Oxford Nanopore Technologies

Digital panels enable targeted enrichment of haematological cancer-associated genomic regions during sequencing

Adaptive Sampling enables targeted enrichment of digitally defined genomic regions and supports interrogation of haematological cancer-associated genes from blood-derived and bone marrow-derived DNA

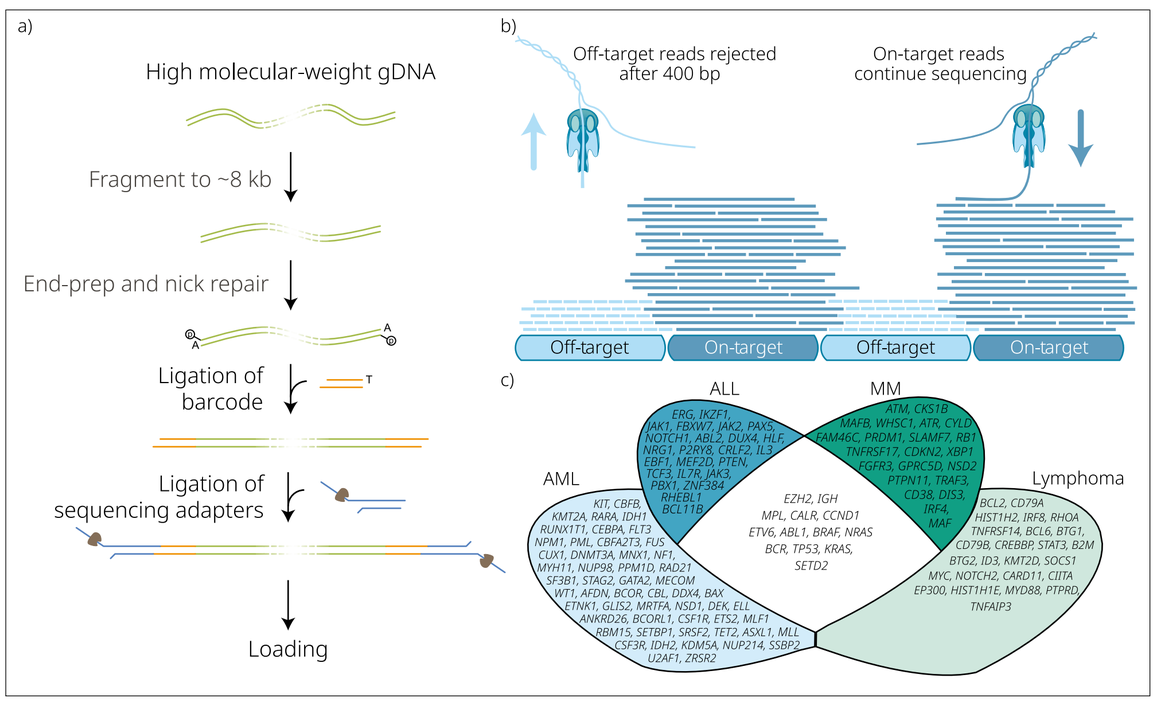

Fig. 1 a) Library preparation with barcoding, b) Adaptive Sampling schematic, c) Digital panel design.

Enrichment of genomic regions associated with pan-haematological cancers using Adaptive Sampling

Adaptive Sampling enables efficient enrichment of regions of interest during sequencing using a digital panel, without additional sample preparation. High molecular-weight DNA is fragmented to ~8 kb and end-prepared before barcode and adapter ligation using the Native Barcoding Kit 24 V14 (**Fig. 1a**). MinKNOW uses a reference FASTA file and a BED file defining the target regions to identify reads for continued sequencing, while off-target reads are rejected after approximately 400 bp (**Fig. 1b**). To demonstrate this approach, we used a BED file targeting 144 genes and regions associated with acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), multiple myeloma (MM), and lymphoma (**Fig. 1c**).

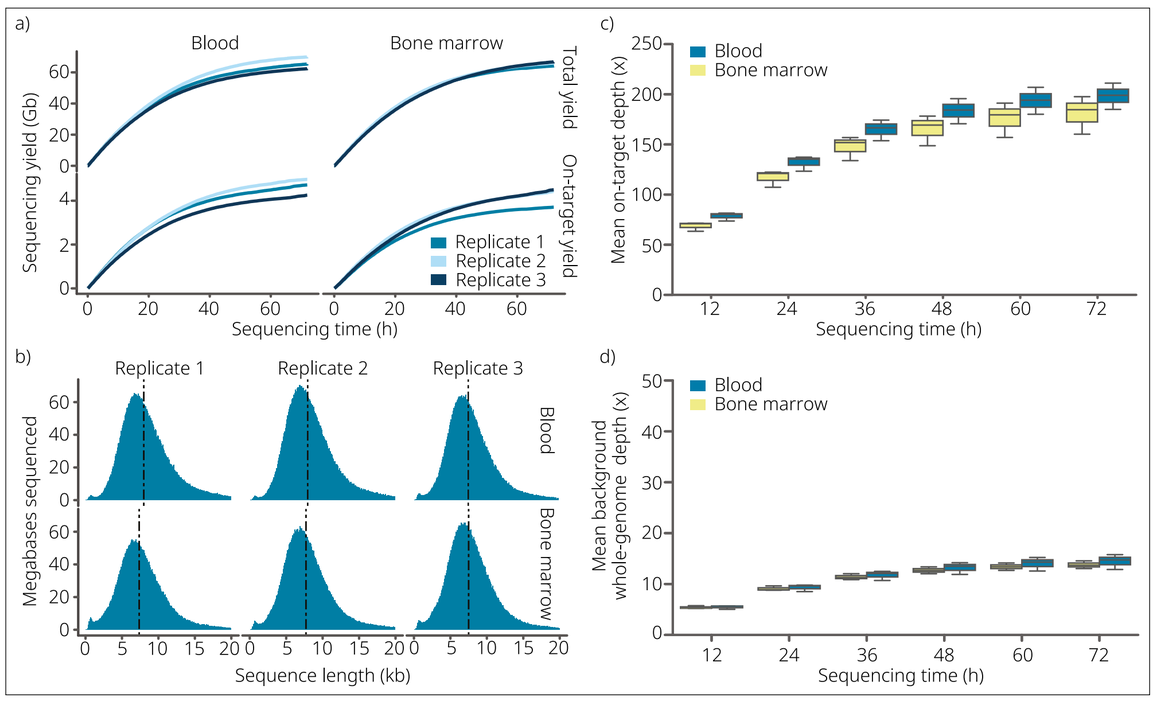

Fig. 3 a) Sequencing yield, b) on-target lengths, c) on-target coverage, and d) background coverage.

Adaptive Sampling of blood-derived and bone marrow-derived DNA provides sufficient enrichment for analysis

Genomic DNA extracted with the Puregene kit (n=3) was sequenced on PromethION Flow Cells (R10.4.1) using Adaptive Sampling with the pan-haematological cancer panel BED file for 72 hours. Comparable sequencing yields were obtained for blood-derived and bone marrow-derived DNA, at 65 ± 4 Gb and 66 ± 1 Gb, respectively (**Fig. 3a**), corresponding to an average of 112 million reads with an on-target read length N50 of 7.7 kb (**Fig. 3b**). Downsampling showed that 24 hours of sequencing was sufficient to achieve >100× coverage at 95% of target sites for both sample types. After 72 hours, mean on-target coverage reached ~198× for blood and ~180× for bone marrow (**Fig. 3c**), with background coverage of ~14× for each (**Fig. 3d**).

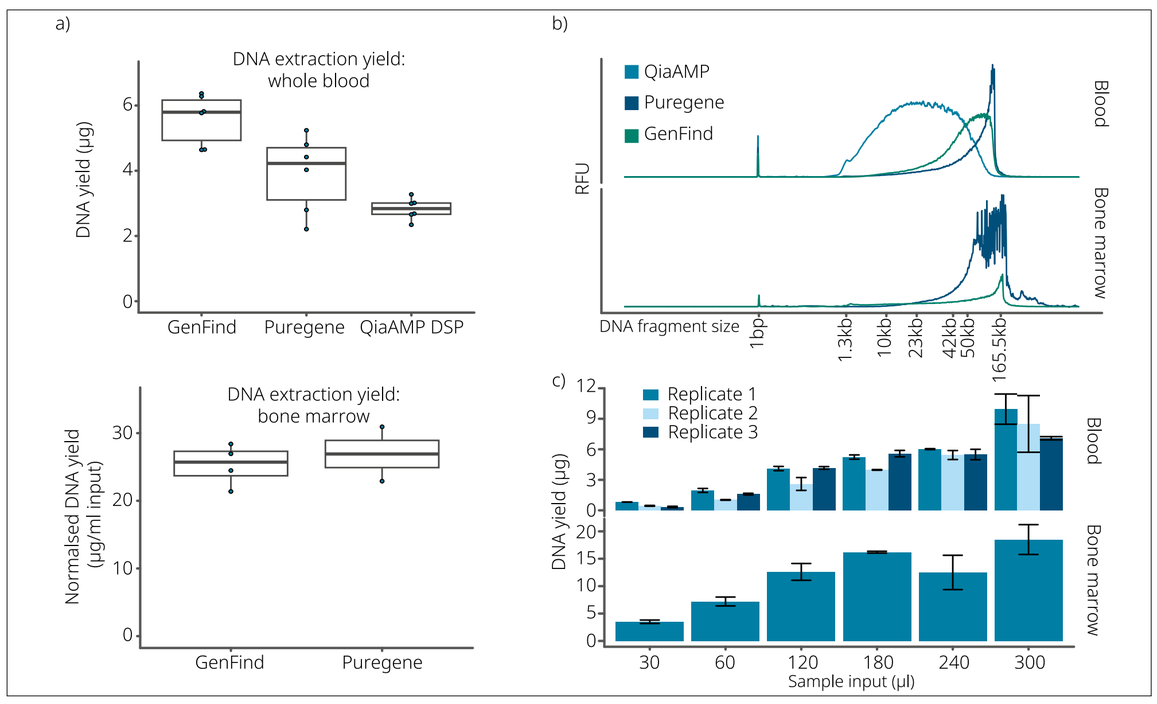

Fig. 2 a) DNA yield from blood and bone marrow, b) Fragment lengths, and c) Sample input volumes.

DNA extraction from whole blood and bone marrow research samples using common extraction kits

Using 200 µl of blood (n=3), the highest gDNA yield was obtained with GenFind (5.8 ± 0.5 µg), followed by Puregene (3.6 ± 1.1 µg) and QIAamp DSP (3.0 ± 0.2 µg). For bone marrow (n=1), GenFind and Puregene produced comparable DNA yield per input volume (25.3 µg/ml and 26.9 µg/ml) (**Fig. 2a**). Femtopulse analysis showed that Puregene gave the longest DNA fragments for both sample types, with mean fragment lengths of 115 kb ± 25 kb for blood and 108 kb ± 6 kb for bone marrow. GenFind produced shorter fragments, while QIAamp DSP generated the shortest DNA from blood (**Fig. 2b**). Reduced inputs of 60 µl for blood and 30 µl for bone marrow each yielded more than 1 µg of DNA, supporting downstream sequencing workflows (**Fig. 2c**).

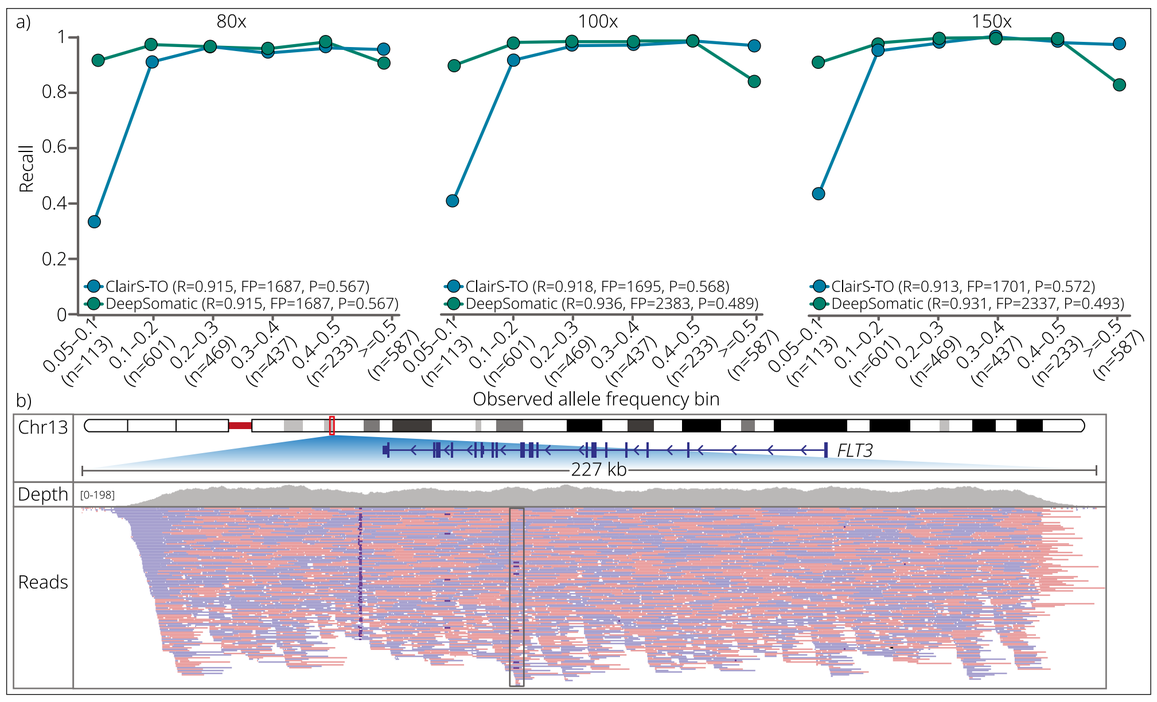

Fig. 4 a) *De novo* SNV calling, b) Horizon Mimix Myeloid Cancer Panel *FLT3* ITD insertion.

Benchmarking of methods to detect single nucleotide variants (SNVs) with digital panel data

For *de novo* SNV analysis, on-target COLO829 reads were basecalled using the super accuracy (SUP) model, then ClairS-TO and DeepSomatic were compared across binned variant allele frequencies (VAFs) for increasing sequence depth. ClairS-TO showed more balanced performance, whereas DeepSomatic recovered more true variants at 5–10% VAF, but with a higher false-positive rate (**Fig. 4a**). To assess structural variant detection, combined on-target and off-target data from the Horizon Mimix Myeloid Cancer Panel gDNA Reference Standard were examined for the *FLT3* internal tandem duplication (*FLT3*-ITD) 300 bp insertion present at 5% VAF. The insertion was visible in the IGV alignment (**Fig. 4b**).

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