From bone marrow to report: an end-to-end NGS workflow for absolute quantification of MRD in ALL

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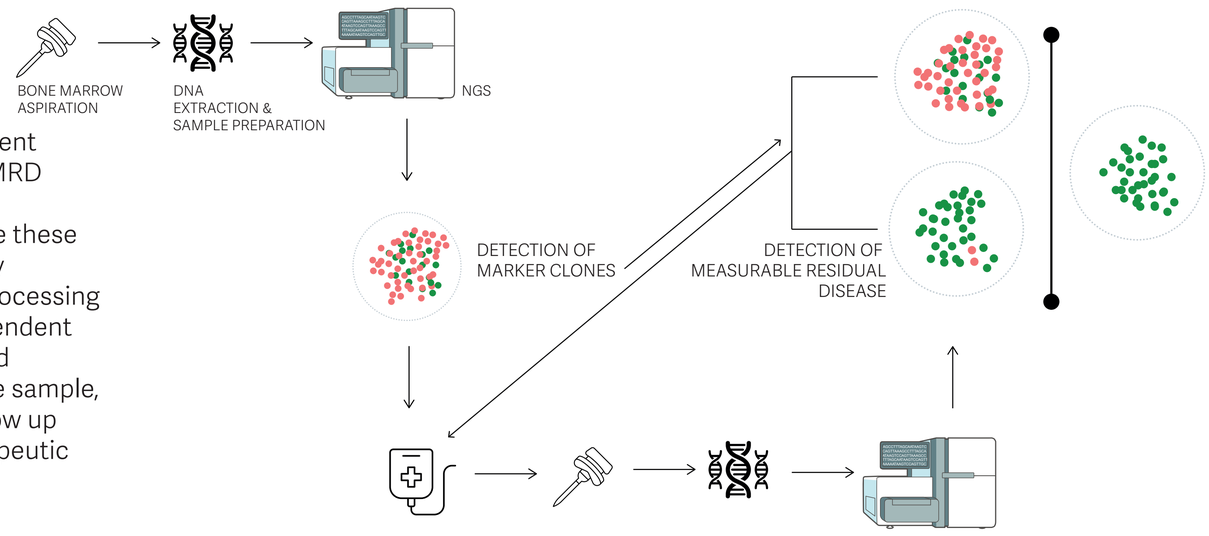
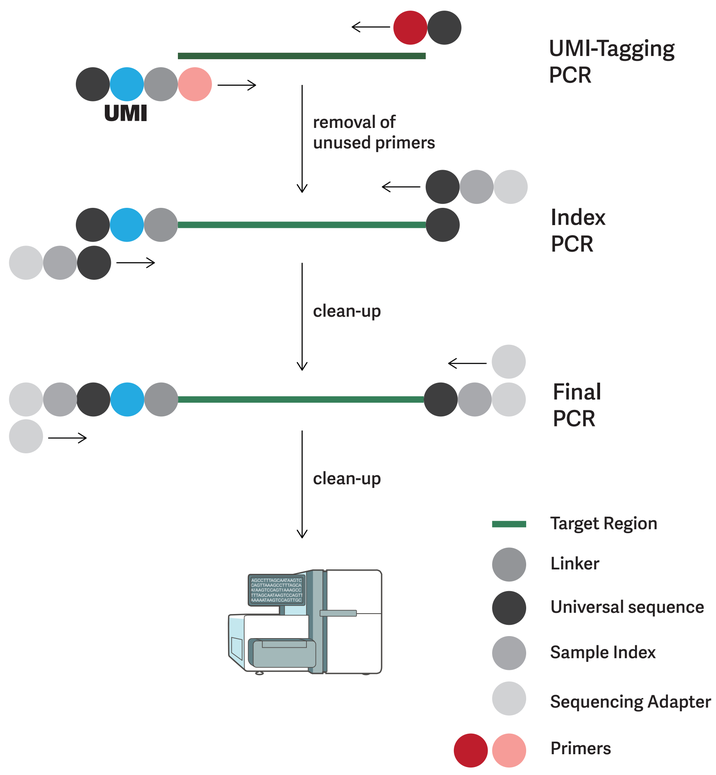
MOTIVATION

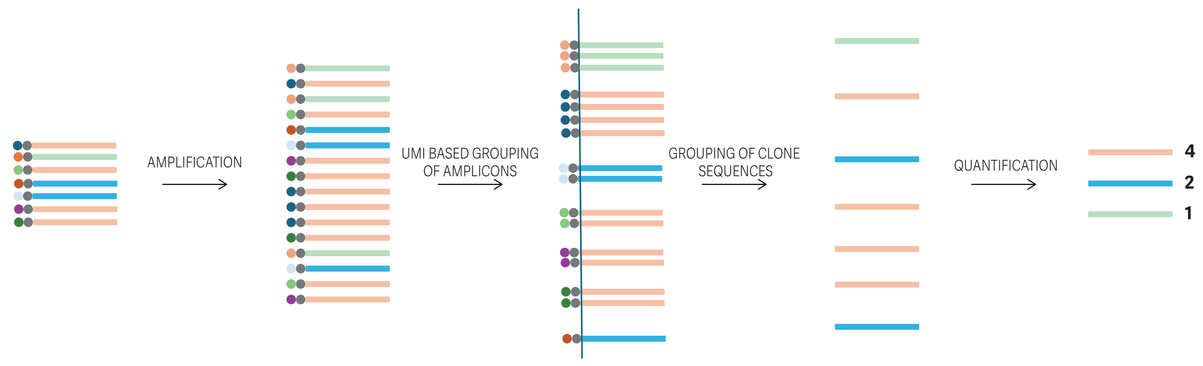
Minimal Residual Disease (MRD) monitoring is crucial in ALL treatment as it strongly predicts patient outcomes and guides therapeutic decision-making. NGS has become increasingly important for MRD detection in ALL. While NGS provides comprehensive marker identification, it has limitations in amplification bias and quantification accuracy. Unique Molecular Identifiers (UMIs) help overcome these challenges by enabling precise counting, reducing bias, and improving error correction, potentially enhancing MRD quantification accuracy. Currently, there is no end-to-end software solution for processing UMI-based T/B-Cell Receptor sequencing data for MRD assessment. QuaSIR is an operator independent nomenclature agnositc framework that processes FASTQs derived from sequencing of UMI-tagged multiplexed PCR of DNA extracted from bone marrow samples. Depending on the timepoint of the sample, QuaSIR then identifies clones that are markers for leukemic blasts, or tracks marker clones in follow up samples. QuaSIR then generates a user-friendly comprehensive report to aid the clinician in therapeutic interventions.



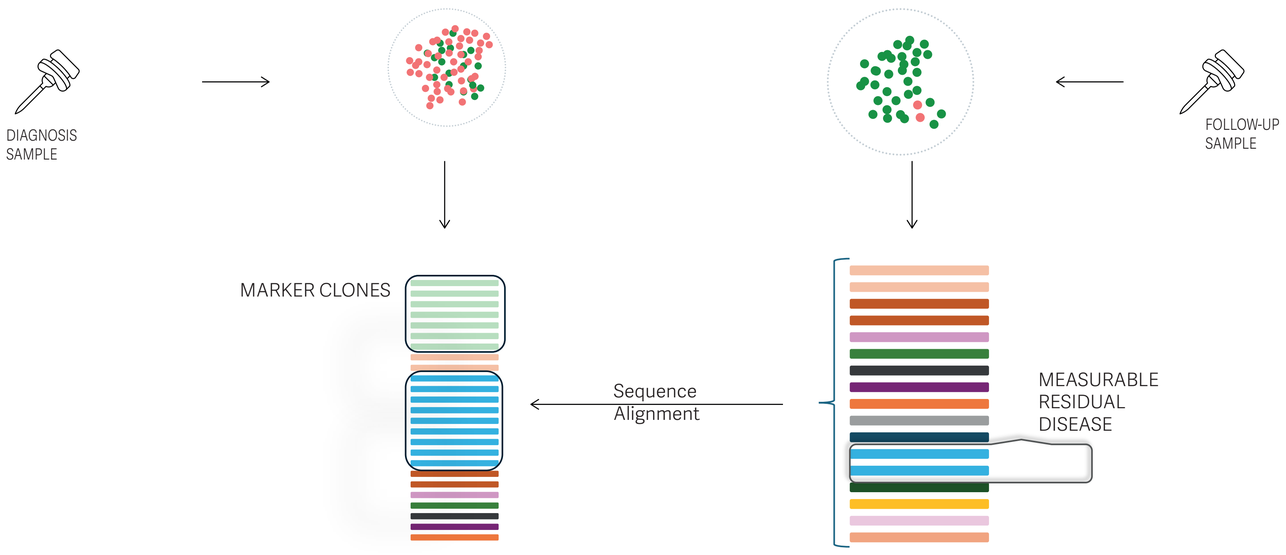
CONCEPT



DIAGNOSTIC ANALYSIS WORKFLOW



FOLLOW-UP ANALYSIS WORKFLOW



WETLAB WORKFLOW

HemaTrack-ALL amplifies clonotype sequences in 3 major steps, each of which is followed by a clean-up procedure

1. UMI-tagging PCR : 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. It is to be noted that 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 for samples from follow-up timepoints.

3. Final PCR: Primers containing only Illumina adapters are added to the purified products of the Index PCR.

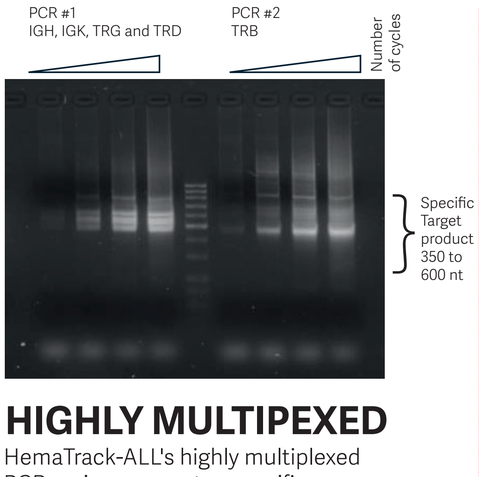
DIAGNOSTIC ANALYSIS WORKFLOW

QuaSIR operates entirely on a sequence basis until the reporting stage. This helps avoid inconsistencies that arise due to mapping artifacts thereby ensuring robust and accurate MRD detection and quantification. QuaSIR involves two rounds of sequence grouping, first on the basis of UMIs, followed by a further gouping of error corrected sequences. This yields quantified clonotype sequences. QuaSIR then uses a Poisson distribution based statistical procedure to determine abundant clonotypes. These clonotypes serve as markers for the follow up timepoints.

FOLLOW-UP ANALYSIS WORKFLOW

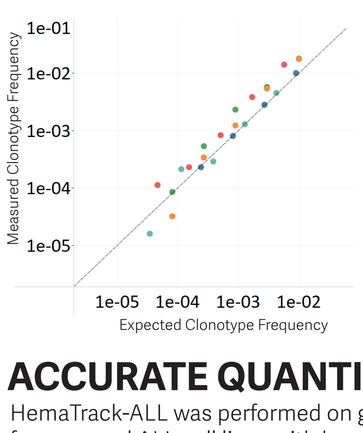
In Follow-Up mode, QuaSIR subjects the sequences to two rounds of grouping as in the diagnostic workflow. Subsequently, QuaSIR aligns the clonotype sequences against those of the marker clones from the diagnostic sample. Using the results of the alignment procedure, QuaSIR then detects and quantifies the residual presence of marker clones. The final MRD call is made according to rules based on UMI presence and read support across the 3 DNA pools derived from the Follow-Up sample.

RESULTS



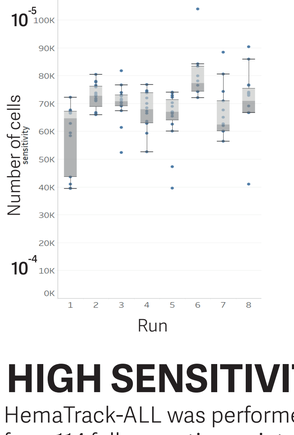
HIGHLY MULTIPEXED

HemaTrack-ALL's highly multiplexed PCR regime generates specific product devoid of primer-dimers. A semi-quantitative PCR with genomic DNA from diluted cell lines (20% REH, 20% PEER) is shown.



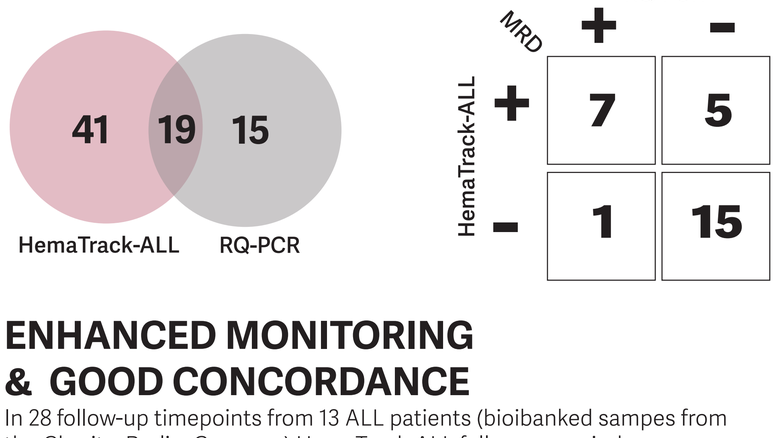
ACCURATE QUANTIFICATION

HemaTrack-ALL was performed on genomic DNA from several ALL cell lines with known clonotypes, pooled together and diluted serially into blood DNA from healthy individuals. The measured frequency of the known clonotypes showed excellent correlation with the expected frequencies.



HIGH SENSITIVITY

HemaTrack-ALL was performed on genomic DNA from 114 follow-up timepoints of biobanked ALL patient samples. Sensitivity was calculated by profiling a reference gene and is given here as the number of cells equivalent.



ENHANCED MONITORING & GOOD CONCORDANCE

In 28 follow-up timepoints from 13 ALL patients (bioibanked sampes from the Charite, Berlin, Germany) HemaTrack-ALL follows more index clonotypes than the gold standard RQ-PCR allowing for enhanced MRD monitoring. HemaTrack-ALL also achieves high levels of sensitivity (88%) and specificity (75%) in comparison to RQ-PCR.

HemaTrack™ is a UMI-based multiplexed TCR/BCR profiling assay for MRD quantification in ALL. QuaSIR™, the analysis workflow arm of HemaTrack™ analyzes TCR/BCR NGS data and generates comprehensive reports to aid in the diagnosis and treatment of ALL. The assay demonstrates linearity in dilution experiments using leukemic cell lines for TCR/BCR profiling. In retrospective analysis of bio-banked ALL samples, the combined HemaTrack™ and QuaSIR™ workflow achieves excellent sensitivity and specificity when benchmarked against RQ-PCR. This integrated workflow enables broader application of NGS-based MRD detection in clinical settings. The utility and accuracy of HemaTrack™ will be evaluated in an upcoming multicenter prospective clinical trial.

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