The MinION Access Project: A first attempt at pre-implantation genetic screening with nanopore sequence sensing

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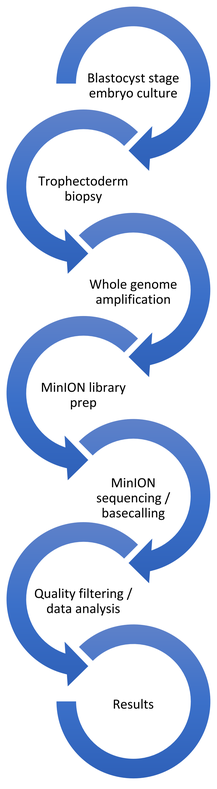
Introduction

Morphological assessment of viable embryos for selection prior to transfer remains unreliable and subjective. Improved selection methods such as pre-implantation diagnostics are likely to increase the current live birth rates in assisted reproductive technology cycles by preventing the transfer of aneuploid embryos. Recent advances in protein nanopore technology have allowed for the development of small USB powered, microfluidic devices that can detect nucleic acid sequences, proteins and small molecules. Here, we present pre-implantation genetic screening data collected as part of the MinION Access Project (MAP) – an early access program designed to acquaint researchers with this new technology.

Methods

Informed consent for the use of embryonic tissue in this research was obtained from parents. A blastocyst stage embryo, previously diagnosed as aneuploid via array comparative genomic hybridization was thawed and briefly cultured, to allow for two additional trophectoderm biopsies. Genomic material in the biopsy samples was isolated and amplified utilizing a SurePlex Amplification kit (Illumina PR-40-415101-00). The entire 25ul reaction generated was utilized for downstream MinION library preparation yielding 223ng and 77ng of prepped genomic material for samples 1 and 2 respectively. Utilizing the MinION flowcell, four sequencing runs of library 1 and one sequencing run of library 2 were performed with a flowcell wash in between samples. Basecalled FAST5 files were aligned to the human genome with the LAST alignment tool. Embryo karyotype was obtained utilizing normalized read counts for each chromosome. Variant calls were generated using SAMtools and annotated using data in the NCBI ClinVar database.



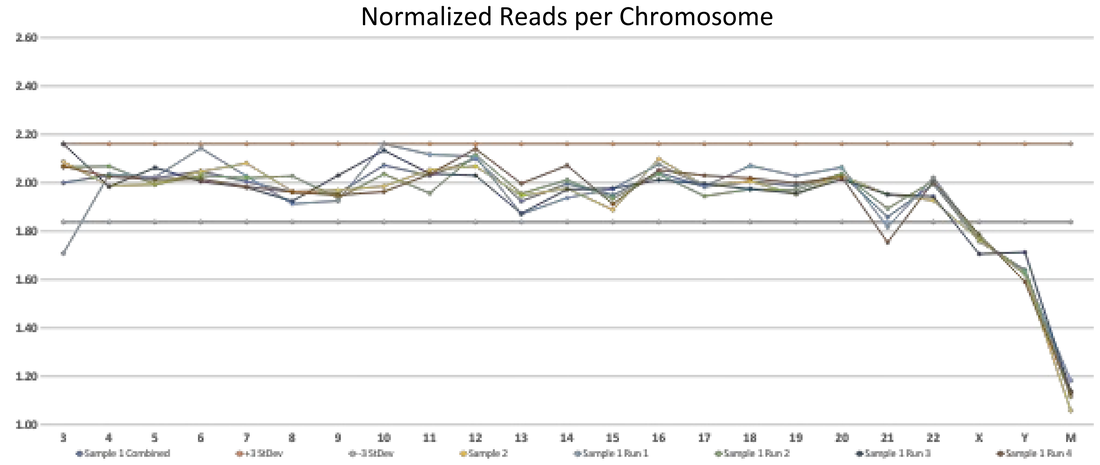
Advantages

- Short run time - ~1 hour each
- Long reads improve alignment accuracy and allow for low genome coverage / short run times
- Low infrastructure requirement, small footprint, ease of use, no maintenance.

Potential Problems

- Low amount of genomic starting material may cause chromosome bias
- The need to utilize an entire WGA for library prep prevents re-runs
- There are no MinION specific (long read) programs for determining karyotype

Results and Discussion



Karyotype

The original aCGH diagnosis of monosomy 16 is not reflected in this data. Chromosomes 1 and 2 had read counts of approximately 8-10 fold higher than all other chromosomes and appear as outliers when graphed. The sex of the embryo (XY) was identified and matched aCGH. Mosaicism and the utilization of different biopsies between aCGH and MinION sequencing may result in the discordant karyotypes. Additionally, mosaicism may be responsible for increased variability in chromosomes 10 and 21.

Variants

Variations were identified from all sequencing data obtained and appeared with equal frequency across all chromosomes. 1183 variants were identified. Of these variants, 34 were associated with a phenotype. Clinically significant variants identified were associated with prostate cancer (5), developmental disorders (5), retinitis pigmentosa (2), growth hormone deficiency (2).

Conclusion and Future Directions

Amplification bias associated with whole genome amplification may prevent accurate analysis of all chromosomes. High quality alignments from long read lengths may allow for improved variant calling. With continued development, this new strategy of pre-implantation genetic screening has the potential to become a new standard method of embryo screening diagnostics.