Third Generation Cytogenetic Analysis (TGCA): Potential Future Diagnostic Application of Long-Read Sequencing

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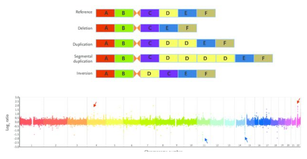
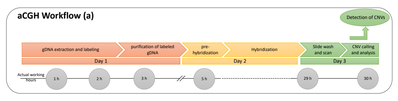
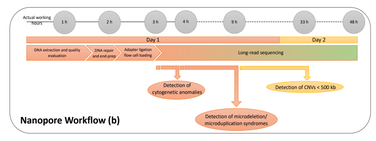
CNVs are primary cause of many rare and common human disease, from Mendelian syndromes to complex disease and malignancies. CNVs range from aneuploidies to submicroscopic alterations and their detection is a key factor for the diagnosis and the appropriate clinical management of such conditions.



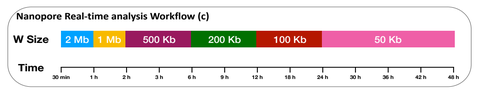
Conventional analysis (CCG, CMA and SGS) have been vastly adopted to detect CNVs in clinical settings but each of these approaches has technical limitations impacting significantly on turnaround time and diagnostic yield. We describe the application of long reads sequencing to the real-time detection of homogeneous or mosaic aneuploidies and recurrent submicroscopic CNVs



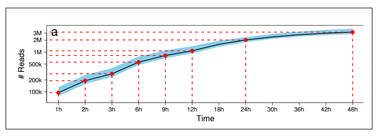
The timeline of the aCGH workflow: gDNA labeling, purification, pre-hybridization, hybridization, wash/scan and analysis.



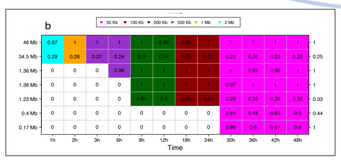
The timeline of the Nanopore workflow: which DNA repair and end-prep, adapter ligation, priming and loading and beginning of the real-time analysis.



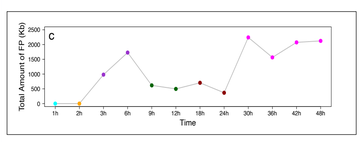
The real-time analysis workflow: running NanoGLADIATOR during the sequencing process by using different window size at different time steps.



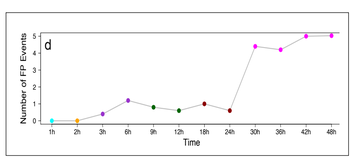
The average number of reads (both pass and fail reads). Blue area surrounding the line represents the standard deviation of number of reads calculated across the seven runs.



The capability of ONT data and NanoGLADIATOR to detect genomic alterations of different size and predict their cellular fraction as a function of sequencing time.



The average size (c) and number (d) of false positive calls made by NanoGLADIATOR at different time points and with different window size.



CONCLUSIONS

We demonstrated that long read nanopore sequencing can be used for the real-time detection of CNVs in patients with genomic disorders, with a higher sensitivity compared to aCGH. High rapidity, accuracy, resolution and sensitivity for CNV detection, make nanopore sequencing an efficient tool that can identify microscopic and submicroscopic anomalies in a single analysis, and these characteristics could foster a widespread acquisition of ONT by diagnostic laboratories in the future

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