Initial Development of a Library Preparation and Analysis Protocol for Preimplantation Genetic Testing of Chromosomal Structural Rearrangements Using Oxford Nanopore Technologies (ONT)

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Objective

The aim of this study was to develop a standard library preparation and analysis protocol for the use of a handheld, nanopore-based DNA sequencer for preimplantation genetic testing of structural rearrangements (PGT-SR) and aneuploidy (PGT-A) in one test protocol.

Methods

Twenty-four samples (9 abnormal Coriell DNA, 2 normal Promega controls, and 13 previously tested PGT samples) were included. Previously published library preparation protocols from Madjunkova et al, 2020 and Wei et al, 2018 were adapted for this study. Fragmentation steps were omitted to preserve longer fragments. The use of combined barcode and sequencing adapter ligation (Wei et al, 2018) was compared to the manufacturer's (ONT) protocol, which performs the barcode and sequencing adapter ligation in two separate steps. Ten samples were also prepped for sequencing with both the PCR Barcoding and Native Barcoding kits from ONT, to compare genome representation between PCR and non-PCR library preparations. Basecalling of Fast5 files from MinION sequencing runs was performed with Guppy (ONT) on an AWS EC2 instance for faster processing time. The resulting Fastq files were then aligned to GRCh38 using Minimap2 (Heng Li, 2018). The resulting BAM files were assessed with BEDtools (Quinlan & Hall, 2010) for minimum coverage. Read counts per chromosome were assessed by z-score method mentioned in Wei et al, 2018.

Discussion

PGT-SR with this method will only be able to detect translocations covered by regions amplified by the SurePlex WGA kit. Correspondingly, detection of balanced translocations will be possible only if the breakpoint falls within the covered region. Therefore, detection of all de novo translocation breakpoints from WGA DNA may not be practical, requiring further enrichment of sample for expected translocation points. PGT-A analysis was considered successful for non-mosaic whole chromosome aneuploidy, as well as gender. With the ease of benchwork, low cost, and small footprint for ONT devices, analysis of nanopore data remains the main barrier for widespread adaptation. Developing standard analysis pipelines for PGT on the ONT platform could open new avenues for PGT-SR and other genetic testing used in reproductive medicine. The handheld, nanopore-based DNA sequencer is a potential asset to the PGT lab. While turnaround time is not critical to a research lab, PGT labs will require to take their time analyzing samples. PGT turnaround times will require a more uniform approach to analysis for this application to be practical.

Results

Over three runs, including 28 samples (1 (4%) failed to produce results (no read for barcode). Three samples (10%) produced results, but with too few reads for analysis, and 24 (86%) yielded results sufficient for analysis. Coverage was sufficient if 10% of bases were covered by at least one read. The combined barcode ligation and sequencing adaptor step produced a higher yield, as well as longer fragments, potentially as a result of fewer washes. Assay sensitivity is unclear at this point, but we do know that the longer reads produced by the MinION are better for the detection of complex chromosome rearrangements. Sufficient reads for PGT-A analysis of 12 samples were acquired with two hours of sequencing, and basecalling was completed during sequencing.

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P-714

Sample Set	Concordant
Normal	100% (6/6)
Abnormal	94% (16/17)
Mosaic	0% (0/1)
Segmental	0% (0/2)
Gender	100% (24/24)
Total	83% (20/24)

XY Normal **XY Abnormal -16** **XY Abnormal +8s**