Haplotype-resolved multi-modal analysis of cancer genomes using nanopore sequencing

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Abstract
Cancer is a complex and dynamic disease driven by somatic genomic and epigenomic alterations that accumulate over time. These changes give rise to heterogeneous collections of cells or clones, each with distinct (epigenomic) profiles within a single tumour. Accurate characterisation of these changes is crucial for understanding the mechanisms driving the disease, identifying potential therapeutic targets, and personalising treatment strategies. Due to the technological constraints of short-read and array-based approaches, cancer research has historically had a strong focus on detecting small genomic changes like SNVs and small indels (SNVAs) as well as the broad characterisation of large-scale copy number changes (CNVAs), mostly ignoring other important variant classes and epigenomic modifications. Here we demonstrate how Oxford Nanopore native long-read sequencing enables the direct detection of not only SNVAs, but also breakpoint-resolved simple and complex structural variants (SVs and CNVs), haplotype phasing of all variant types, and identification of DNA modifications like 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) from a single tumour-normal dataset.

We used well-characterised cancer cell lines and in-silico benchmarking datasets to assess somatic SNV and SV calling performance using different sequencing depths, and also demonstrated how Nanopore long reads enable haplotype and clone-specific CNV calling. Finally, we use twelve matched tumour-normal pairs from four different tissues to showcase a comprehensive tumour-normal analysis using Nanopore sequencing. This includes the characterisation of complex patterns of somatic SVs in the different cancer samples, the identification of 5mC methylation patterns in specific cancer regions, and the expression of characteristic differences in 5hmC levels between tumour and normal samples as well as the detection of microsatellite instability and other hall marks of cancer.

1. Background
Cancer is a complex disease driven by somatic genomic and epigenomic alterations that accumulate over time. Detection of genomic and epigenomic changes in cancer is crucial for understanding the disease, identifying potential therapeutic targets, and personalising treatment strategies. Due to the technological constraints of short-read and array-based approaches, cancer research has historically focused on detecting small genomic changes like SNVs and small indels (SNVAs) as well as the broad characterisation of large-scale copy number changes (CNVAs), mostly ignoring other important variant classes and epigenomic modifications. Here we demonstrate how Oxford Nanopore native long-read sequencing enables the direct detection of not only SNVAs, but also breakpoint-resolved simple and complex structural variants (SVs and CNVs), haplotype phasing of all variant types, and identification of DNA modifications like 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) from a single tumour-normal dataset.

2. Whole-genome-matched tumour-normal nanopore sequencing
By comparing the genetic makeup of tumour and normal cell populations, it is possible to identify genetic and epigenetic alterations that are specific to the tumour and not present in normal tissue, as well as cancer-specific regions that are uniquely methylated.

3. Native nanopore sequencing gives consistent performance
We sequence twelve matched tumour-normal pairs from four different tissues and perform gemmese as well as somatic genomic and epigenetic variant calling, and obtained consistent yields of data and read length N50s across the sample.

4. Detecting hereditary-cancer-related variation and MSI
First, we screened all samples for pathogenic germline mutations and identified a 10 kb deletion associated with Lynch syndrome in sample B0707 (left panel). Reduced DNA repair activity caused by Lynch syndrome can lead to a shortening of microsatellites in tumour cells (microsatellite instability or MSI). Thus, microsatellites are considered phenotypic markers in some cancers. To illustrate Oxford Nanopore’s ability to detect MSI, we compared microsatellite lengths between tumour and normal samples and confirmed a clear MSI signal only in B0707 (right panel).

5. Comprehensive characterisation of genetic variation in cancer
Shallow nanopore sequencing is an efficient approach for detecting large copy-number changes. We performed ~1 WGS nanopore sequencing per sample to identify characteristic whole-chromosome gains as well as micro copy number amplifications on chromosomes 7 and 12.

6. Genome-wide case-control pair resolution 5mC and 5hmC profiling in cancer
Regulation of methylation is a critical mechanism of cancer progression. Nanopore sequencing can profile 5mC and 5hmC within the genome, allowing for the detection of differences in methylation patterns between cancer and normal tissues.

Conclusions
There is a vast array of genetic aberrations that have been linked to solid tumour formation and disease progression. Navigating this complex mutational landscape poses significant difficulties due to the high level of heterogeneity seen both between and within solid tumours. We demonstrate that single technology can provide valuable insight into the mutational landscape of solid tumours by identifying relevant aberrations using a matched tumour-normal approach. Resolving haplotypes in the germline samples identifies mutations associated with hereditary cancers, while regions of differential methylation were called between the tumour-normal pairs. Changes in methylation within the early tumour microenvironment are thought to be one of the first signals in tumour progression. Unique 5mC and 5hmC methylation patterns were seen between both tissue types and tumour-normal samples.

References

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