Translocation detection in cancer using low-pass Pore-C sequencing

Oxford NANOPORE Technologies
APPLICATIONS

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Background

Complex chromosomal rearrangements, including translocations and extrachromosomal DNA (ecDNA) (Figure 1), play a critical role in oncogenesis and are often identified as recurrent genetic aberrations in hematologic malignancies and solid tumors[1]. Translocation detection is usually done by karyotyping of an individual's metaphase chromosomes. However, detection of balanced rearrangements, which have no gain or loss of genetic material, is often cytogenetically cryptic and can even be undetectable by methods based on copy number variation. For ecDNA, fluorescence microscopy-based detection tools are limited by their low signal-to-noise ratios and difficulty in generalizing the method to diverse ecDNA sequences.

Rearrangement detection using whole-genome sequencing (WGS) data is a viable approach for de novo translocation detection. However, its reliance on breakpoint-spanning reads requires both high sequencing depth (30-60x) and long reads to achieve high sensitivity, especially in repetitive regions of the genome.

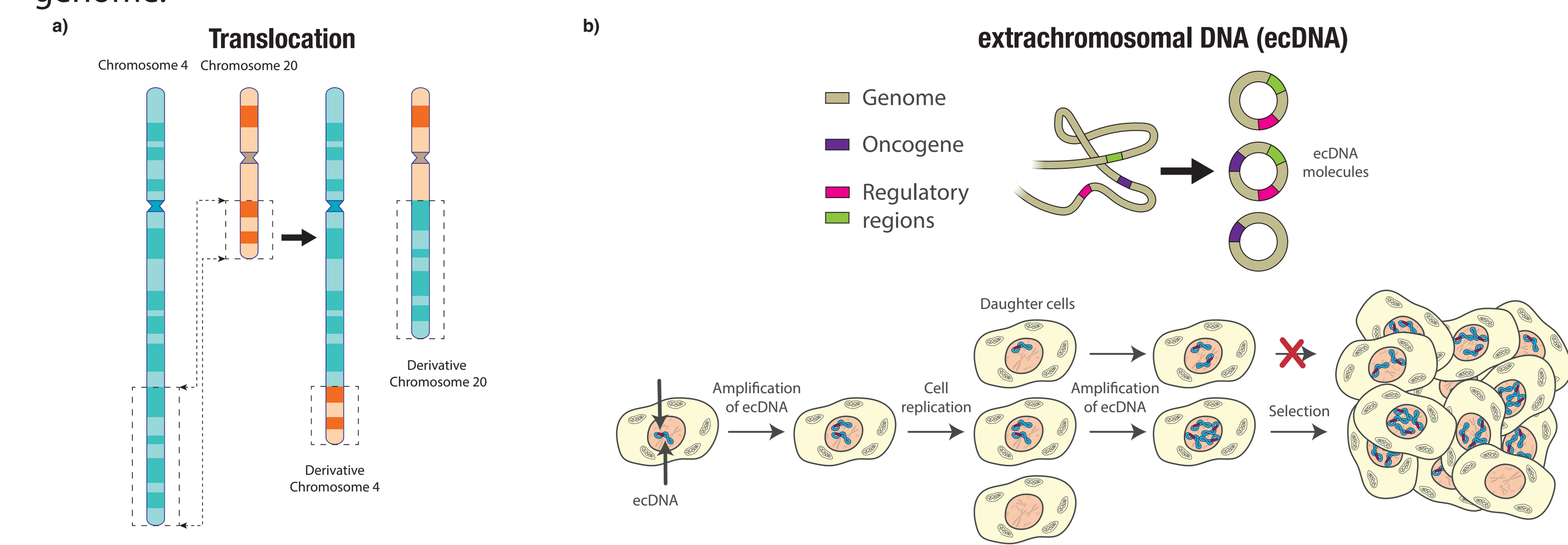

Figure 1. Translocations and extrachromosomal DNA genomic aberrations. a) Balanced translocation between q arms of chromosomes 4 and 20. b) Extrachromosomal DNA amplicon genomic composition. ecDNA amplicons proliferation in cancer cells and its impact on tumor heterogeneity.

Pore-C[2], developed by Oxford Nanopore Technologies, is a chromatin conformation capture tool that has a simpler and faster sample prep workflow than that of Hi-C. Importantly, after proximity ligation and purification, the long DNA concatemers containing multiple restriction enzyme fragments can be sequenced directly with the LSK114 library prep kit (Figure 2a,b).

Here, we describe a Pore-C workflow for translocation detection at low sequencing depth. Pore-C does not require breakpoint-spanning reads for translocation calling. Instead, a Pore-C library produces sequencing reads containing multiple short genomic segments that are in close physical proximity to each other in the sample. When the reads are aligned to a reference genome, translocation breakpoints present as an enrichment of trans contacts between two chromosomes. This dramatically reduces sequencing depth requirements and improves mapping in low complexity regions (Figure 2c).

In addition to translocations, low-pass Pore-C libraries are also capable of detecting ecDNA in cancer samples. ecDNA molecules are often present at a high copy number in cells and due to their mobility can physically interact with any chromosome, leading to a characteristic pattern of extensive contacts between the specific sequences that comprise the ecDNA molecule and the full chromosomes.

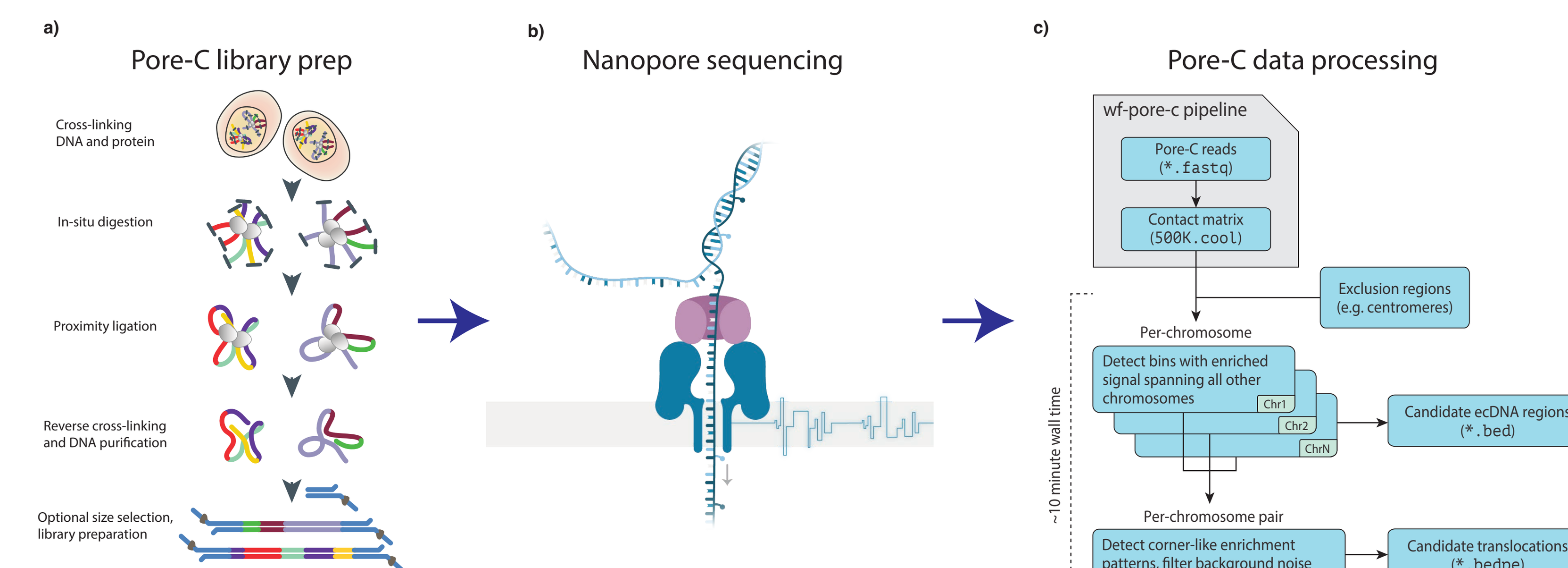

Figure 2. Pore-C based workflow for translocation and ecDNA detection. a) Main steps in Pore-C library preparation protocol. b) Nanopore sequencing of Pore-C library c) Computational processing of sequenced Pore-C reads including EPI2ME Labs wf-pore-c workflow as well as novel method for translocations and ecDNA inference in low resolution trans-chromosomal Pore-C contact maps.

Low-pass Pore-C sequencing of 12 multiplexed cancer samples

To demonstrate the suitability of low-pass Pore-C for translocation detection we prepared, barcoded, and sequenced Pore-C libraries of 12 distinct cancer cell lines including lung cancer (A549), leukemia (THP-1, GDM-1), melanoma (COLO829), breast cancer (HCC1806), neuroblastoma (CHP212) and 6 others from NIGMS Human Genetic Cell Repository on a single Q20+ PromethION flow cell. We obtained >90Gb of yield with an average concatemer read length N50 of >5Kbp.

Sequenced reads were basecalled and demultiplexed using `dorado` and processed with `EPI2ME` Labs wf-pore-c workflow. We observed an average genome-wide read depth coverage of >2x per barcode, allowing for both manual and automated analysis of chromosomal contact maps (Figure 3).

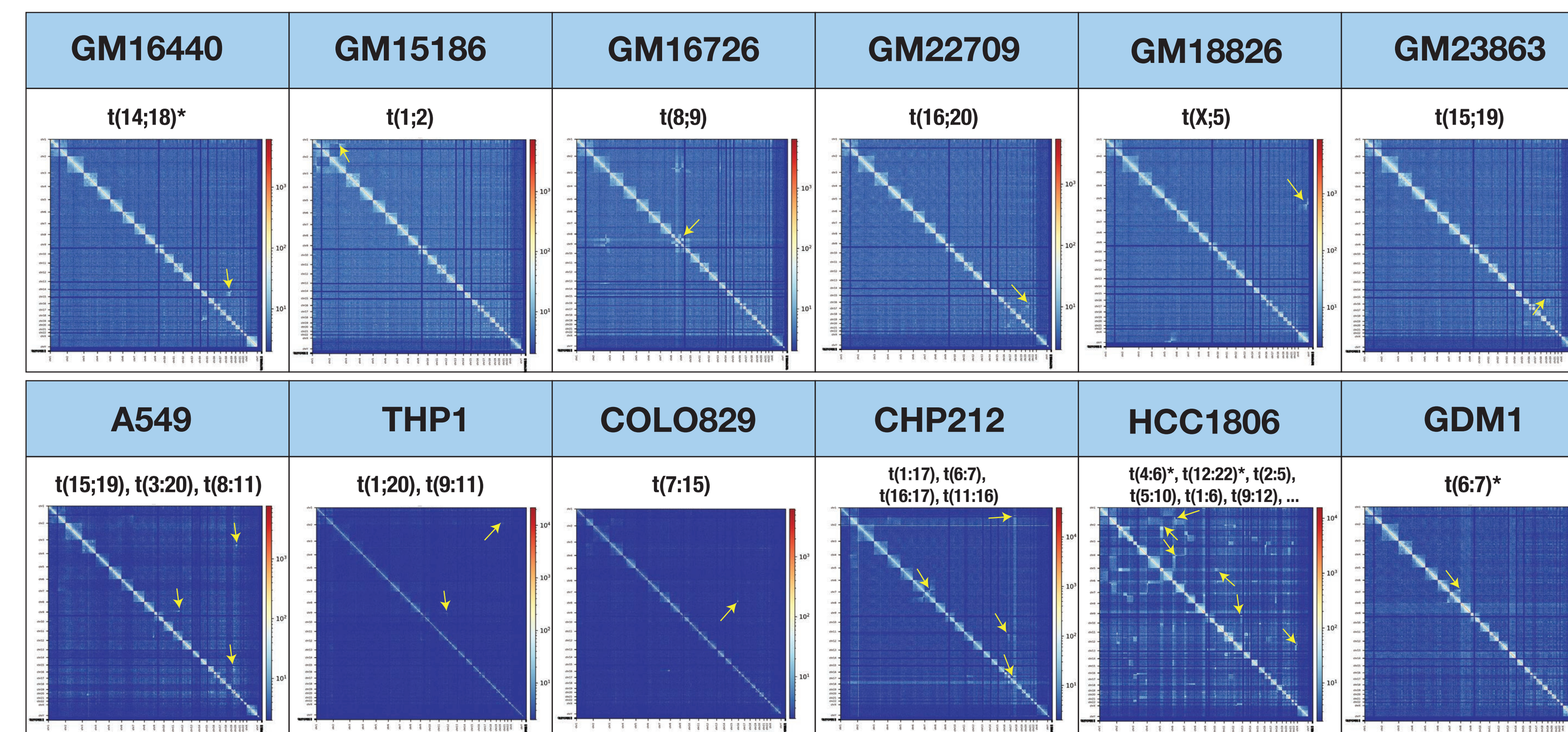

Figure 3. Genome-wide contact maps for low-pass Pore-C sequencing of 12 barcoded and multiplexed cancer cell lines with translocations highlighted with yellow arrows.

Detection of balanced and unbalanced translocations

We developed a novel method to automate the detection of translocations in low-pass Pore-C data using image processing algorithms. Briefly, for each inter-chromosomal contact map we first apply a triangle threshold, followed by the Harris corner detector[3] to identify top candidate bins containing a translocation breakpoint. Each candidate bin is then filtered using a heuristic scoring approach to identify whether the degree of contact enrichment and the surrounding contact pattern is consistent with the presence of a translocation breakpoint. This approach allows for detection of multiple balanced and unbalanced translocations for every chromosomal pair, enabling the identification of complex cross-chromosomal rearrangement events (Figure 4).

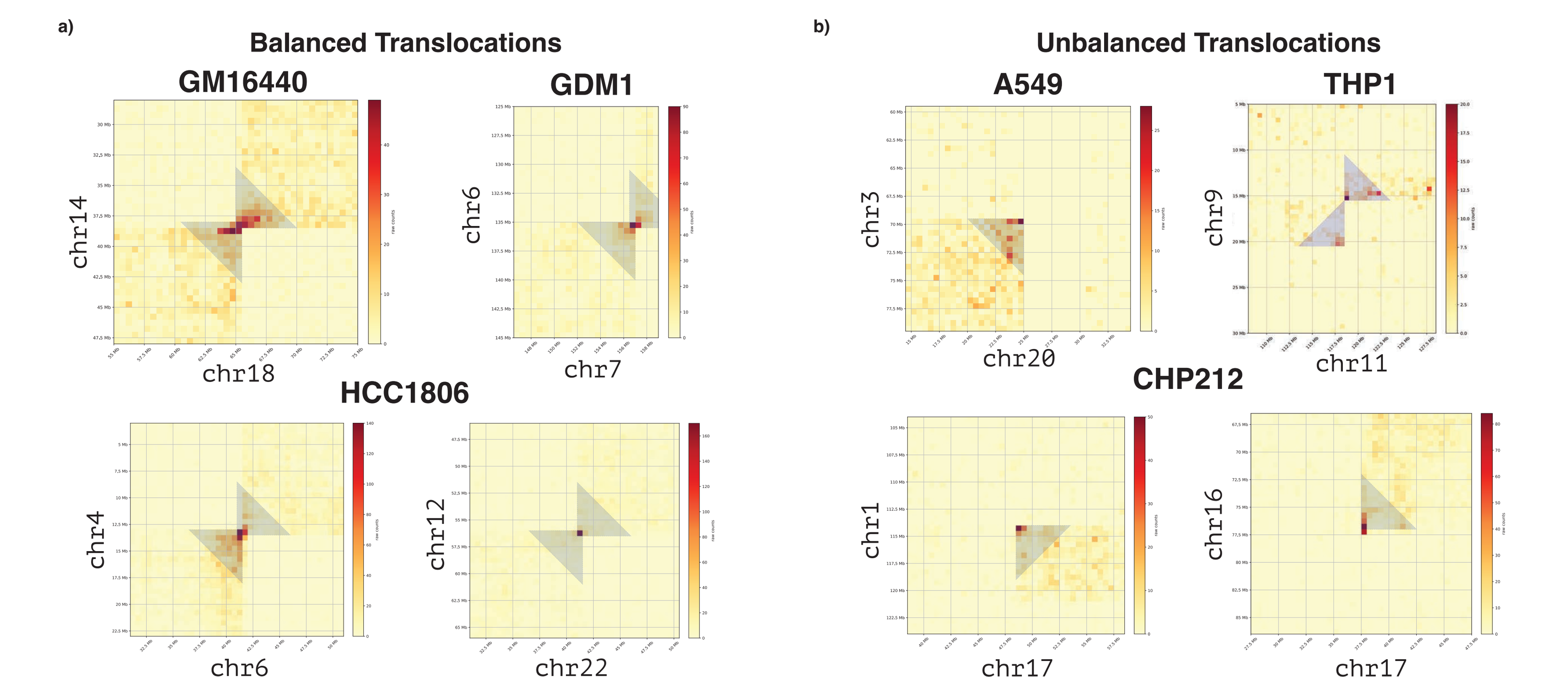

Figure 4. Examples of computational predictions (blue triangles) of translocations inferred on low-pass Pore-C inter-chromosomal contact map. a) Balanced / reciprocal translocations exhibit a butterfly / symmetrical angle pattern. b) Unbalanced translocations with a singluer angular pattern around the breakpoint.

Detection of extrachromosomal DNA

We also developed a novel method for automated detection of ecDNA in low-pass Pore-C data. We focus on identifying chromosome- and genome-wide interaction strips (Figure 5) indicative of the presence of ecDNA amplicons. Briefly, we first apply a triangle threshold to contact maps, followed by iterative morphological closing with a horizontal footprint, followed by small horizontal "island" removal. Finally we use the Hough line transform to detect horizontal lines. This approach enables the identification of genomic regions comprising ecDNA amplicons without any restriction on the number of ecDNAs.

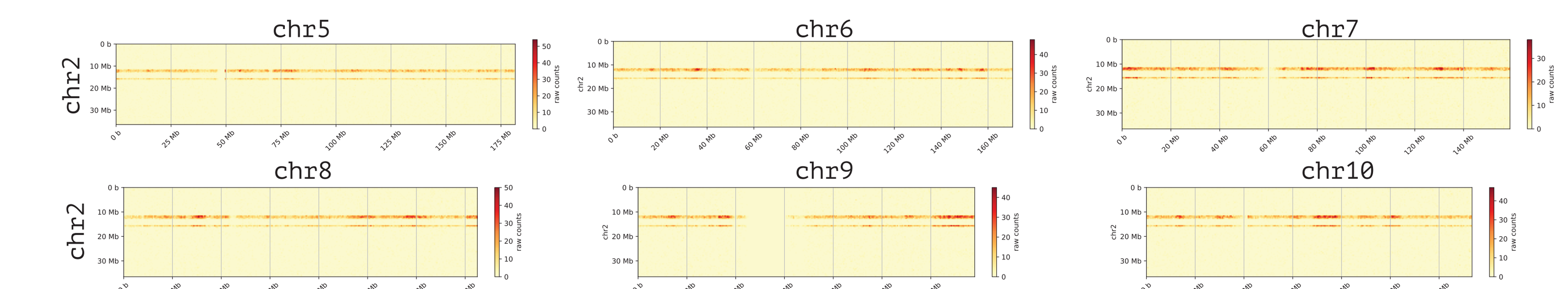

Figure 5. Example of several Pore-C inter-chromosomal contact maps with computationally predicted ecDNA-comprising fragments (chr2:11.5-13Mbp and chr2:15.5-16.5Mbp) in CHP212 cell line.

Nanopore adaptive sampling confirms low-pass Pore-C results

Translocations and ecDNA molecules detected with low-pass nanopore Pore-C sequencing can be confirmed or refined using nanopore adaptive sampling. This is an on-instrument sequencing mode that enriches for reads from certain target regions without the need for any additional sample prep steps (Figure 6a). Regions highlighted by Pore-C as potentially containing translocation events or ecDNA sequences can be subsequently targeted using adaptive sampling to identify the base-pair-resolved translocation breakpoints and ecDNA boundaries (Figure 6b).

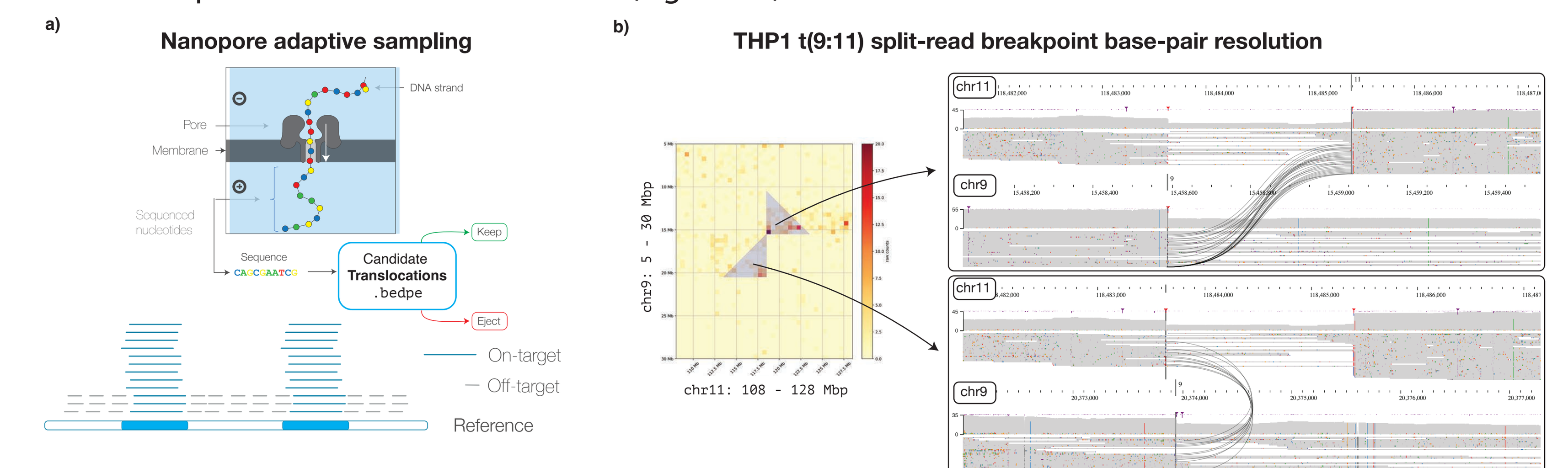

Figure 6. Nanopore adaptive sampling translocation breakpoint base-pair resolution. a) Adaptive sampling workflow with target regions comprised of low-pass Pore-C translocation and ecDNA predictions. b) Example of base-pair resolved breakpoint for 2 translocations t(9;11) predicted for THP1 cell line low-pass Pore-C data.

Conclusions

Low-pass Pore-C enables the detection of translocations and extrachromosomal DNA amplicons in cancer samples in an unbiased manner and does not require prior knowledge of either the translocation or ecDNA structure. Combined with its simple sample-preparation workflow and multiplex capability, it can serve as a cost-effective and comprehensive tool for cancer genomic studies. The ability to perform adaptive sampling to target the regions identified by low-pass Pore-C provides a unique and cost-effective capability to confirm and fully resolve translocation breakpoints and ecDNA boundaries at a base-pair resolution.

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References

1. Rabbitts, Terence H. "Chromosomal translocations in human cancer." Nature 372.6502 (1994): 143-149.
2. Deshpande, Aditya S., et al. "Identifying synergistic high-order 3D chromatin conformations from genome-scale nanopore concatemer sequencing." Nature Biotechnology 40.10 (2022): 1488-1499.
3. Harris, Chris, and Mike Stephens. "A combined corner and edge detector." Alvey vision conference. Vol. 15. No. 50. 1988.

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