A novel assay based on Oxford Nanopore technology for potential mass screening of Klinefelter syndrome

Anne Kristine Schack[1,2], David Galevski[4], Aleksandar Nikov[4], Gjorgji Madjarov[2,4,5], M. Carmen Garrido Navas[6,7,8], Chris Kyriakidis[2], Zoran Velkoski[2], Lukasz Krych[1,3]

[1] Food Microbiology & Fermentation, Department of Food Science, University of Copenhagen, 1958 Frederiksberg C, Denmark; [2] gMendel ApS, 2100 Copenhagen, Denmark; [3] GenXone S.A., 60-476 Poznan, Poland; [4] Netcetera, Zypressenstrasse 71, 8040 Zürich, Switzerland, 5 University Ss Cyril & Methodius, Skopje 1000, N. Macedonia; [6] GENYO Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government, Liquid Biopsy and Cancer Interception Group, PTS Granada, Granada, Spain; [7] Genetics Department, Faculty of Sciences, Universidad de Granada, Granada, Spain; [8] CONGEN, Genetic Counselling Services, C/Albahaca 4, Granada, Spain

Klinefelter syndrome (KS) is the most common and most underdiagnosed sex chromosomal abnormality with prevalence of 1 in 500 male births. Affected KS males carry an additional X chromosome (47,XXY) leading to a variety of clinical features, most often characterized through small testes, tallness, gynecomastia and infertility. Furthermore, individuals affected by KS are more exposed to an increased comorbidity, language deficits, cognitive impairment involving psychological and/or social difficulties and other medical problems, of varying severity. In most cases subjects affected with KS remain undiagnosed until developing obvious, often irreversible symptoms. Multiple studies demonstrate that if treated timely (e.g. hormonal therapy and early fertility management) boys affected with KS can have normal life, with 25% chances to achieve fertility.

The key element in successful treatment of KS is a timely diagnosis. In order to ensure early diagnosis of KS, the disease should be included in postnatal screening programs. Nevertheless, the majority of chromosomal aberration detection relies on tedious, time-consuming technologies such as manual karyotyping (FISH). In order to facilitate implementation of a mass screening program of KS a novel diagnostic solution is of burning need.

Here we present research demonstrating a cost-effective, end-to-end assay with potential diagnostic utility for postnatal detection of Klinefelter Syndrome (KS) based on Oxford Nanopore technology. The DNA library was prepared in less than 8 hours prior to loading on the GridIONx5 (ONT) Flo -Cell. Furthermore, we present a platform called Phivea®, capable of real-time detection of chromosomal aberrations based on cutting-edge Machine Learning technologies, that employs customized Deep Learning architectures at all levels of the learning process.

Our approach allows for simultaneous assessment of a total of 384 samples, which is equivalent to 96 subjects, each in 4 technical replicates, reaching analytical specificity and sensitivity-score at 99.1% and 97%, respectively. Furthermore, our technology demonstrates the potential to detect mosaicism in an individual, with a Limit of Detection (LoD) of 25.3%. When a Klinefelter sample was mixed with a female (XX) sample, LoD was 27.9%, showing further potential of the approach to be used as a non-invasive prenatal screening tool.

We believe that the research suggests the technology could be applied for detection of any other genetic disorder.