Klinisk Institut

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Can nanopore long-read sequencing replace current cytogenetic methods in clinical genetic diagnostics?

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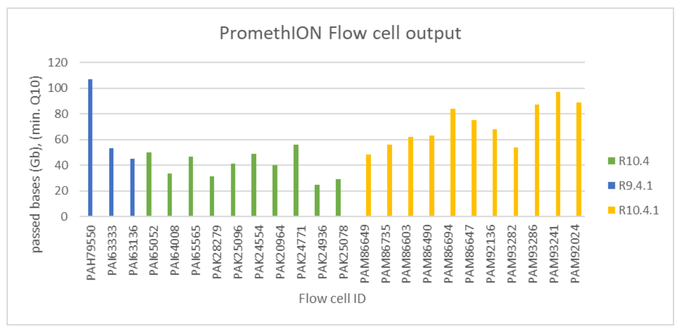
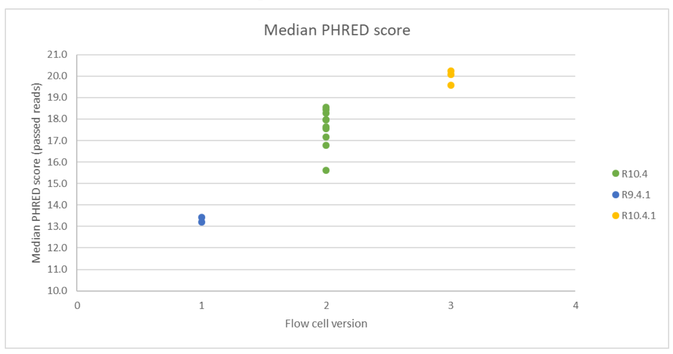
INTRODUCTION: Balanced translocations are one example of structural variations that can still only be detected by traditional cytogenetic methods, which have a low resolution. Long-read sequencing combines the advantages of traditional cytogenetic methods with the base-pair-level resolution of newer molecular methods. We carried out a number of pilot studies focusing on different types of structural variants to explore the capability of long-read sequencing to detect structural variants and fine map the involved breakpoints. A broad spectrum of variants has been sequenced, including balanced and unbalanced translocations, tandem duplications, deletions, and inversions. The study is ongoing, but preliminary results have demonstrated that long-read sequencing data enables fine mapping of break points that are inaccessible by other methods.

METHODE:

DNA extraction on samples (blood and celllines) was extracted using Monarch® Genomic DNA Purification Kit. All samples are sequenced on the PromethION24 device using different flow cell versions (R9.1.1, R10.4 and R10.4.1). All samples are basecalled with Super High Accuracy (default parameters).



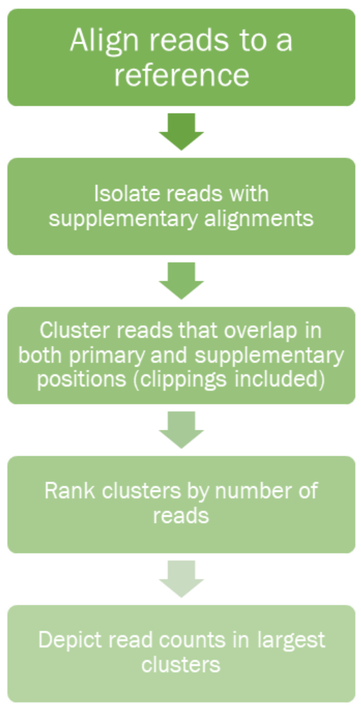
QC and Flow cell performance with Q20 as mean PHREAD score for R10.4.1 Flow Cells. We used PycoQC and mosdeph tolls for QC analysis.

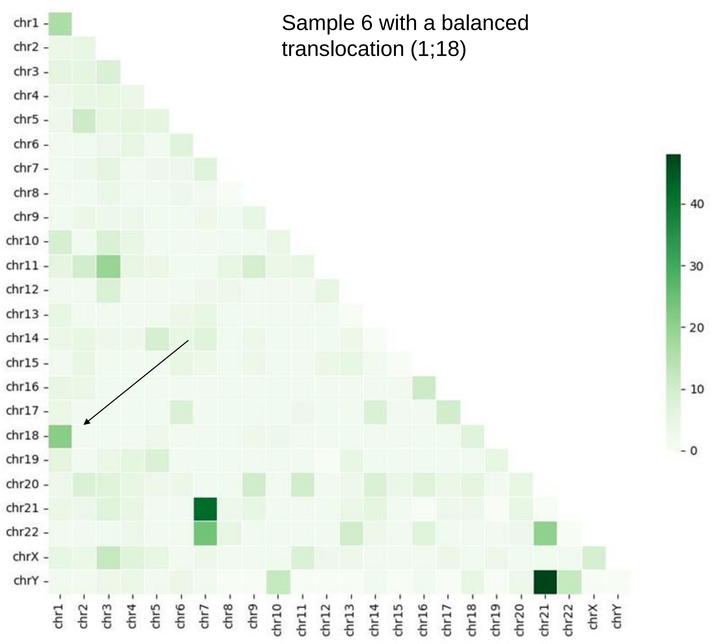


Detection of chromosomal translocations

Second approach: Build a new tool.

Reads are aligned using winnowmap or minimap2 with Telomere-to-telomere (T2T) as reference.





Sample 6 with a balanced translocation (1;18)

RESULTS:

The table shows 8 humane genomes sequenced on the PromethION24 device with mosdepth between ~16.7 and 25.9.

Sample	Flow Cell	Mosdepth (total)
1	R9.4.1	28.73
2	R9.4.1	21
3	R9.4.1	17.7
4	R10.4	22.87
5	R10.4	25.24
6	R9.4.1	16.78
7	R10.4.1	25.88
8	R10.4.1	22.83

CONCLUSION AND PERSPECTIVE

Using Nanopore Long-read sequencing for detection of structural variants and fine mapping of breakpoints has great potential. To optimize the new translocation tool we are working on filtering normal variation and different other approaches.

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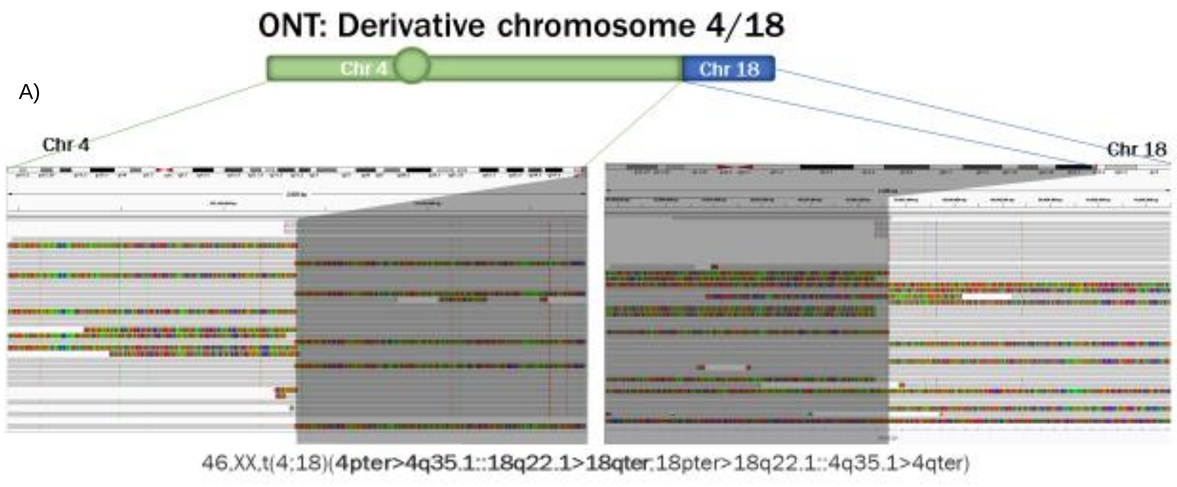
A)



B)

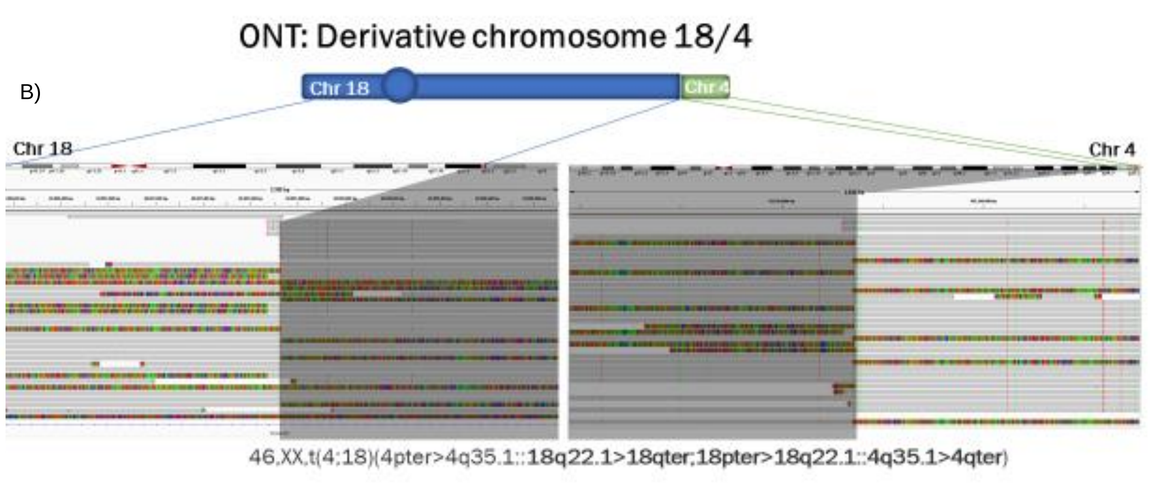
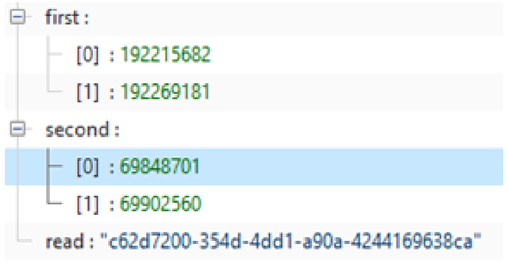
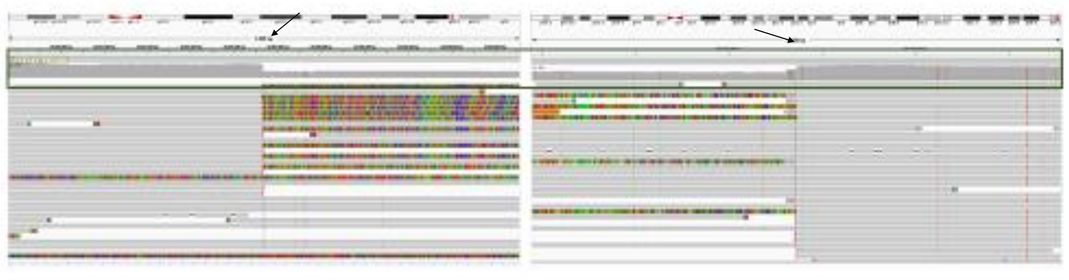


Figure 1: Sample 1; Balanced translocation (4;18). Visualisation of breakpoints using Integrative Genomics Viewer (IGV) and suplementary allignemnt reads. A) Shows the derivative cromosome 4 and B) hows the derivative cromosome 18. Sample1; Coriell sample GM11966.

Sample 1: Read counts in largest cluster between chromosome 4 and 16 was 24 reads. ‘first’ (chr 4) read coordinates. ‘second’ (chr 18) read coordinates.



Chr 18 Chr 4



Sample 2: Unbalanced translocation (4;18). Visualisation of breakpoints using Integrative Genomics Viewer (IGV) and suplementary allignemnt reads. Inheriated the derivative comosome 18/4 shown in *Figure 1 B*

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