Oxford Nanopore Technologies

Multiomics whole-genome characterisation of a cancer genome using Oxford Nanopore sequencing

Comprehensive identification of somatic and single nucleotide variants (SNVs), structural variants (SVs), and copy number variants (CNVs), as well as epigenetic modifications (5mC and 5hmC) and chromatin accessibility from a single dataset

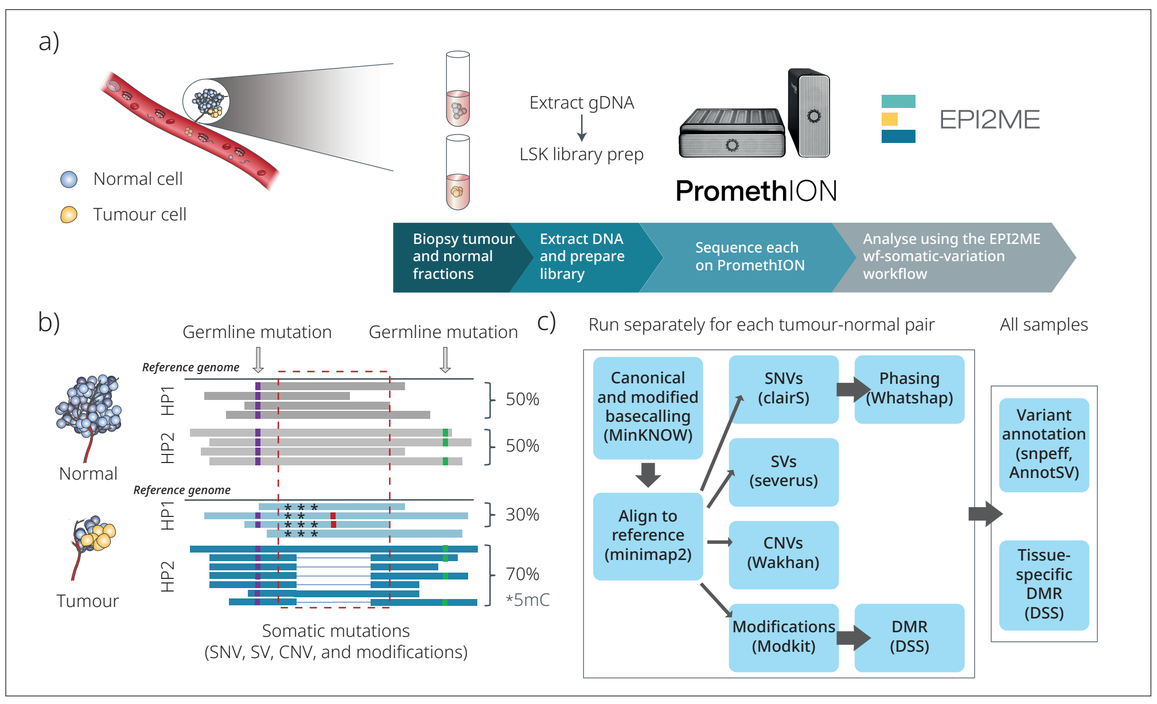

Fig. 1 a) Tumour-normal sequencing b) germline vs somatic variants c) analysis workflow.

End-to-end tumour-normal and tumour-only Oxford Nanopore sequencing workflow

Cancer is a complex disease driven by somatic genomic and epigenomic alterations that accumulate over time. Detection of these alterations in cancer is crucial for understanding the disease, identifying potential therapeutic targets, and personalising treatment strategies. Here we demonstrate a workflow (**Fig. 1a**) for tumour-normal and tumour-only sequencing (**Fig. 1b**) using the well-characterised COLO829 cell line. Analysis was carried out using a pre-release version of wf-somatic-variation (**Fig. 1c**). To further enrich the analysis, we performed chromatin stencilling treatment prior to sequencing, where artificially introduced 6mA DNA modifications highlight chromatin accessibility, as well as full-length transcriptome sequencing.

Fig. 3 Visualisation of a) SV and b) CNV calls from tumour-normal and tumour-only data.

Purity estimation and joint haplotype-resolved somatic SV and CNV calling

Large-scale somatic genomic aberrations are a hallmark of cancer. Here we analysed somatic SVs using Severus (**Fig. 3a**) and CNVs using Wakhan (**Fig. 3b**). For CNV detection, Wakhan leverages somatic SV signatures identified by Severus to guide genomic segmentation. Wakhan also estimates tumour ploidy, purity, and haplotype-specific CNVs, including annotation of regions with loss of heterozygosity (LOH) and likely subclonal CNV events. This approach provides an unprecedented view into genome instability in tumour samples by integrating long-read breakpoint analysis with copy number profiling. Most notably, we observed high consistency between tumour-normal and tumour-only modes.

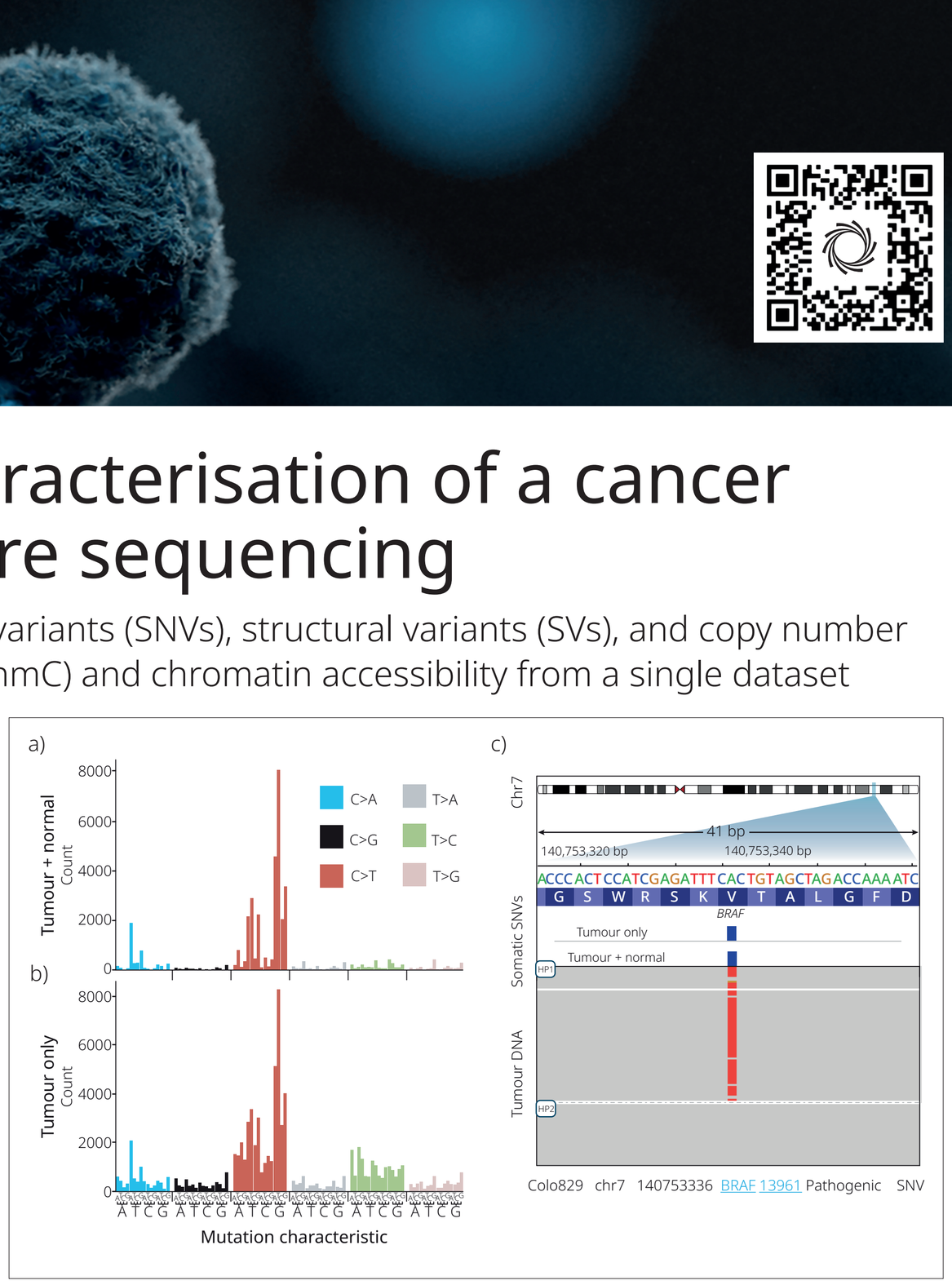

Fig. 2 Mutational signatures (COLO829) for a) tumour-normal b) tumour only and c) pathogenic SNV.

Accurate identification, phasing and annotation of somatic SNVs

First, we identified somatic SNVs in both tumour-normal and tumour-only modes with ClairS and ClairS-TO, respectively. We observed high concordance between both analysis modes, with mutation characterisation signatures displaying high levels of consistency (**Fig. 2a,b**). We note that ClairS-TO identified and annotated more somatic small variants, which is expected given the absence of a matching normal sample for germline variant filtration. **Fig. 2c** shows an example of a pathogenic ClinVar-annotated somatic SNV in the *BRAF* gene in COLO892, identified in both tumour-normal and tumour-only analysis modes and phased using long-read data only.

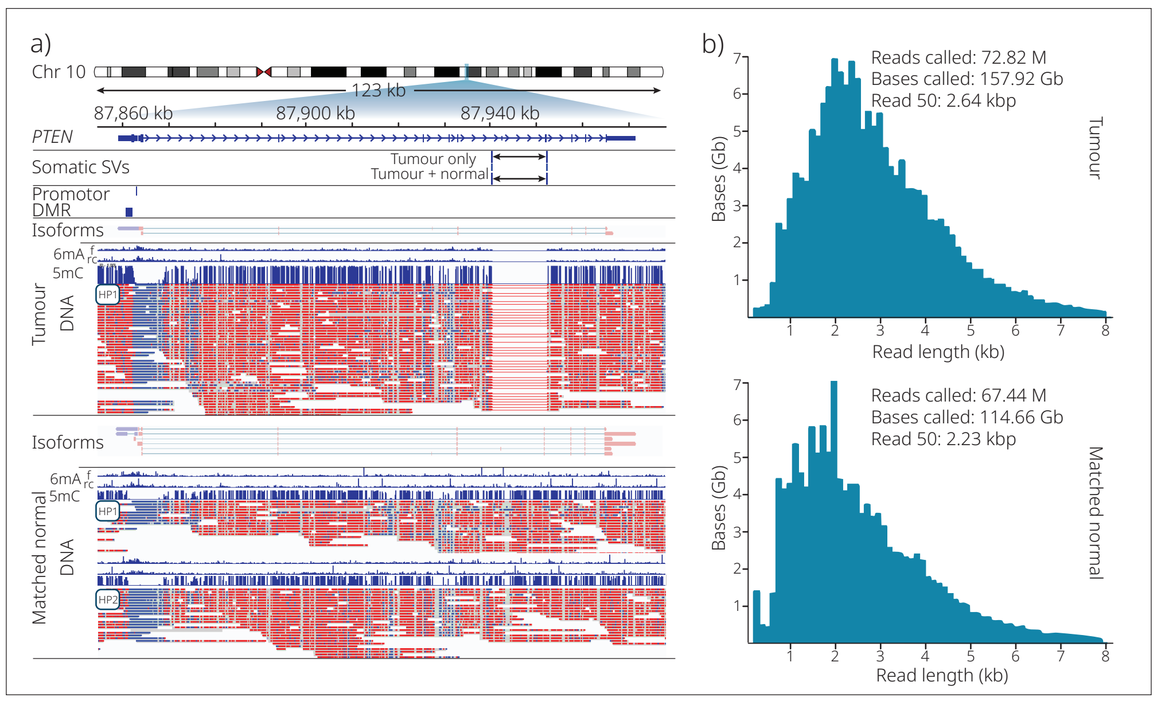

Fig. 4 a) Visualisation of multiomics data for *PTEN* b) cDNA read length distributions.

Multiomics analysis of *PTEN* in COLO829 including full-length transcriptome sequencing

Fig. 4a shows an integrated multiomics analysis for the tumour suppressor gene *PTEN*. We identified a somatic SV — a deletion affecting exons 5 and 6 — along with gene-spanning LOH, characterised by two copies of the H1 haplotype and loss of the H2 haplotype. Full-length transcripts from cDNA sequencing (run statistics shown in **Fig. 4b**) reveal isoform changes between tumour and normal samples and confirm the loss of exons 5 and 6. We also observed a 5mC differentially methylated region near the *PTEN* promoter when comparing tumour and normal. Overlaid chromatin stencilling data shows that, despite being reduced in size, the tumour retains open chromatin around the *PTEN* promoter.