HemaTrack ALL: UMI-corrected Nanopore Sequencing for Highly Sensitive Measureable Residual Disease (MRD) Monitoring in ALL

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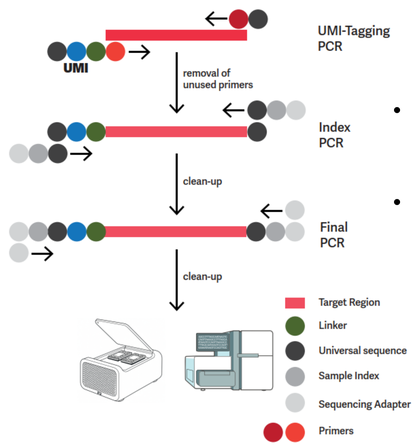
Background

- **Measurable residual disease (MRD)** is a key parameter for risk-adapted stratification in acute lymphoblastic leukemia (ALL)
- **Limitations of standard methods**: flow cytometry and real-time quantitative PCR (RQ-PCR) show constraints in standardization, sensitivity, or scalability, although RQ-PCR remains clinical gold standard
- **Next-generation sequencing (NGS)** of immunoglobulin (IG) and T-cell receptor (TR) rearrangements can address limitations → establishment of NGS-based MRD primarily on Illumina platforms
- **Oxford Nanopore Technologies (ONT):** higher raw error rate has so far prevented its use for MRD detection, but offers the potential for flexible sample throughput in short turnaround times

Aim

- Develop and validate a **nanopore-based assay with Unique Molecular Identifier (UMI) - mediated error correction** for highly sensitive, quantitative, and cost-efficient MRD monitoring in ALL, using Illumina-based NGS as the NGS reference and RQ-PCR as clinical gold standard

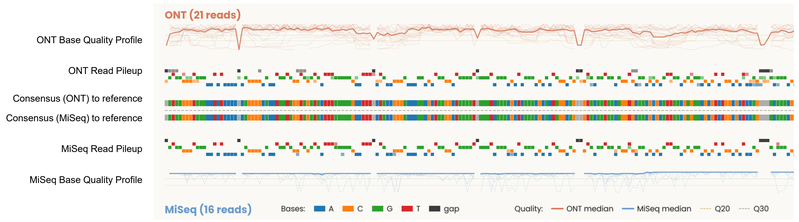
Method



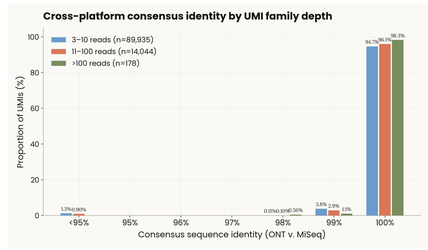
- UMI-tagging of immunoglobulin (**IGH, IGK**) and T-cell receptor genes (**TRD, TRG, TRB**) in a multiplexed format
- Automated analysis pipeline (**QuaSIR**) optimized for ONT data
- Same workflow for patient samples **at diagnosis** (1 µg input DNA in 2 reactions) or at a **follow-up timepoint** (3.5 µg input DNA in 2 reactions as triplicates + reference gene for quantification)

Results

1) Sequencing analysis



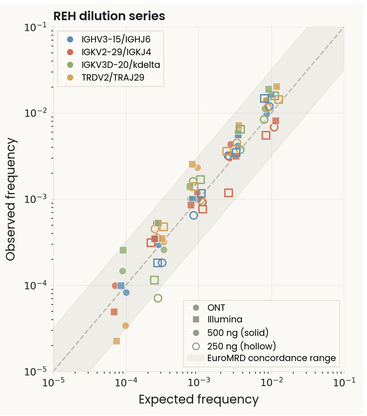
1a) Comparison of ONT and Illumina read alignments and base quality profiles for an exemplary family of reads sharing the same UMI. Despite substantial ONT noise, UMI-based error correction leads to identical consensus sequences on both platforms.



→ High-fidelity consensus sequences based on UMI families corrects platform-specific sequencing errors

1b) Cross-platform consensus identity by UMI family depth of 104157 paired UMI families from 11 diagnostic samples. 94.9% of paired UMI families produce identical consensus sequences.

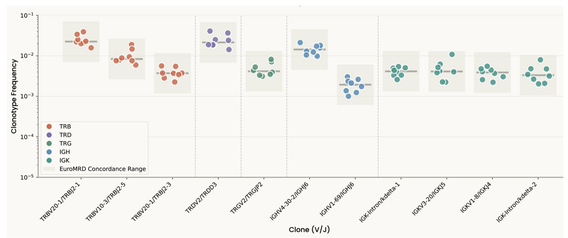
2) Analytical performance



2a) Accuracy analysis: genomic DNA of ALL cell line REH serially diluted into DNA from healthy individuals. Clonotype frequencies were analysed with HemaTrack ALL (MiSeq & ONT)

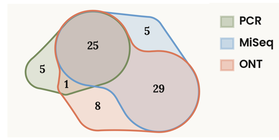
→ Accurate and linear quantification of ALL cell line in matched Illumina–ONT datasets down to 1 in 10,000 cells (10^{-4})

→ Precise quantification by ONT



2b) Precision analysis: genomic DNA of ALL cell line NALM-6 (1%) and MOLT-3 (1%) spiked into DNA from healthy individuals. Clonotype frequencies were analysed with HemaTrack ALL (ONT) in 8 independent experiments.

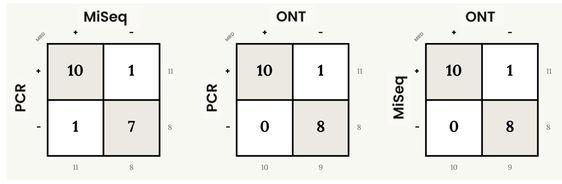
3) Clinical performance



3a) Overlap of detected diagnostic markers from 11 patients across the three methods. Shared and method-specific counts are indicated.

Clinical cohort of biobanked ALL samples with prior RQ-PCR-based MRD assessment

→ HemaTrack ALL identifies more informative rearrangements than PCR



3b) 2x2 contingency tables comparing MRD status (positive + / negative -) between methods across 19 biobanked follow-up timepoints from 11 ALL patients

» HemaTrack ALL achieves high sample-level MRD concordance across technologies

Conclusion

- HemaTrack ALL is a **robust end-to-end solution for accurate MRD monitoring** in ALL.
- UMI-corrected nanopore sequencing exploits the specific advantages of ONT sequencing, such as **higher flexibility** in batch sizes**, shorter turnaround times, and reduced costs**.

Outlook

- A prospective multi-center clinical study has recently been initiated in collaboration with Tata Translational Cancer Research Centre (TTCRC), Kolkata, India.
- In Europe, HemaTrack ALL is currently being evaluated under RUO (Research Use Only) conditions by selected beta testers.

Acknowledgements

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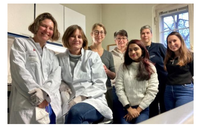

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