Quantitative Sequencing of Immune Repertoires (QuaSIR)

A software suite for accurate ALL-MRD quantification from UMI-tagged NGS data

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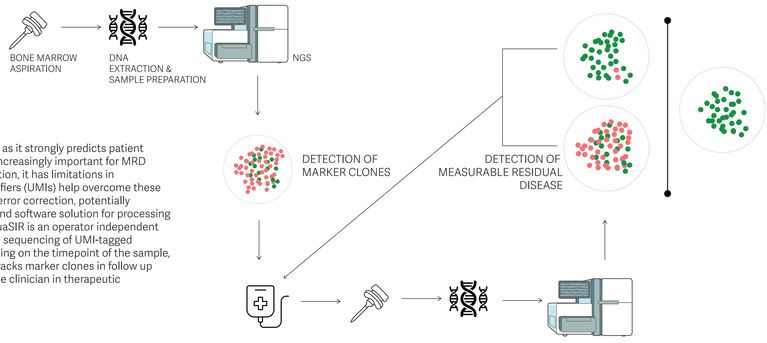
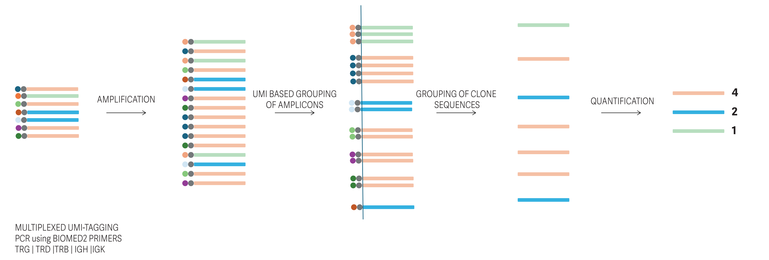
1. MOTIVATION

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 medical report to aid the clinician in therapeutic interventions.

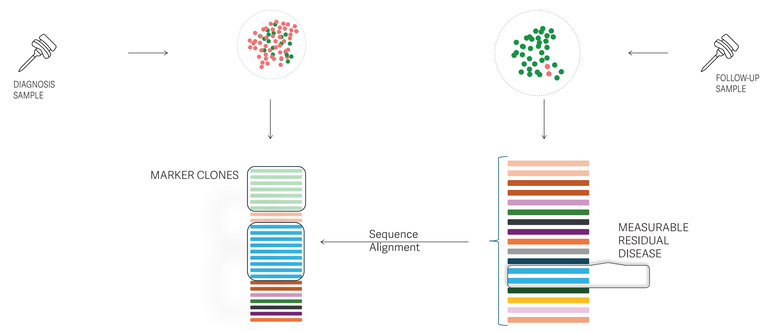


2. CONCEPT



DIAGNOSTIC 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.

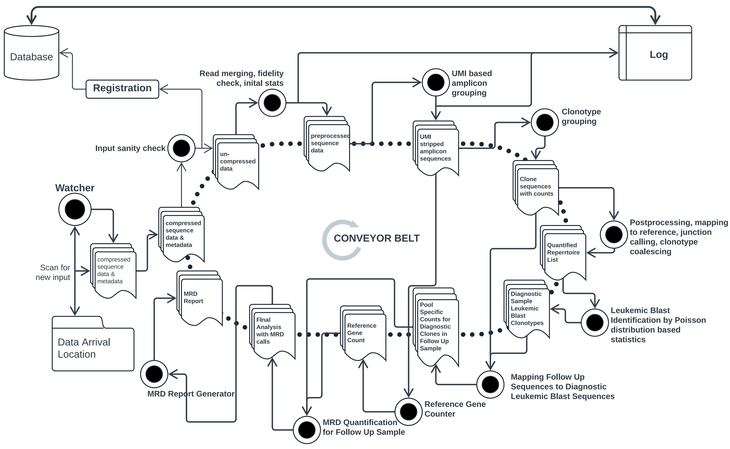


FOLLOW-UP WORKFLOW

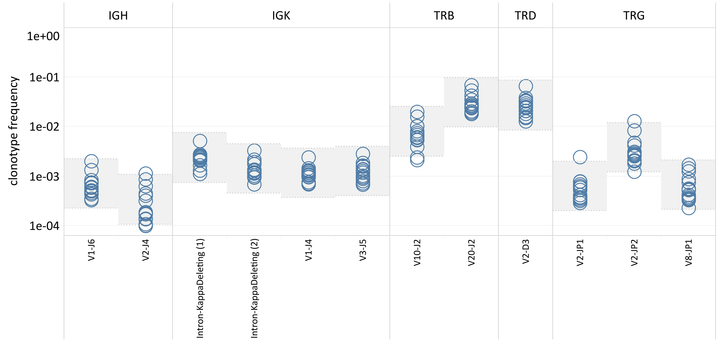
In Follow-Up mode, QuaSIR subjects the sequences to two rounds of grouping as in the diagnostic workflow. Subsequently, QuaSIR aligns the clonotype sequences against those of the marker clones from the diagnostic sample. Using the results of the alignment procedure, QuaSIR then detects and quantifies 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.

3. IMPLEMENTATION

QuaSIR has an extremely flexible architecture. Each component of the workflow is engineered as an independent module allowing it to be deployed either linearly or asynchronously depending on the data processing load. This enables rapid troubleshooting of the workflow and allows it to be easily maintained.

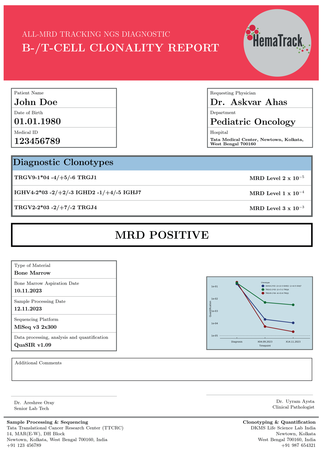


4. RESULTS



Clonotype frequency of 12 marker clonotypes of a healthy donor DNA sample with spiked-in ALL cell line DNA (1:1 NALM-6 / MOLT-3) at low concentration to mimic a patient MRD sample.
(grey shaded area indicates interval of concordance according to EURO-MRD guidelines)

QuaSIR robustly, reproducibly and consistently identifies the marker clonotypes in 15 independent experiments.



A draft medical report for MRD status to be used in the multicenter prospective study that starts in March 2025

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