HemaTrack-ALL: Taking advantage of Unique Molecular Identifiers and NGS for accurately monitoring MRD in ALL

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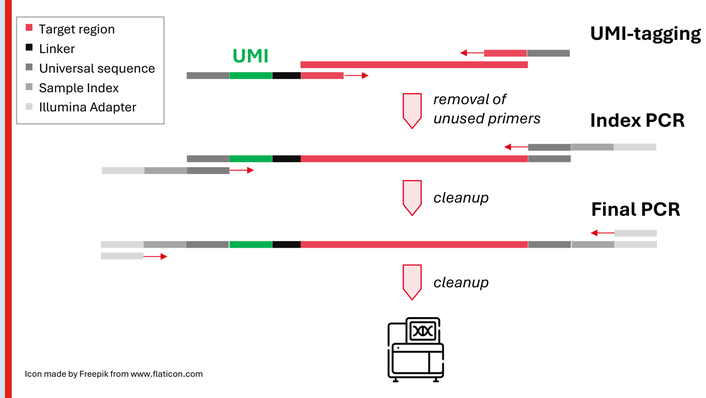
Introduction

Measurable Residual Disease (MRD) is the single best predictor for survival outcomes in Acute Lymphoblastic Leukemia (ALL). PCR-based MRD assays have emerged as gold standard in Europe promising higher specificity and reproducibility than flow cytometry-based approaches. The use of NGS may outperform laborious patient-specific assay development in qPCR-based quantification approaches, as it provides high level of specificity due to single nucleotide resolution while delivering sufficient data to achieve high sensitivity. However, biases in PCR amplification may severely distort accuracy of quantification.

To address this issue, we developed a robust NGS-based approach for MRD monitoring in ALL, that overcomes existing limitations by incorporating Unique Molecular Identifiers (UMIs).

Methods



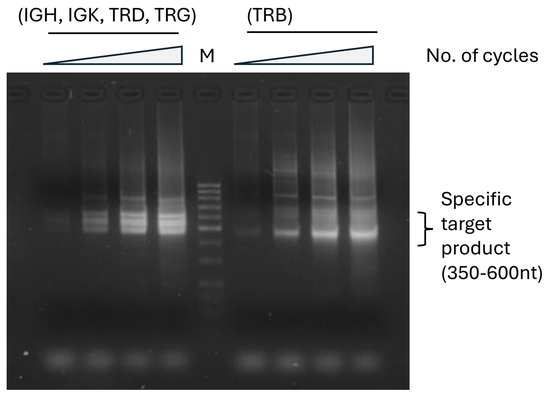
Schematic workflow of HemaTrack-ALL.
HemaTrack amplifies clonotype sequences in three major steps whereas each step is followed by a clean up procedure:

1. **UMI-tagging step:** UMIs are appended with two cycles of PCR containing 500ng of genomic DNA in two highly multiplexed reactions targeting IGH, IGK, TRG and TRD with primer set 1 and optionally, TRB with primer set 2. A third reaction amplifies a reference gene in a singleplex reaction. Noteworthy, the same workflow is applied to patient samples at diagnosis or at a follow-up timepoint except that reactions containing primer set 1 or 2 are performed in triplicates in case of follow-up samples.
2. **Index PCR:** Universal primers containing sample barcodes and Illumina adapter sequences are added and a second PCR is performed for a limited number of cycles. Triplicate reaction are pooled at this step in case of samples from follow-up timepoints
3. **Final PCR:** Primers containing only Illumina adapters are added to the purified products of the Index PCR.

Results

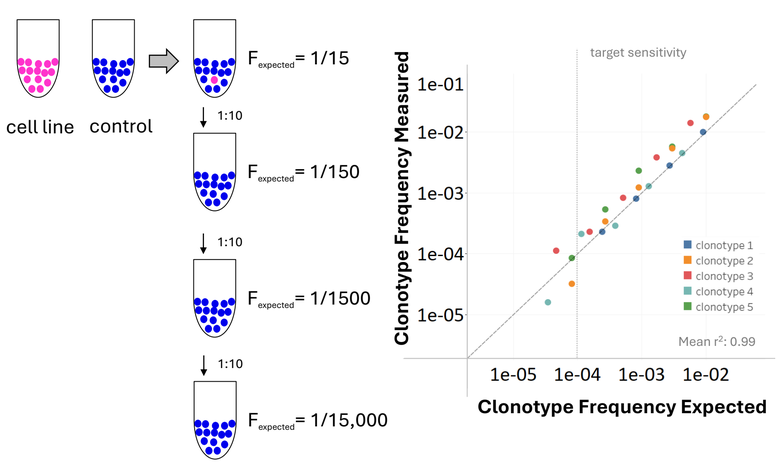
Assay specifications

Highly multiplexed PCR workflow



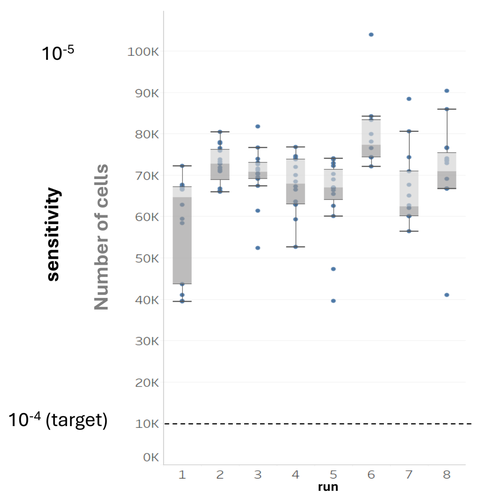
HemaTrack-ALL highly multiplexed PCR regime generates specific product devoid of primer-dimers.
A semi-quantitative PCR with genomic DNA from diluted ALL cell lines (#1: 20% REH; #2: 20% PEER) is presented as indicated with the four lanes for each sample.

Accurate quantification



HemaTrack-ALL quantifies accurately. HemaTrack-ALL was performed on genomic DNA of several ALL cell lines with known clonotypes and frequencies were diluted serially into blood DNA from healthy individuals.

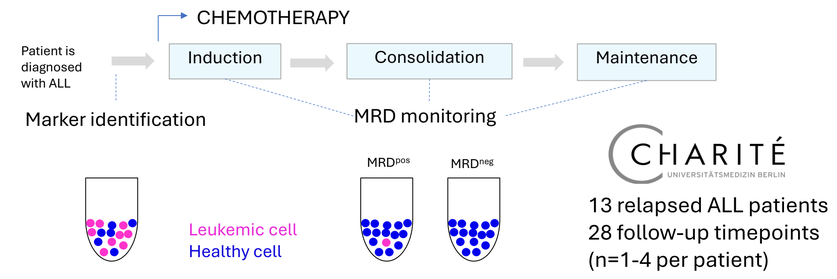
High sensitivity



HemaTrack-ALL exhibits high sensitivity. Sensitivity of HemaTrack-ALL was performed in follow-up timepoints of ALL patients (n=114). Genomic DNA from follow-up samples of ALL patients (previously biobanked at the TMC Kolkata, India) were subjected to HemaTrack-ALL. Up to 14 different samples were included in a MiSeq run. The sensitivity was calculated by means of a reference gene and is given here as the number of cell equivalents. The box marks the interquartile range (IQR) while the whiskers extends to all datapoints that are within 1.5x IQR.

Comparison with gold standard

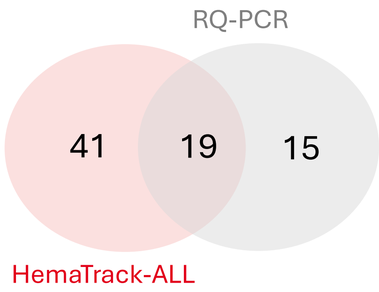
Patient cohort and samples



13 relapsed ALL patients
28 follow-up timepoints
(n=1-4 per patient)

ALL study cohort. HemaTrack-ALL was performed on samples from relapsed ALL patients for which RQ-PCR results were available for comparison purposes.

HemaTrack-ALL monitors more clonotypes



HemaTrack-ALL follows more index clonotypes than the gold standard (RQ-PCR).
Upper panel: Number of index clonotypes identified and monitored by either or both (intersection) methods.
Lower panel: Average number of clonotypes used for MRD monitoring by the respective methods.

RQ-PCR	**2.62**
HemaTrack-ALL	**4.62**

Good concordance with RQ-PCR in MRD calls

		RQ-PCR	
	MRD	+	-
HemaTrack-ALL	+	7	5
	-	1	15
		88%	75%
		sensitivity	specificity

HemaTrack-ALL achieves high level of sensitivity and specificity in comparison to gold standard (RQ-PCR). 2x2 contingency table comparing the MRD statuses from 28 follow-up timepoints called by both methods.

Conclusion

HemaTrack-ALL is an innovative NGS-based, end-to-end workflow which features the identification of markers of ALL blasts and their subsequent high sensitivity quantitative tracking. The assay presents with a straightforward way of quantification, meets the clinical requirements in terms of accuracy and sensitivity and will be further evaluated in real-live clinical routine in a prospective clinical trial in India next year.

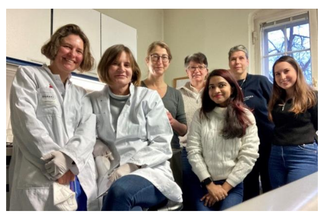
Outlook

HemaTrack-ALL completed one small research test phase and is currently evaluated in a large retrospective study (216 samples in total) in Kolkata, India.
In 2025, HemaTrack-ALL will be assessed in prospective trial format in three cancer centers across India (TMC Kolkata, SJMCH Bengaluru and WIA Chennai) in a large cohort.

Acknowledgements

Team TTCRC

Team Charité



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