Abstract citation ID: deac107.491
P-533 Single-cell long-read nanopore sequencing as a fast and cost-efficient method for aneuploidy detection and potentially PGT-A, a pre-clinical study
A. Oberle1, L. Carli2, A. Ennemoser2, M. Hengstschläger1, M. Feichtinger3
1Wunschbaby Institut Feichtinger, Department of Genetics, Wien, Austria
2Wunschbaby Institut Feichtinger, Embryology, Wien, Austria
3Wunschbaby Institut Feichtinger, Head of Institute, Wien, Austria

Study question: Is long-read nanopore sequencing feasible and reproducible as routine technique for aneuploidy detection, potentially allowing fresh embryo transfer PGT-A cycles?

Summary answer: Pre-clinical analysis of euploid and aneuploid DNA and single cells using long-read nanopore sequencing resulted in high concordance and reproducible results for aneuploidy detection.

What is known already: PGT-A is mostly performed using short-read next-generation sequencing, which requires high initial investment costs and high running expenses. Third-generation sequencing is a novel sequencing technology with the potential of fast, easy, and cost-effective sequencing analysis, possible even for small and less well-financed clinics. Long-read nanopore sequencing for PGT was mainly shown for structural variants and monogenic disease, which comes with high costs and is far from clinical routine. PGT-A from trophectoderm biopsy samples using nanopore sequencing was so far demonstrated in a small pilot study and further pre-clinical and clinical studies are needed to transfer the technology into clinical routine.

Study design, size, duration: In this pre-clinical study, euploid and aneuploid DNA and single cells, as well as three to 20 cells were analyzed for aneuploidy using two different whole genome amplification (WGA) kits and long-read nanopore sequencing. In total, 44 different samples were analyzed after multiple displacement amplification (MDA) using REPLg WGA kit (QIAGEN) and so far, 15 samples were analyzed using PicoPlex WGA kit (Takara) from April 2021. To confirm reproducibility, certain WGA samples were sequenced repetitively.

Participants/materials, setting, methods: Different euploid and aneuploid gDNA samples were diluted to 4.5 pg DNA per sample. Four different human fibroblast cell lines were diluted to approx. 20 cells, or single cells/three cells were picked using micromanipulation technique. DNA and cells were amplified, prepared for sequencing, and sequenced on MinION sequencer from Oxford Nanopore Technology. Data were analyzed using a custom pipeline consisting of pre-processing, alignment and copy-number calling. QC values and whole chromosome aneuploidies were determined automatically.

Main results and the role of chance: From 44 different single cells (n = 14), few cells (n = 9) or diluted gDNA (n = 21) samples amplified using MDA, 42 samples showed good quality sequencing results. Two samples resulted in QC failure, yielding a sample-success-rate (SSR) of 95.5%. Overall, sample sensitivity was 100%, specificity 95.2% with positive predictive value (PPV) of 95.5% and negative predictive value (NPV) of 100%. Per chromosome sensitivity was 100%, specificity 99.8% with PPV of 93.3% and NPV of 100%. Using PicoPlex WGA, 15 samples were analyzed so far: 7 gDNA and 8 single cell samples. The study is ongoing. All samples analyzed resulted in high quality sequencing data with the correct karyotype, leading to 100% SSR, 100% sensitivity, specificity, PPV, NPV per samples as well as per chromosome.

Repetitive sequencing (n = 3) of four MDA amplified single cells showed identical sequencing results, indicating high reproducibility of library preparation, and sequencing. A 5p deletion in one cell line was correctly identified in all single cell analyses from either MDA or PicoPlex amplification, indicating sufficient resolution even for segmental aneuploidies. The whole workflow is feasible in under 24 hours. These results indicate high accuracy and high reproducibility of nanopore sequencing technology for single cell aneuploidy detection, possibly transferable for PGT-A.

Limitations, reasons for caution: The results of this pre-clinical study look very promising and workflow for sample preparation, sequencing and data analysis is fast, cost-efficient, and feasible for PGT-A applications. Nevertheless, no polar body, blastomere or trophectoderm biopsy sample was used and a clinical trial using real clinical samples is needed to confirm applicability.

Wider implications of the findings: Implementing novel technologies into clinical routine requires extensive systematic pre-clinical and clinical testing. To our knowledge, this is the first study that systematically analyzes sensitivity, specificity, PPV and NPV for aneuploidy detection using long-read sequencing technology. The fast workflow principally allows day 3/5 fresh embryo transfer after polar body biopsy.

Trial registration number: Not applicable