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H029

Tracking Measurable Residual Disease in Acute Myeloid Leukemia with Error Corrected Sequencing

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Introduction

Measurable or minimal residual disease (MRD) detection has proven to be useful in the clinical management of patients with leukemia and can facilitate the development of new therapies. DNA-based focused target enrichment strategies are an attractive solution to detect MRD using next generation sequencing (NGS) as they can be applied to a broader population of patients as compared to a single gene. However, NGS approaches have a relatively high error rate; approximately one erroneous base call per 100-1,000 sequenced nucleotides. To circumvent this limitation we present our MyMRD® Myeloid Gene Panel Assay, which uses a proprietary probe design targeting 53 select genes with an innovative approach of duplexed sequencing and tag-based error correction method that improves sequencing accuracy. In this RUO assay, unique molecular identifiers (UMIs) are physically incorporated into sequencing libraries. During the error correction process, duplicate reads (determined by UMIs) allow for the assessment of whether a variant is an error or a true mutation, thereby enabling accurate tracking of measurable residual disease.

Materials and Methods

A commercially-available myeloid reference standard DNA containing 13 hotspot mutations (11 single nucleotide variants [SNVs] and 2 insertion or deletions [INDELs]), and an in-house FLT3-ITD positive cell line, were diluted in background DNA (GIB2, NA24385) to a variant allelic frequency (VAF) of 5e-04 . The study also included a set of negative control samples and a dilution series of positive control samples to estimate the panel-wide LoB and LoD, respectively. All contrived DNA was then enzymatically fragmented using the Twist Library Preparation Enzymatic Fragmentation Kit 2.0. UMIs were then ligated to the fragmented DNA to facilitate error correction. Illumina-style adapters were then incorporated to generate NGS libraries, which were quality checked and hybridized with MyMRD® Myeloid probes. Enriched libraries were sequenced on the NovaSeq™ 6000 sequencer, and sequencing data was analyzed using proprietary MyMRD® Myeloid analysis software.

Genes Targeted					
ASXL1	*CREBBP*	*EZH2*	*KRAS*	*PTEN*	*STAG2*
BCL2	*CSF3R*	*FLT3*	*MPL*	*PTPN11*	*SUZ12*
BCOR	*CTCF*	*GATA1*	*MYD88*	*RAD21*	*TET2*
BCORL1	*DDX41*	*GATA2*	*NF1*	*RUNX1*	*TP53*
BRAF	*DHX15*	*IDH1*	*NOTCH1*	*SETBP1*	*U2AF1*
BRINP3	*DNMT3A*	*IDH2*	*NPM1*	*SF3B1*	*U2AF2*
CALR	*EP300*	*IKZF1*	*NRAS*	*SMC1A*	*WT1*
CBL	*ETNK1*	*JAK2*	*PDS5B*	*SMC3*	*ZRSR2*
CEBPA	*ETV6*	*KIT*	*PHF6*	*SRSF2*	

MyMRD Myeloid Wet Lab Flowchart

MyMRD Myeloid Bioinformatics Flowchart

Results:

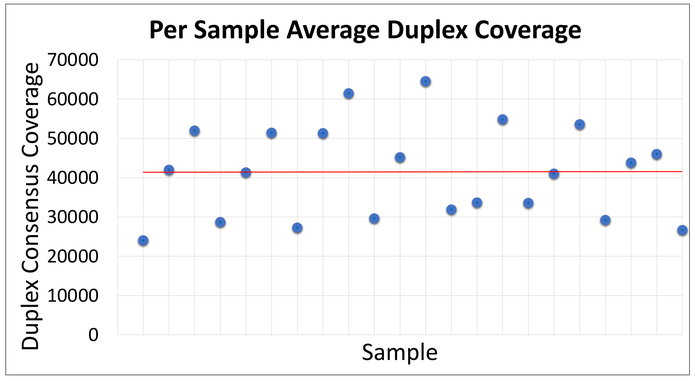

Figure 1: This scatter-plot shows the average duplex coverage (y-axis) across all 22 samples (x-axis) in this study (two separate NovaSeq runs). We see a mean average duplex coverage of 41,461x (red line). Across the 22 samples, we observed a maximum duplex coverage of 97,113x (mean=62,884x) and a minimum of 3,457x (mean=5,566x).

Results:

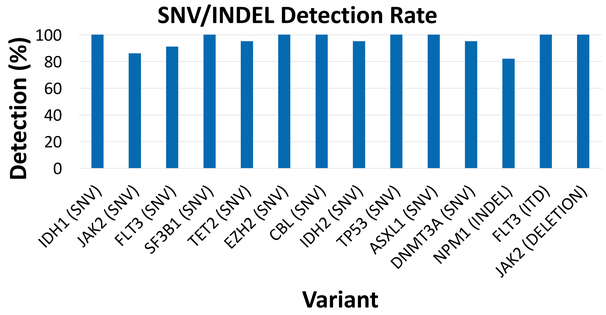

Figure 2: This figure shows a bar plot representing the detection rate of variants at a VAF of 5e-04. Nine of the SNVs interrogated were detected ≥95% of the time. Two other SNVs were detected at 91% and 86% frequency. The three INDELS we evaluated (JAK2 F537-K539>L deletion, a 30 bp FLT3 ITD, and the NPM1 W288fs*12 insertion), were detected at 100%, 100%, and 82% frequency, respectively. Overall, in the 22 samples evaluated with the MyMRD® Myeloid Gene Panel Assay, one or more hotspot mutations were detected at 5e-04 VAF with 96% frequency

Observed VRF (%)	Required Depth of Coverage for >95% Confident in T.P.	% of Targets >= DoC
0.05% (5e-4)	>=18,325x	99.9%
0.035% (3.5e-4)	>=30,000x	98.6%
0.025% (2.5e-4)	>=36,675x	88.1%
0.01% (1e-4)	>=91,750x	0.012%

Figure 3: This figure shows the percentage of targets (single nucleotide positions) that have the required depth of coverage (DoC) to make high confidence (true positive (T.P.)) calls at each specified variant read frequency (VRF**)** for a high-performing sample with average duplex coverage of 61,426x.

a)

N	96
Rank Position (α=0.05)	91.7
LoB	**3.00E-04**

b)

y	x	LoD
1.645	-3.374	**4.23E-04**

Figure 4: (a) The estimated limit of blank (LoB) for SNVs. The LoB estimated to be 3e-04 and was estimated using the non-parametric analysis of 96 data points across 24 unique SNVs (analysis method from CLSI-EP17-A for LoB). **(b)** The estimated limit of detection (LoD) for SNVs. The LoD was estimated to be 4.23e-04 and was calculated from a probit regression model (using 20 SNVs) at the VAF at which a positive call is made at a detection rate of 95%.

Conclusions

- The MyMRD® Myeloid Gene Panel Assay demonstrates excellent accuracy and high sensitivity in the detection of AML driver mutations at MRD levels.
- Its ability to reliably detect multiple mutations within one sample enables comprehensive myeloid studies.
- By leveraging both the raw (i.e. no-UMI parsing) and the duplex-consensus reads, we are able to make low-frequency variant calls across the majority of positions targeted.
- Depending on depth of coverage, complexity and quality of sequenced region, the assay can achieve detection at or below 1e-04 for some SNVs.
- We have seen ultra-high depths of duplex coverage (>60,000x) in some samples, leading to even greater SNV sensitivity below the estimated panel-wide LoD of 4.23e-04.

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