Nanodigmbio

μCaler AML MRD

Comprehensive Solution

- Precise coverage
- Low background noise
- Higher sensitivity
- Lower sequencing cost
- Stable and efficient
- High-speed and convenient

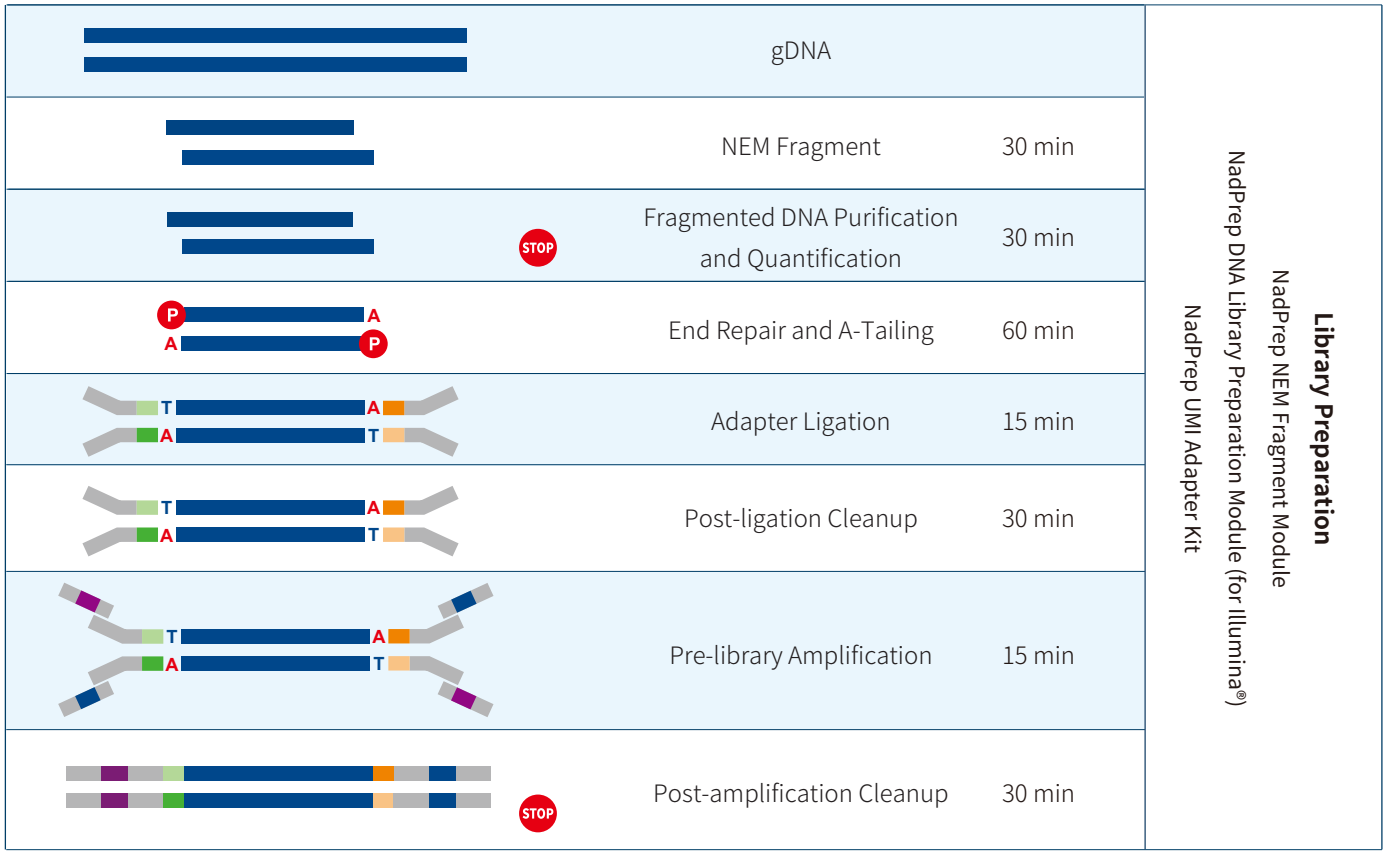
µCaler AML MRD Comprehensive Solution

Background

Acute Myeloid Leukemia (AML) accounts for 25.3% of all leukemias, and it is a clonal disorder characterized by the excessive proliferation of hematopoietic cells in bone marrow, leading to the rapid growth of abnormal cells in the bone marrow and blood, thereby interfering with hematopoiesis. Studies conducted both domestically and internationally have shown that Minimal Residual Disease (MRD) detection can be used for treatment response evaluation, relapse monitoring, treatment selection, and early intervention, making it a crucial step in reducing leukemia relapse and improving outcomes. The MRD detection methods for AML patients mainly include multiparameter flow cytometry (MFC), real-time quantitative PCR, digital PCR, and NGS. MFC is simple and fast but lacks the ability to determine specific leukemia subtypes at a low sensitivity.

Real-time quantitative PCR and digital PCR have good specificity, high sensitivity, and are both relatively affordable, but they are only applicable to less than 40% of AML patients. On the other hand, NGS can simultaneously detect multiple mutation sites, observe clonal evolution, and facilitate high-throughput operations, making it to be considered as the "ultimate solution" applicable to all AML patients. However, NGS is associated with high sequencing costs and susceptibility to background noise, which needs to be overcome. Therefore, Nanodigmbio has designed and developed the **µCaler AML MRD comprehensive solution,** which combines the exclusive patented µCaler hybrid capture system with Unique Molecular Identifier (UMI). This solution enables ultra-high detection sensitivity and completes the entire experimental process within same day.

Workflow



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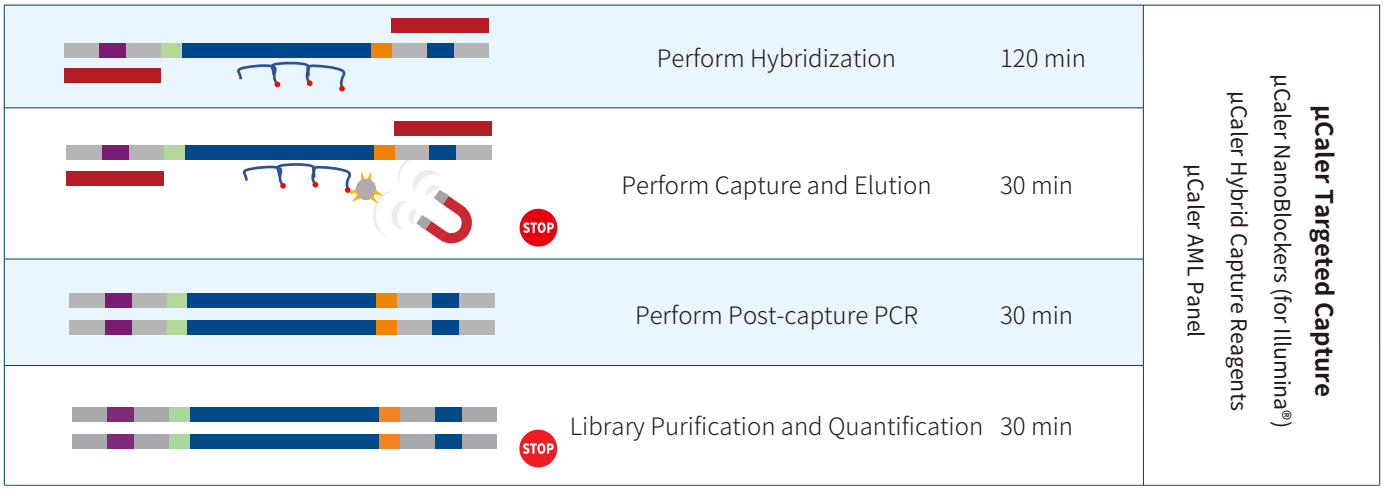


Introduction

µCaler AML MRD comprehensive solution is based on liquid-phase hybridization target enrichment technology and specifically designed for adult Acute Myeloid Leukemia (AML) MRD research. This comprehensive solution selects MRD targets that cover approximately 90% of AML cases, allowing for the analysis of various mutations, including base substitutions, insertions/deletions, and gene fusions. By combining UMI and the µCaler hybrid capture system, it achieves ultra-high detection sensitivity and can complete the entire experimental process within same day.

NadPrep NEM Fragment Module utilizes a nucleic acid endonuclease for fragmentation of genomic DNA. The cleavage products with enzymatic digestion exhibit high end-sequence integrity, and the enzymatic process does not involve polymerase reactions. The original nucleic acid sequence information and base modifications are preserved throughout the entire fragmentation process, avoiding the introduction of background noise generated during replication. When combined with the NadPrep Library Preparation Module coupled with UMI, this enables ultra-low-frequency mutation analysis of gDNA samples.

NadPrep cfDNA Library Preparation Kit (for Illumina®) is designed for preparation of high-quality libraries from double-stranded DNA (dsDNA) on Illumina platforms. This A-T ligation-based kit offers a stable and efficient library preparation solution for genome sequencing and compatible with hybridization capture based targeted sequencing on Illumina platforms. This kit includes UMI adapters, which can enhance the performance of mutant detection with ultralow-frequency. The library prepared with this adapter module contains a 10 nt-unique dual index, which is compatible with both Illumina and MGI sequencing platforms. When sequencing the library on the Illumina platform, it supports index readout modes of either 8 nt or 10 nt-dual index. When the library is directly circularized and sequenced on the MGI platform, it supports index readout mode of 10 nt-dual index.

µCaler Hybrid Capture Reagents is designed for targeted enrichment of small Panel, integrated with upgraded and optimized hybrid capture and elution processes, and equipped with µCaler Panel designed based on innovative protocols, which can complete the whole process of capture-library preparation in same day.

µCaler NanoBlockers (for Illumina®) are optimized blockers for Illumina® platforms based on µCaler Hybrid Capture System. The µCaler NanoBlockers (for Illumina®) facilitates better binding of the library's adapter sequences to the Illumina sequencing platform. This reduces non-specific binding between adapters, resulting in improved on-target rates and increased data utilization. µCaler NanoBlockers (for Illumina®) can be used to block the adapters with 10 nt-dual index in the library.

µCaler AML Panel v1.0 is designed for detecting common mutations in adult acute myeloid leukemia. The panel covers approximately 42.5 Kb region of the genome, targeting 32 genes. It enables enrichment of various types of mutation information, including base substitutions, insertions/deletions, and gene fusions, making it suitable for MRD monitoring.

Feature

- **Precise coverage:** ~90% of cases have mutations, with ~60% of cases having three or more mutations
- **Low background noise:** Accurate and reliable with a high signal-to-noise ratio
- **Higher sensitivity:** High conversion rate enables lower detection limits
- **Lower sequencing cost:** >70% on-target rate, saving 80% of sequencing volume
- **Stable and efficient:** Avoids inconsistent sequencing data and reduces rework
- **High-speed and convenient:** Simplified experimental process, easy operation, and completion of the entire workflow within same day

Performance

Precision coverage

- Reference was made to multiple databases (COSMIC & TCGA) and guidelines to select regions more likely to contain mutations.
- ~90% of cases have at least one mutation, and ~60% of cases have three or more mutations (AML_OHSU_2022 cohort).
- Covers approximately 42.5 Kb of the genome, allowing detection of base substitutions, insertions/deletions, and gene fusions in 32 genes associated with AML.

Gene list

ASXL1 Exon 12,13	*BRINP3* Exon 3,8	*CBL* Exon 8,9	*CEBPA** Full CDS	*DNMT3A* Exon 8-23	*EZH2* Exon 4-6,8,13-20	*FLT3* Exon 14,15,20	*GATA2* Exon 3-6	*HNRNPK* Exon 4-6,10,12,15,16	*IDH1* Exon 4	*IDH2* Exon 4
JAK2 Exon 14	*KIT* Exon 8,17	*KMT2A*† Intron 8-10	*KRAS* Exon 2,3	*MYH11*† Intron 32	*NPM1* Exon 10,11	*NRAS* Exon 2,3	*PHF6** Full CDS	*PTEN* Exon 5,7	*PTPN11* Exon 3,13	*RAD21** Full CDS
*RUNX1** Full CDS	*SF3B1* Exon 14,15	*SMC1A* Exon 2,9,11,13,15-17,22		*SMC3* Exon 9,10,13,19,24,25,27		*SRSF2* Exon 1	*STAG2* Exon 4,5,7-9,14,16,18,19,24,26-30		*TET2** Full CDS	*TP53** Full CDS
U2AF1 Exon 2	*WT1* Exon 6-9									

Note: * Indicates that the gene is covered across the entire coding sequence (CDS) region; † Indicates that the gene is covered in fusion-related intronic regions.

Low background noise

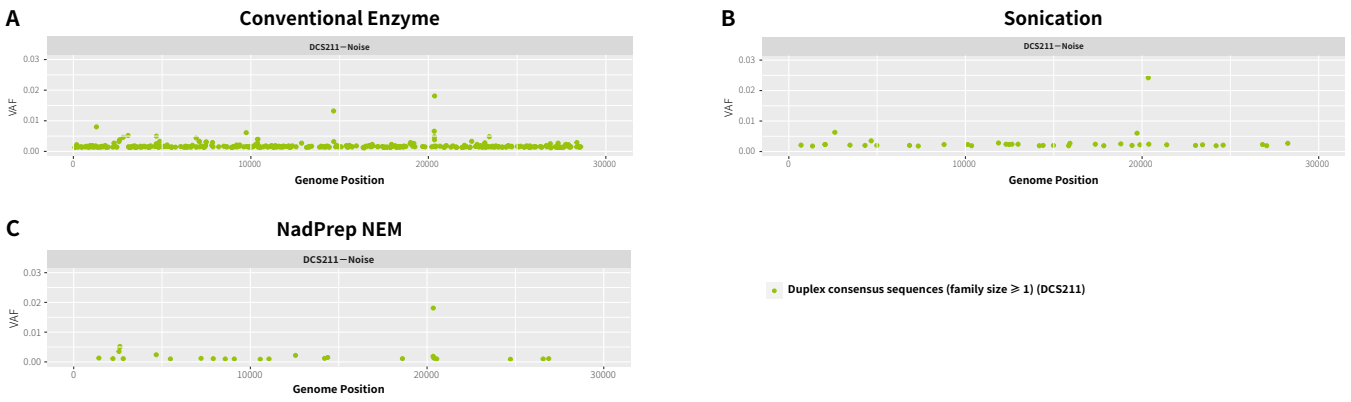


Figure 1. Analysis of background noise after DCS211 filtering using different fragmentation methods. The different methods are **A.** Conventional enzymatic fragmentation; **B.** Sonication and **C.** NadPrep NEM fragmentation, respectively. NadPrep NEM fragmentation significantly reduces background noise compared to the other two methods from the source.

Higher sensitivity

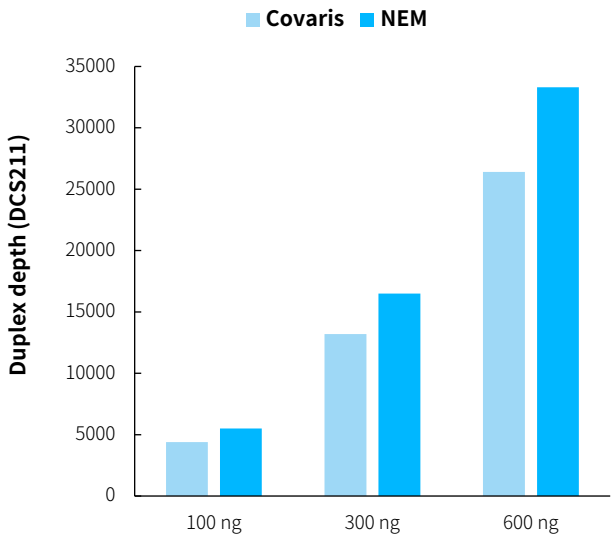


Figure 2. Duplex depth (DCS211) detected by µCaler AML MRD comprehensive solution and sonication-based library preparation under different input conditions. The µCaler AML MRD comprehensive solution user manual was used as a reference, and the filtering was performed based on Duplex Consensus Sequences (DCS211). The sequencing mode is Illumina Novaseq 6000, PE150.

Lower sequencing cost

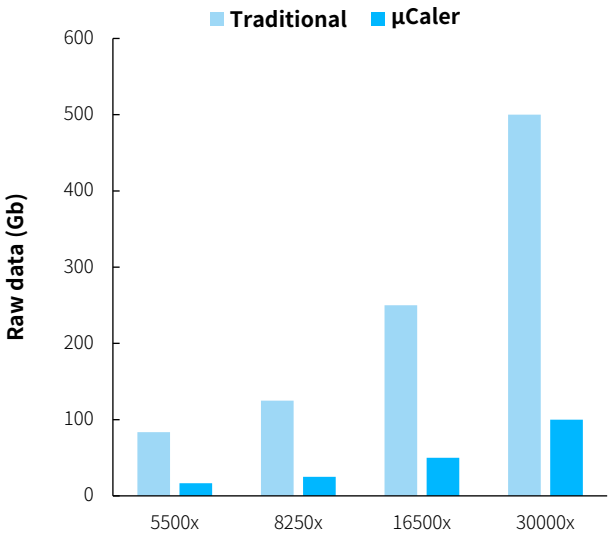


Figure 3. Minimum amount of raw data required for µCaler AML MRD comprehensive solution and traditional hybridization capture method to achieve a specific depth (satisfying Duplex Consensus Sequences analysis).

Note: Traditional indicates the conventional hybridization capture method, calculated based on the on-target rate of 15%.

Stable and efficient

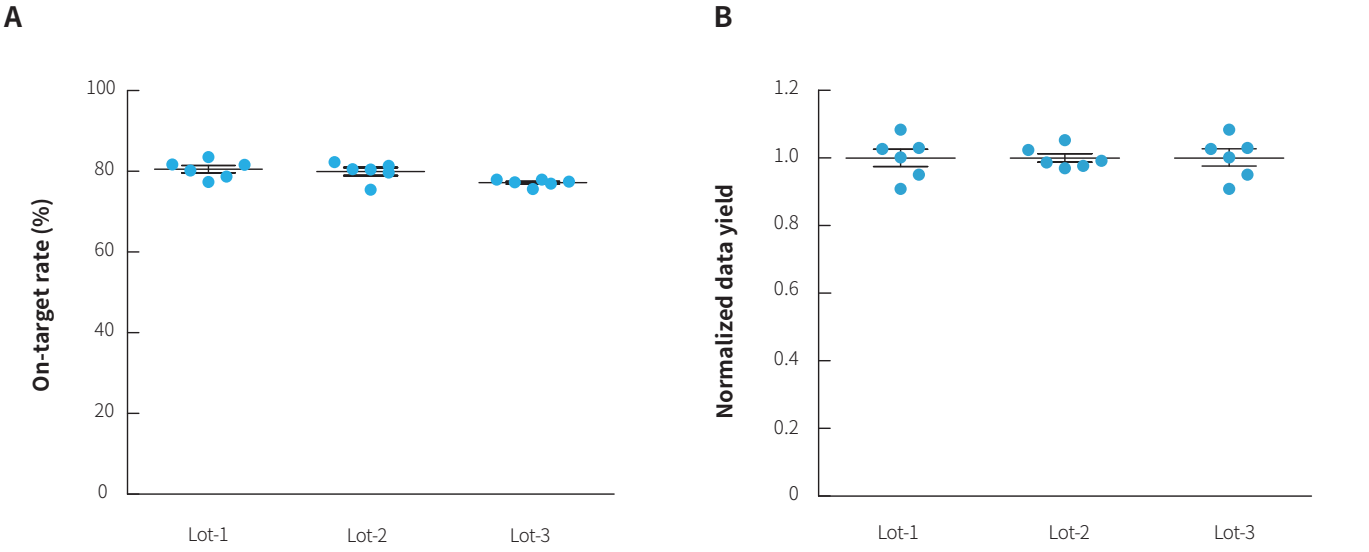
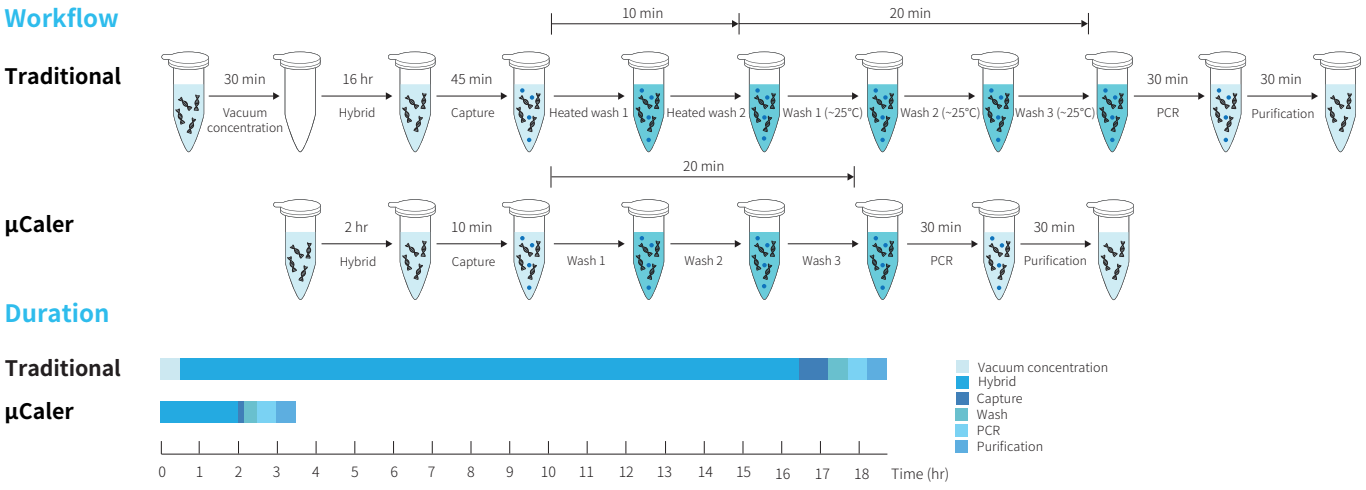


Figure 4. Stability performance of µCaler AML MRD comprehensive solution in different experimental batches. A. On-target rate; **B.** Uniformity of data yield. The captured library were prepared from gDNA samples according to the user manual of µCaler AML MRD comprehensive solution. Using BWA to alignment to the reference genome hg19 and on-target rate was calculated by the number of reads.

High-speed and convenient



Capture performance

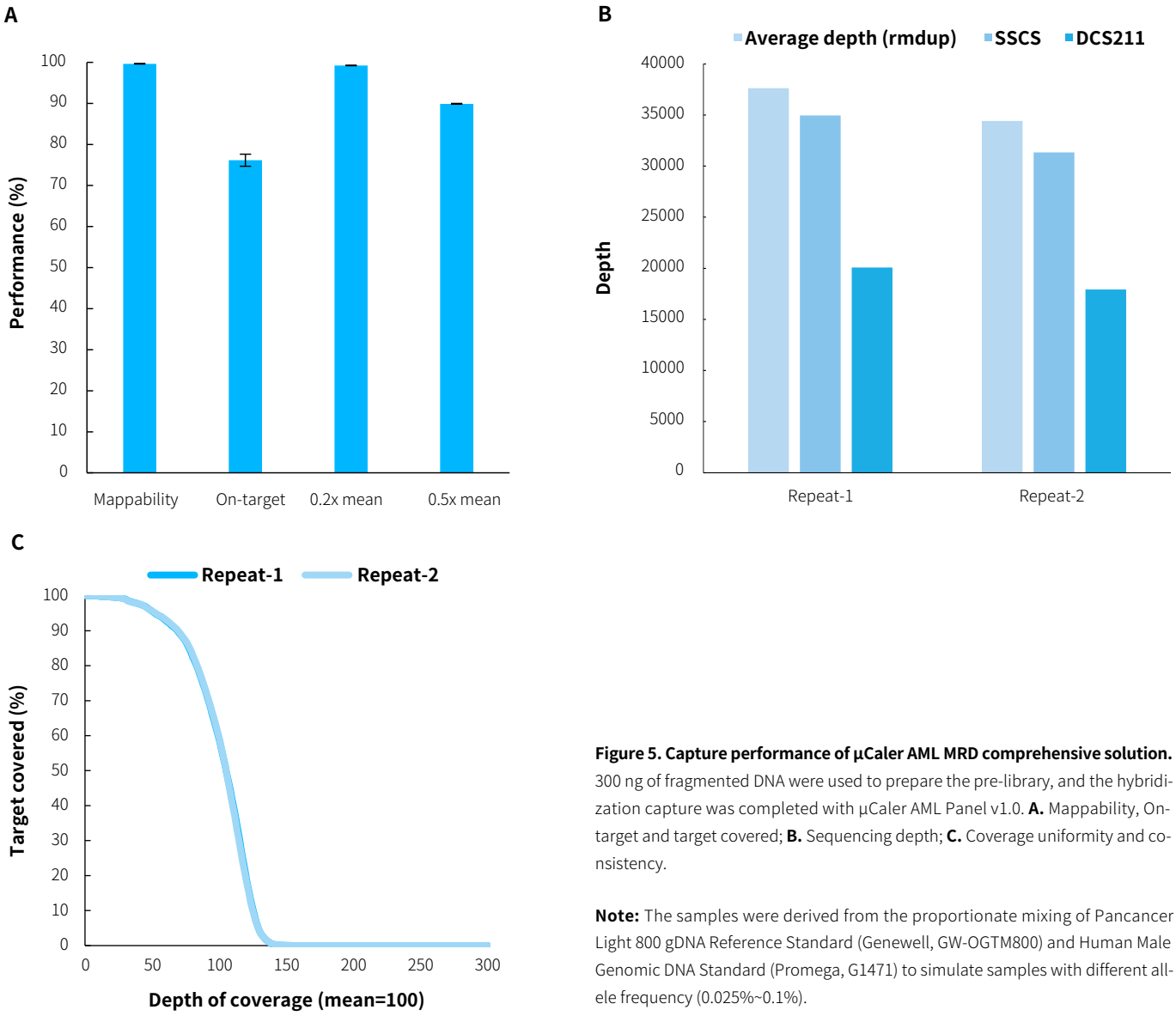


Figure 5. Capture performance of µCaler AML MRD comprehensive solution. 300 ng of fragmented DNA were used to prepare the pre-library, and the hybridization capture was completed with µCaler AML Panel v1.0. **A.** Mappability, On-target and target covered; **B.** Sequencing depth; **C.** Coverage uniformity and consistency.

Note: The samples were derived from the proportionate mixing of Pancancer Light 800 gDNA Reference Standard (Genewell, GW-OGTM800) and Human Male Genomic DNA Standard (Promega, G1471) to simulate samples with different allele frequency (0.025%~0.1%).

Analysis of mutation in standard

The samples were derived from the proportionate mixing of PancancerLight 800 gDNA Reference Standard (Genewell, GW-OG-TM 800) and Human Male Genomic DNA Standard (Promega, G1471) to simulate samples with different allele frequency (0.025% ~ 0.1%). The detection of different mutation sites in the simulated samples is as follows:

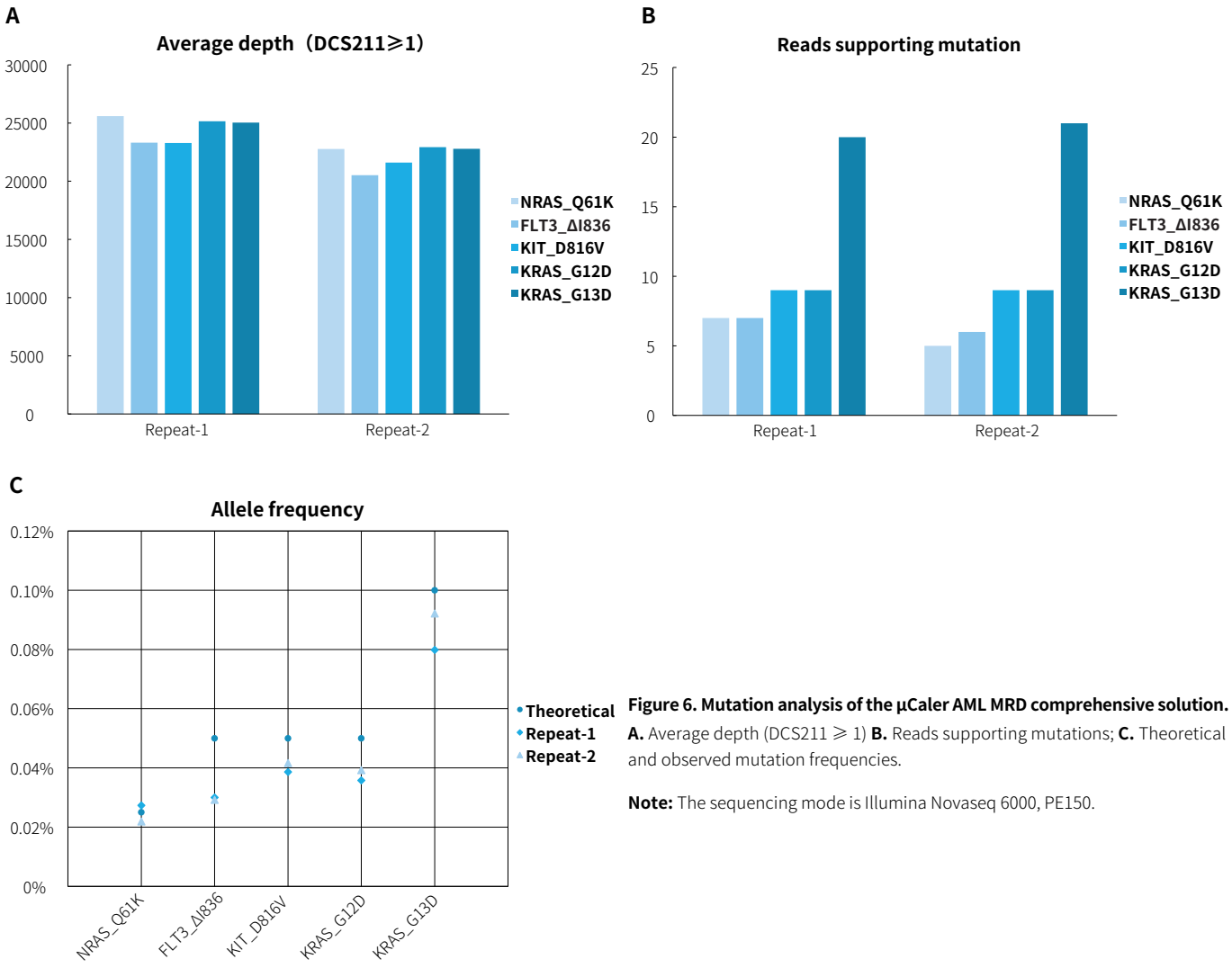


Figure 6. Mutation analysis of the µCaler AML MRD comprehensive solution. **A.** Average depth (DCS211 ⩾ 1) **B.** Reads supporting mutations; **C.** Theoretical and observed mutation frequencies.

Note: The sequencing mode is Illumina Novaseq 6000, PE150.

Ordering Information

Type	Product	Detail	Catalog#
NEM Fragment Module	NadPrep NEM Fragment Module, 24 rxn	24 rxn	1002801
Lib Prep Module	NadPrep DNA Library Preparation Kit (for Illumina®) G24	24 rxn	1002101
	NadPrep DNA Library Preparation Kit (for Illumina®) E96	96 rxn	1002103
Adapter Module	NadPrep UMI Adapter Kit Set A1 (with 10 nt Index), 24 rxn	Index # 1-12	1103111
	NadPrep UMI Adapter Kit Set B1 (with 10 nt Index), 96 rxn	Index # 1-24	1103121
	NadPrep UMI Adapter Kit Set B2 (with 10 nt Index), 96 rxn	Index # 25-48	1103122
Blocker	µCaler NanoBlockers (for Illumina®), 16 rxn	16 rxn	1106102
	µCaler NanoBlockers (for Illumina®), 96 rxn	96 rxn	1106101
Hybrid Capture	µCaler Hybrid Capture Reagents, 16 rxn	16 rxn	1105102
	µCaler Hybrid Capture Reagents, 96 rxn	96 rxn	1105101
Panel	µCaler AML Panel v1.0, 16 rxn	16 rxn	1101412
	µCaler AML Panel v1.0, 96 rxn	96 rxn	1101411

Statement

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