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Oxford NANOPORE Technologies APPLICATIONS

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

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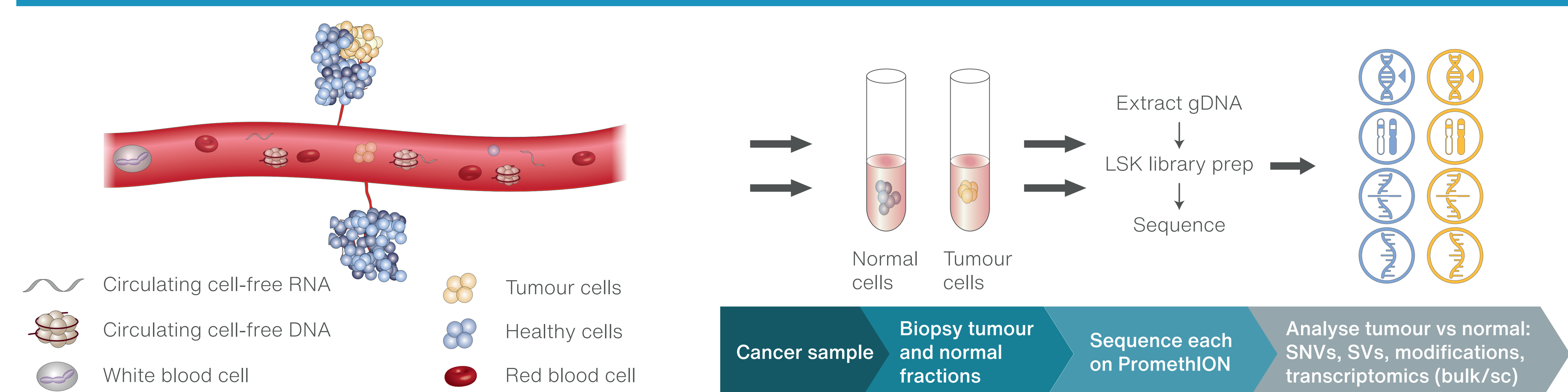
Abstract

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 (epi)genomic 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 SNPs and small indels (SNVs) as well as the broad characterisation of large-scale copy number changes (CNVs), mostly ignoring other important variant classes and epigenetic modifications. Here we demonstrate how Oxford Nanopore native long-read sequencing enables the direct detection of not only SNVs, but also break point-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-hydroxy-methylcytosine (5hmC) from a single tumour-normal dataset.

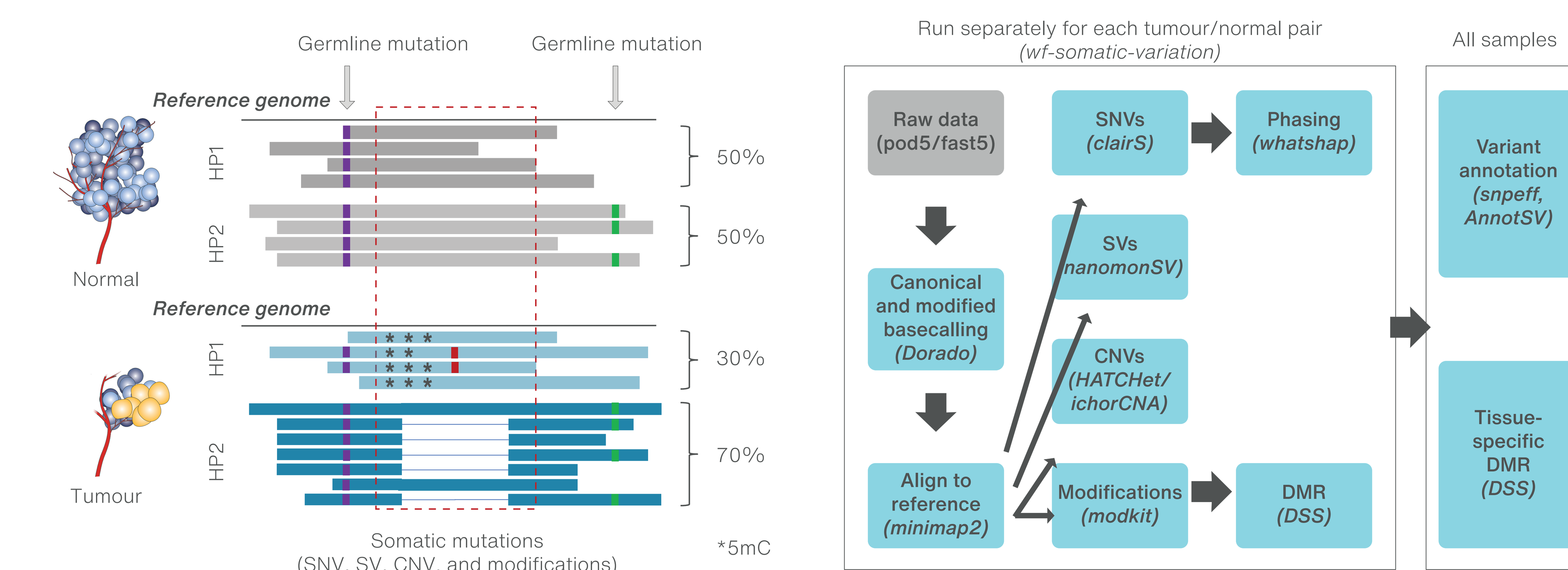
We use well characterised cancer cell lines and in-silico benchmarking datasets to assess somatic SNV, and SV calling performance using different sequencing depths, and also demonstrate how Nanopore long reads enable haplotype and clone specific CNV calling. Finally, we use twelve matched tumour-normal 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 (e.g. in promoter regions) that are unique to certain tissues, exploring 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



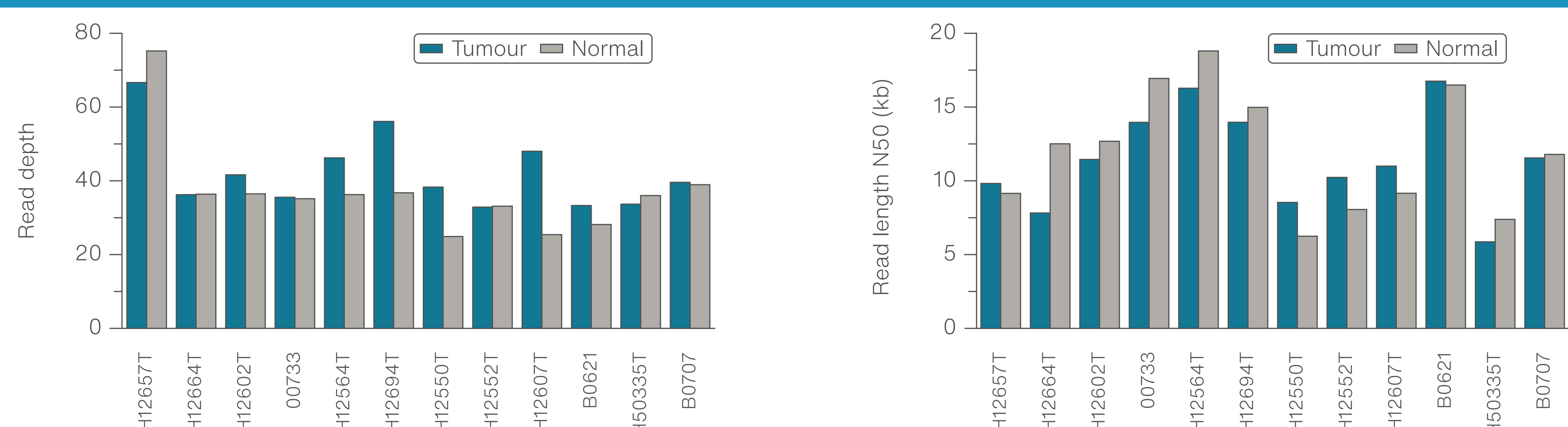
Cancer is a complex and dynamic disease driven by somatic genomic and epigenomic alterations that accumulate over time. Detection of genomic and epigenomic changes in cancer is crucial for understanding the disease, identifying potential therapeutic targets, and personalising treatment strategies[1]. A common way to analyse cancer data is tumour-normal sequencing. This involves the comprehensive analysis of genetic material from both cancerous (tumour) and healthy (normal) cells of an individual.

2. Whole-genome-matched tumour-normal nanopore sequencing



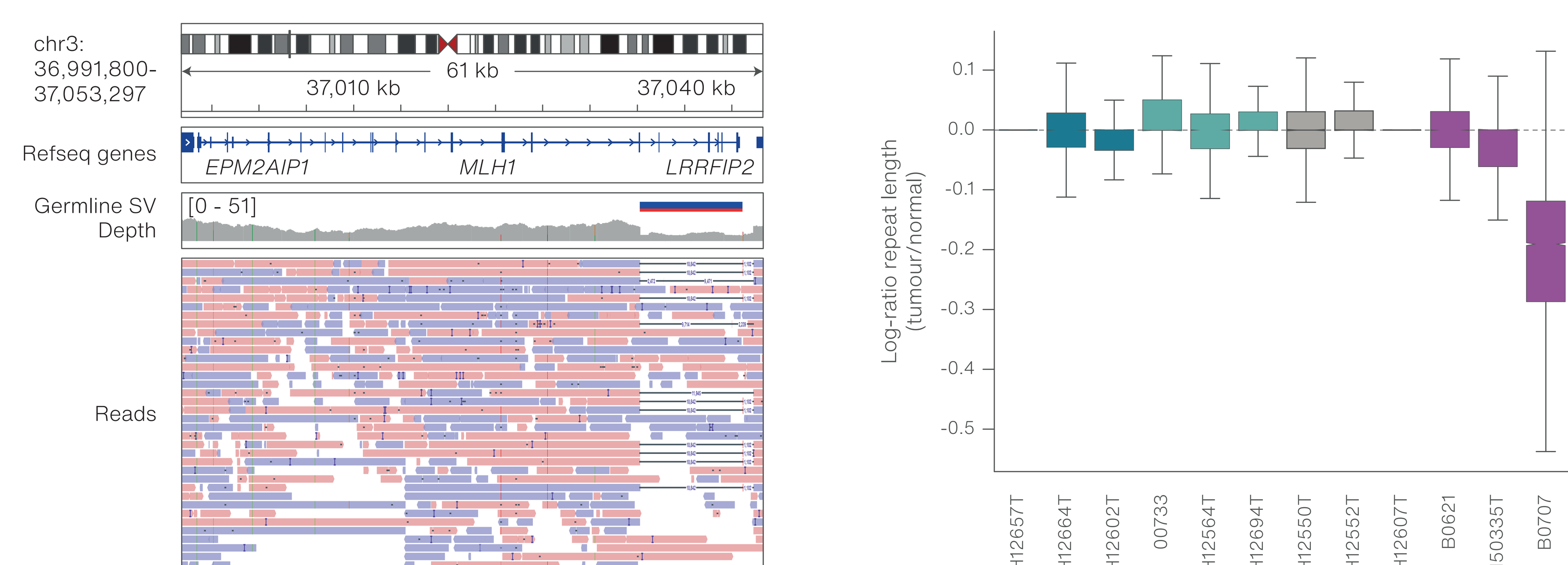
By comparing the genetic makeup of the tumour and normal cell populations, it is possible to identify genetic and epigenetic alterations which are specific to the tumour and not present in the normal tissue, as well as germline mutations[2]. Native long reads enable direct detection of SNVs, complex structural variants (SVs and CNVs), haplotype phasing, and identification of DNA modifications like 5-methylcytosine (5mC) and 5-hydroxy- methylcytosine (5hmC) from a single dataset using *wf-somatic-variation* (https://github.com/epi2me-labs/wf-somatic-variation) and other open-source analysis tools.

3. Native nanopore sequencing gives consistent performance



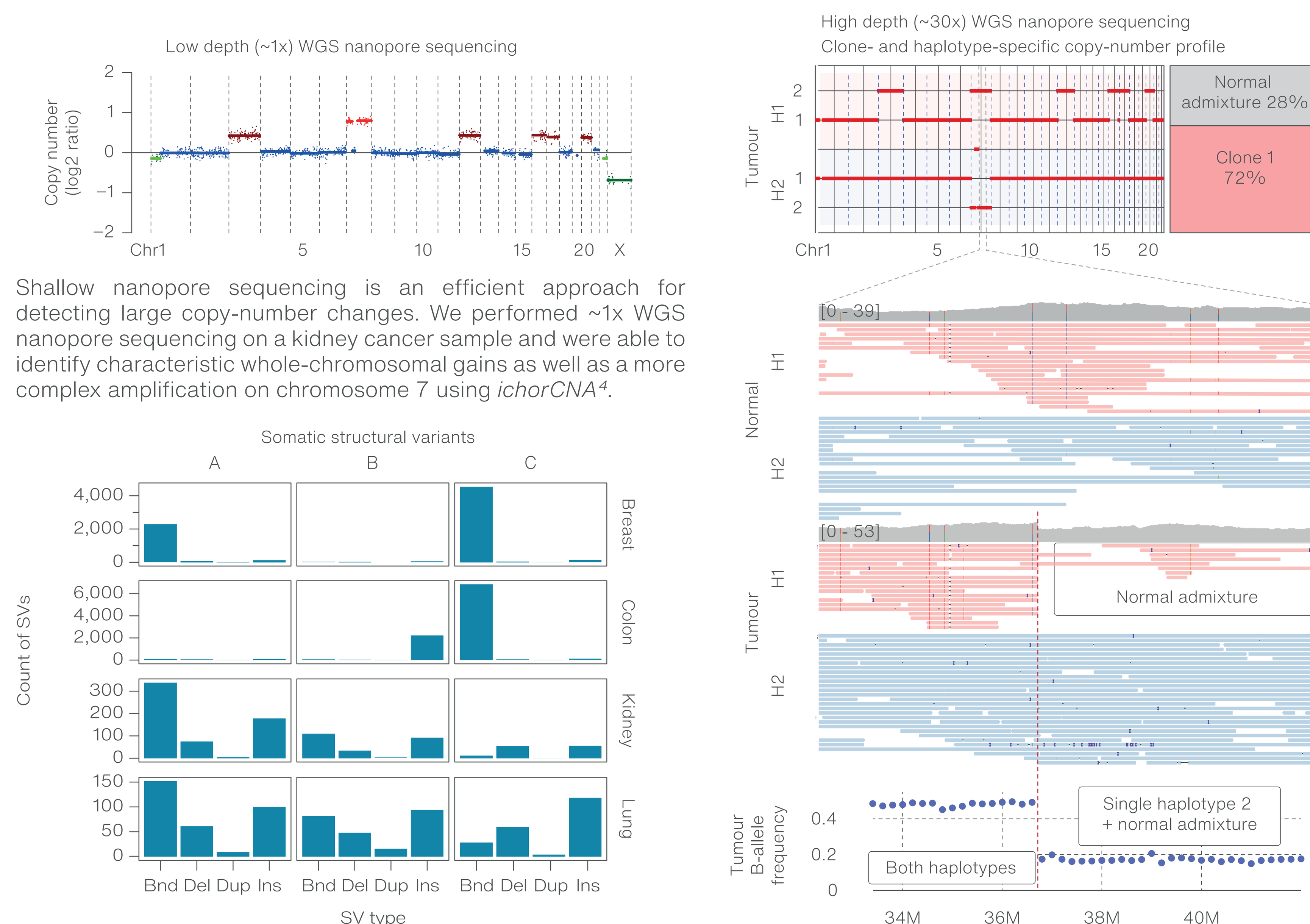
We sequence twelve matched tumour-normal pairs from four different tissues and performed germline as well as somatic genetic and epigenetic variant calling, and obtained consistent yields of data and read length N50s across the samples.

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)[3]. 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



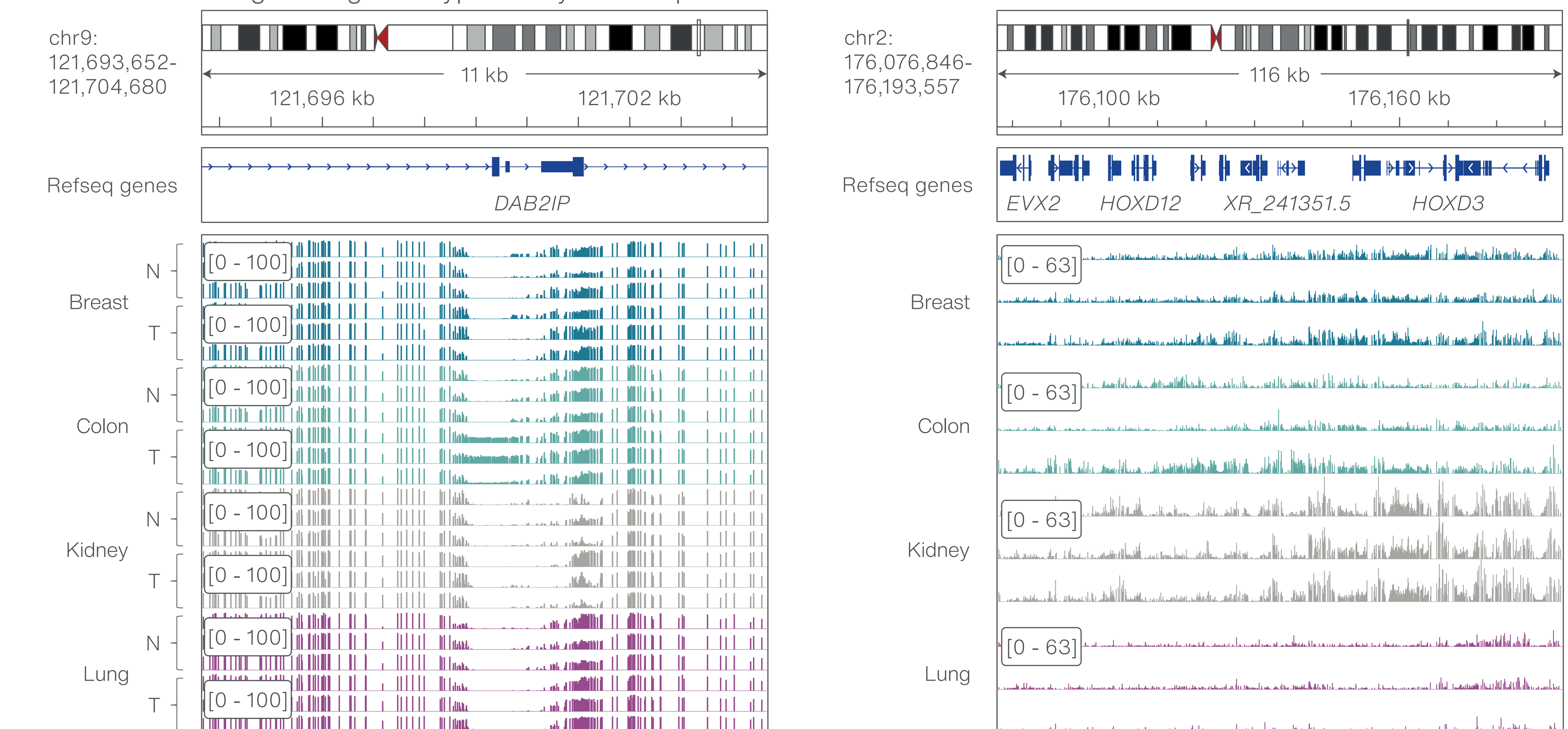
Shallow nanopore sequencing is an efficient approach for detecting large copy-number changes. We performed ~1x WGS nanopore sequencing on a kidney cancer sample and were able to identify characteristic whole-chromosomal gains as well as a more complex amplification on chromosome 7 using *ichorCNA*[4].

Using *HATCHet*[5] on the full high-depth dataset of the same sample we inferred genome-wide clone- and haplotype-resolved CNV profiles as well as exact break points for the rearrangement on chromosome 7, which revealed a large region of loss-of-heterozygosity, another common event in cancer.

To investigate smaller structural changes, we performed somatic SV calling on all samples and found high variability in the number and types of SVs across samples, highlighting the complexity present in cancer.

6. Genome-wide base-pair resolution 5mC and 5hmC profiling in cancer

Regulation of methylation is a critical mechanism of cancer progression[6]. Nanopore sequencing can profile 5mC and 5hmC methylation without the need for bisulfite treatment. When comparing average 5mC levels we found genome-wide hypomethylation in cancer tissues alongside regional hypermethylation of promoters.



The left panel shows the DAB2IP promoter region. This gene plays a key role in colorectal cancer[7] and is only methylated in our colon tumour samples. 5hmC is known to be highly tissue specific and strongly reduced in cancer tissue. This is confirmed by our observed average 5hmC frequencies across the genome (upper panel) and per-base frequencies across the HOXD3 gene (right panel). HOXD3 showed high levels of 5hmC in kidney and lower levels in lung, matching expected expression in these tissues.

Conclusions

There is a vast array of genomic 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 a 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 pairs. Low pass and high depth copy number analysis was used to detect large scale chromosome rearrangements. Resolving these copy number changes in a haplotyped manner not only identified haplotype specific breakpoints but provided an indication of tumour burden. The ability to gain this level of insight into a diverse range of potential driver mutations, from a potentially diverse range of solid tumour samples using a single, non-targeted, whole genome sequencing approach provides members of the cancer research field with a powerful tool to help further the understanding, and hopefully prevention or treatment, of cancers in the future.

References

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Workflow

Data

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