Oxford Nanopore Adaptive Sampling for tumour-only SNV, SV, and CNV profiling in one assay

Oxford Nanopore Adaptive Sampling turns a BED file into a software-defined digital panel during sequencing, focusing coverage on regions of interest while preserving low-pass off-target reads across the genome from the same run.

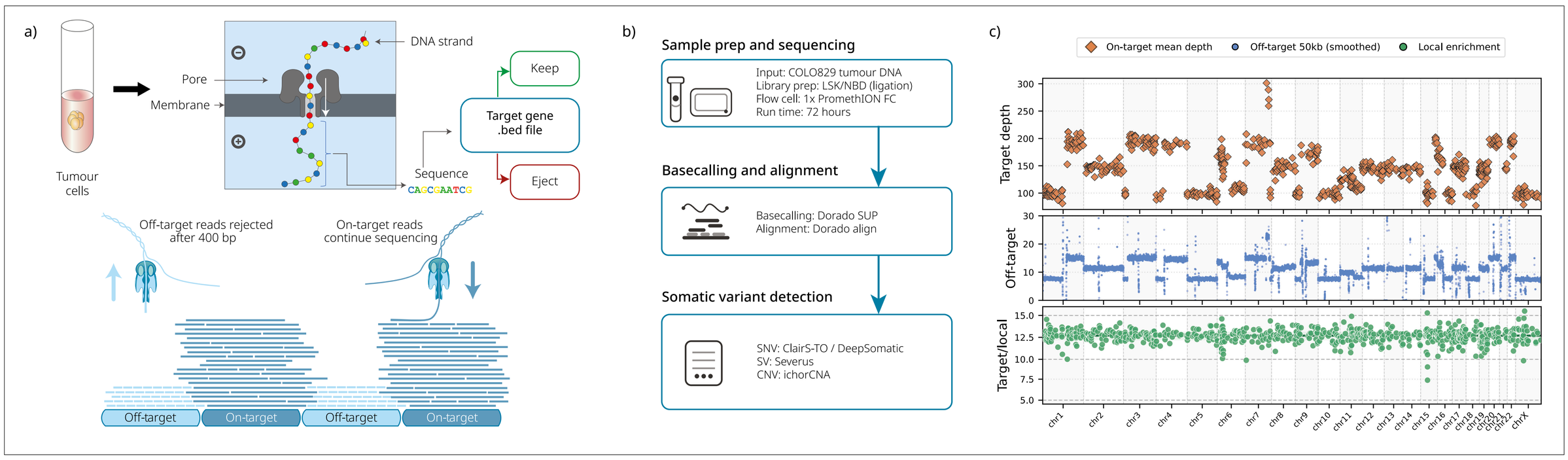

Fig. 1 A digital panel enriched during sequencing: a) real-time targeting, b) workflow overview, and c) resulting coverage enrichment.

Adaptive Sampling maps each DNA molecule in real time against a user-defined BED file, keeping on-target reads and ejecting off-target reads to enrich regions of interest while retaining genome-wide low-pass coverage

Tumour-only workflows often face a trade-off. Highly targeted approaches can improve sensitivity at low allele fraction, while whole-genome sequencing provides a broader view on structural variants (SVs) and copy number variants (CNVs). Oxford Nanopore Adaptive Sampling is designed to deliver both. As a DNA molecule begins moving through a pore, the partial sequence is mapped in real time against a target BED file (**Fig. 1a**). Reads that overlap the target are kept and sequencing continues. Reads outside the target are ejected, freeing the pore to capture another molecule. This focuses sequencing capacity on regions of interest without PCR or pulldown enrichment, generating high depth of coverage of native, on-target long reads, but also low-pass whole-genome coverage from short reads. In this study, a single COLO829 tumour DNA sample was sequenced for 72 hours on one PromethION Flow Cell. Basecalling and alignment were performed with dorado, creating one aligned dataset for downstream analysis (**Fig. 1b**). Synthetic mixtures and purity titrations generated *in silico* from the same dataset were then used to explore performance under various conditions. The resulting coverage profile shows the practical value of the workflow: deep enrichment where targeted variant calling matters, with enough genome-wide signal retained for copy-number inference (**Fig. 1c**). Because the panel is defined by a BED file, the same setup can be employed for other cancer research applications without changing library preparation.

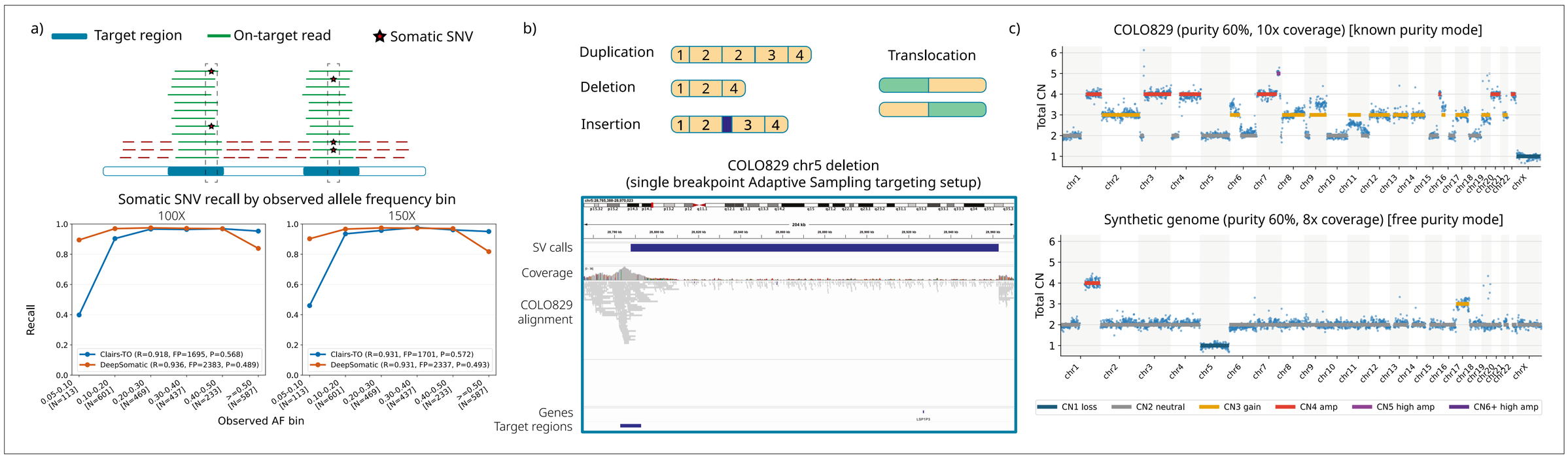

Fig. 2 Integrated SNV, SV, and CNV profiling from one dataset: a) SNV performance, b) SV recovery and breakpoint evidence, and c) CNV inference.

From a single dataset, Adaptive Sampling supports on-target somatic SNV calling, recovery of diverse SV classes, and stable genome-wide CNV inference

On-target reads enable confident tumour-only somatic single nucleotide variant (SNV) analysis using ClairS-TO and DeepSomatic (**Fig. 2a**). At matched on-target coverages of 80×, 100×, and 150×, both methods showed high recall for variants at allele fractions of 0.10 and above, while the 0.05–0.10 range remained more difficult. DeepSomatic improved recall at low allele fraction, whereas ClairS-TO produced fewer false positives. For SV, Severus was applied to the full aligned dataset, including both on-target and off-target reads (**Fig. 2b**). Using the full BAM improved recovery when only one breakpoint overlapped the digital panel or when supporting evidence extended into off-target regions, and representative translocations, deletions, duplications, and insertions were recovered. CNVs were inferred from the off-target low-pass signal with ichorCNA (**Fig. 2c**). Across COLO829 *in silico* mixtures spanning 2-15X effective genome-wide coverage and 20-100% tumour purity, segmentation was strong overall for the highly aberrant COLO829 genome, while the less-aberrant synthetic ladder was near-perfect across metrics. Taken together, these results support a single-sample, tumour-only workflow that combines targeted depth, structural resolution, and genome-wide copy-number signal in one assay, and can be retargeted by swapping the BED file for disease-focused research panels.

PO_1343(EN)_V1_19May2026