fragments present in nRich^{DX}, Qiagen, and Thermo Fisher samples with high accuracy >96.0% . We also observed high quality scores of >14.0 for all samples. The capability to sequence small DNA fragments such as cfDNA on the MinION raises the potential for further in-depth studies of cfDNA found in human biofluids. The sequencing data can also be used for single nucleotide variant (SNV) analysis of multiple sites, providing more information about the sample than other analytical methods available. Our results also highlight the potential for applying the MinION device for studies of cfRNA found in human biofluids. We plan to use the MinION device in future studies to also evaluate the impacts of nRich^{DX}'s large volume capabilities on sequencing depth.