

## Myeloid MRD Panel

### Features

#### Don't miss key genomic insights

- Detect key variants across 45 hotspot exons in 13 key AML-associated genes (including longer, ultra-low frequency *FLT3*-ITDs) for the clearest picture of your sample's MRD status

#### Ultra-low variant detection down to a possible 0.01% VAF

- Confidently detect MRD with SureSeq's unparalleled bait design and sequence identification strategy for sensitive detection, including for key targets like *NPM1*

#### Personalize your workflow

- Choose between two workflow options to tailor your approach for your sensitivity, sample batching and sequencing requirements

#### Get the most out of your MRD data

- Our complimentary analysis software, Interpret, gets you started with an 'out of the box' bioinformatic analysis pipeline without adding to your lab's bioinformatics burden



### Introduction

Acute myeloid leukemia (AML) is an aggressive hematopoietic stem cell malignancy, and the most common form of acute leukemia in adults – with an incidence rate of 2–6 cases per 100,000 globally<sup>1</sup> and following treatment, ~50% of patients with AML who achieve complete remission (CR) will relapse.<sup>2</sup>

In AML, measurable residual disease (MRD) assesses the presence of leukemic cells, which may remain post-treatment, through quantification of AML associated biomarker(s). Currently, the detection of MRD in AML utilizes multiple techniques such as immunophenotypic multiparameter flow cytometry (MFC) and PCR-based approaches. Recent developments in next-generation sequencing (NGS) technology have allowed for highly sensitive detection of multiple biomarkers of MRD at extremely low levels.

Published analyses have documented the prognostic relevance of MRD in acute myeloid leukemia (AML), which has highlighted that those who were defined as MRD negative had a better 5-year survival rate compared to those defined as MRD positive.<sup>3</sup>

Being able to detect even a few residual leukemic cells can provide a more comprehensive picture of the current AML status of a subject. For clinical research, this strengthens the ability to analyze and understand disease features such as the early identification of relapse, therapeutic response and tailoring future treatment approaches.

### Expert-led, evidence-based content

Understanding the full AML MRD profile may be limited by a focus on individual biomarkers as this does not address the broader genomic heterogeneity present in AML. The **SureSeq™ Myeloid MRD Panel** gene content has been driven by recommendations from leading cancer experts and the European LeukemiaNet (ELN) MRD Working Group<sup>4</sup> to incorporate a key range of AML-associated biomarkers, allowing for the rapid generation of extensive genomic profiles.

Gene	Exons	Gene	Exons
<i>CSF3R</i>	Exons 13–17	<i>FLT3</i>	Exons 13–15 and 20
<i>MPL</i>	Exon 10	<i>IDH2</i>	Exons 4 and 5
<i>SF3B1</i>	Exons 13–16	<i>TP53</i>	Exons 2–11 (inc. NM_001276695:ex10, NM_001276696:ex10)
<i>IDH1</i>	Exon 4	<i>CALR</i>	Exon 9
<i>KIT</i>	Exons 2, 8–11, 13 and 17	<i>RUNX1</i>	Exons 4–8
<i>NPM1</i>	Exon 11	<i>CEBPA</i>	Exon 1
<i>JAK2</i>	Exons 12 and 14		

Table 1: The SureSeq Myeloid MRD Panel targets SNVs, indels and ITDs across 45 hotspot exons in 13 genes recommended ELN guidelines and leading cancer experts for AML MRD.

### Detect key variants across 45 hotspot exons in 13 key AML-associated genes

OGT's unparalleled bait design process, combined with unique detection algorithms incorporated into our complimentary NGS analysis software, Interpret, allows for the sensitive detection of key AML-biomarkers, as low as 0.01% VAF, even for key targets like *NPM1* (Table 2). Informed by our unique expertise, the sensitive detection capabilities of the SureSeq Myeloid MRD Panel expand your MRD detection so you have a better understanding of the current AML status of your samples and genomic insights are not missed.

*FLT3* internal tandem duplications (ITDs) are present in approximately 25% of AML cases and are an important prognostic marker.<sup>5,6</sup> However, the inherent repeat content and length of these ITDs make them challenging to target and thus detect and characterize; PCR-based approaches can suffer from template bias which may negatively impact the ability to detect longer *FLT3*-ITDs.<sup>7</sup> By leveraging our expertise in hybrid capture technology and sequence identification analysis, the SureSeq Myeloid MRD Panel can detect large *FLT3*-ITDs (Table 3), in excess of 300bp, so you don't miss actionable insights..

Gene	HGVSc	Total read depth	% VAF	Gene	HGVSc	Total read depth	% VAF
<i>CSF3R</i>	c.2047G>A	18859	0.085	<i>SF3B1</i>	c.1997A>C	22929	0.214
<i>FLT3</i>	c.2503G>T	18097	0.072	<i>TP53</i>	c.638G>T	22935	0.161
<i>IDH1</i>	c.395G>A	12347	0.146	<i>TP53</i>	c.742C>T	12459	0.12
<i>IDH2</i>	c.419G>A	14948	0.04	<i>TP53</i>	c.742C>T	11713	0.145
<i>IDH2</i>	c.419G>A	15961	0.038	<i>TP53</i>	c.646G>A	15908	0.025
<i>IDH2</i>	c.429G>C	18894	0.085	<i>TP53</i>	c.509C>T	29214	0.065
<i>JAK2</i>	c.1849G>T	22111	0.045	<i>TP53</i>	c.108G>A	18552	0.075
<i>JAK2</i>	c.1849G>T	20462	0.039	<i>TP53</i>	c.637C>T	24779	0.165
<i>JAK2</i>	c.1849G>T	16146	0.056	<i>TP53</i>	c.375G>A	18692	0.144
<i>JAK2</i>	c.1849G>T	22038	0.023	<i>TP53</i>	c.108G>A	16731	0.155
<i>KIT</i>	c.2447A>T	17901	0.045	<i>NPM1</i>	c.860_863dup	23029	0.035
<i>RUNX1</i>	c.486G>T	14968	0.114	<i>NPM1</i>	c.860_863dup	19603	0.020
<i>RUNX1</i>	c.593A>T	13320	0.03	<i>NPM1</i>	c.860_863dup	14517	0.028
<i>RUNX1</i>	c.1389C>G	11805	0.051	<i>NPM1</i>	c.860_863dup	16778	0.018
<i>SF3B1</i>	c.2098A>G	14196	0.303	<i>NPM1</i>	c.860_863dup	16629	0.036
<i>SF3B1</i>	c.2098A>G	16019	0.05	<i>NPM1</i>	c.860_863dup	21049	0.100

Table 2: Example SNVs and Indels detected from a cohort of 36 orthogonally validated research samples. These include SNVs in key genes *CSF3R*, *FLT3*, *IDH1*, *IDH2*, *NPM1*, *JAK2*, *KIT*, *RUNX1*, *SF3B1*, *TP53* that range from 0.303 - 0.018% VAF

Gene	Variant		Expected frequency: 0.04%		Expected frequency: 0.05%		Negative control	
	HGVSc	Expected Length (bp)	Read depth	Observed VAF (%)	Read depth	Observed VAF (%)	Read depth	Observed VAF (%)
<i>FLT3</i>	ITD300	300	13,119	0.05	12,208	0.04	21,686	0.00

Table 3: Detection of a 300 bp ITD, in Myeloid Reference DNA Standard (Horizon Discovery) with expected frequency ranges of 0.04%-0.05%.

### Exceptionally high coverage uniformity essential for MRD detection

OGT specializes in the development of superior hybrid capture technology that excels in the detection of complex structural variants and eliminates inaccurate calls caused by alternative PCR-based approaches. The hybridization-based approach utilized in the SureSeq Myeloid MRD Panel combined with our proprietary bait designs allows for high coverage uniformity and thus better sequencing depth across all targets, which is essential for detecting low frequency variants in MRD.

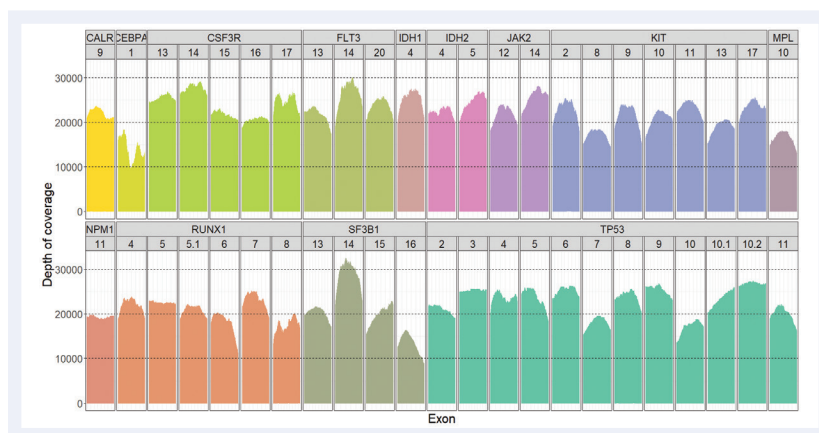


Figure 1: Example coverage profile of target regions in the SureSeq Myeloid MRD Panel.

*NPM1* is the most commonly mutated gene in adult AML, present in approximately 25–35% of cases<sup>8</sup>, making it an essential biomarker to help further understand MRD in AML. Our bait designs and use of unique molecule identifiers (UMIs) delivers exceptional coverage uniformity, enabling reliable detection as low as 0.01% VAF of all target regions for key AML-biomarkers recommended by leading KOLs and ELN guidelines<sup>4</sup> even for key targets such as *NPM1* (Figure 1).

### Adaptable workflows to meet your labs sensitivity, sample batching and sequencing requirements

The SureSeq Myeloid MRD Panel combines the superior performance of hybridization-based enrichment with the streamlined and automatable Universal NGS Complete Workflow Solution to deliver unparalleled results with minimal hands-on time (Figure 2).

By developing and optimizing two separate workflows which provide different target sensitivities (0.1% and 0.01% VAF respectively) we allow our users to adapt their MRD NGS workflows allowing our highly targeted panel to effortlessly adapt to your lab's sensitivity, sample batching and sequencing requirements.

The incorporation of Unique Molecular Identifiers (UMIs) prior to sample amplification allows true variants to be distinguished from PCR artefacts for highly sensitive and reliable results.

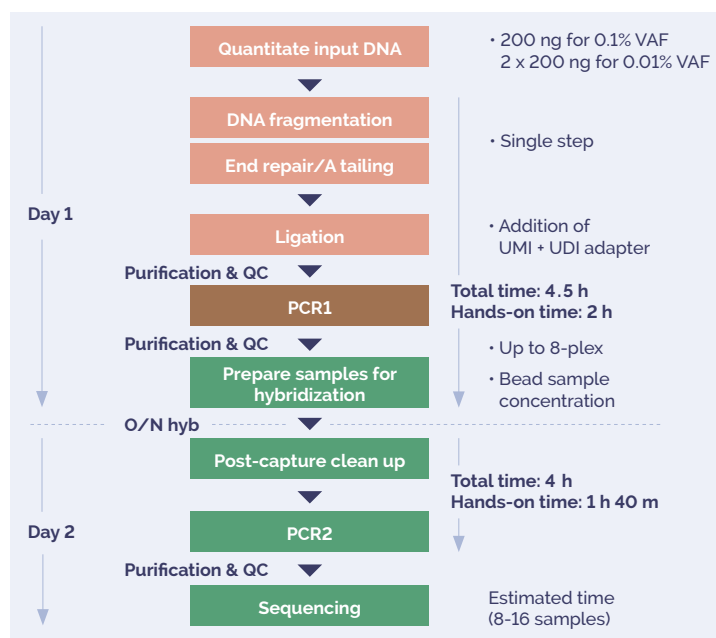


Figure 2. The streamlined SureSeq Myeloid MRD Panel workflow, available for 0.1% and 0.01% VAF detection.

### Seamless MRD data analysis without adding to your lab's bioinformatics burden

We ensure you can get started with MRD analysis without adding to your lab's bioinformatics burden. Our complimentary analysis software, Interpret, provides an 'out of the box' bioinformatic analysis pipeline so you can get up and running with your analysis as quickly as possible.

OGT's powerful and easy-to-use Interpret NGS Analysis Software facilitates analysis and visualization of a wide range of somatic variants including structural aberrations. Following the fast and accurate detection of all SNVs, indels and ITDs, Interpret displays all variants in our user-friendly variant browser, for translation of all your myeloid MRD data into meaningful results.

You can tailor your bioinformatics pipeline to your bespoke requirements features such as visualization of changing MRD dynamics over time (Figure 3). Depending upon your requirements Interpret can be deployed locally or in the cloud to suit your analysis infrastructure.

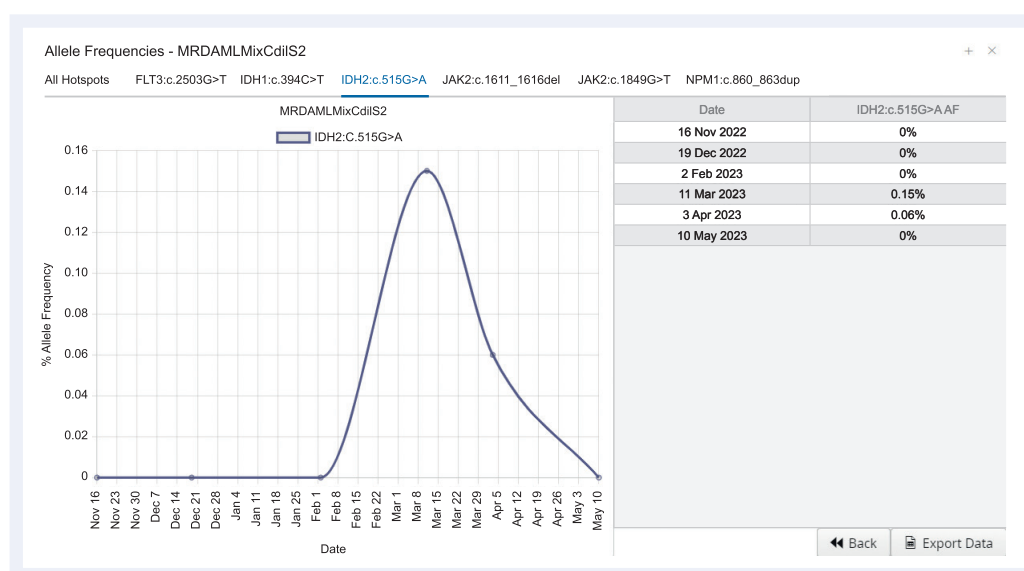


Figure 3. Interpret NGS Analysis Software enables easy visualization of genetic changes across multiple timepoints in MRD sample studies.

### Why partner with OGT?

With over 25 years' experience providing *in vitro* diagnostics and clinical genomics solutions at OGT, we've always been driving the advancement of genomic technologies.

We understand the challenges facing our partners in clinical and research laboratories around the globe, including issues such as time efficiencies. To fully realize the potential of NGS for MRD assessment, we have combined our decades of in-house experience in molecular biology and clinical hematology with insights from leading cancer experts and ELN recommendations, to target a comprehensive range of disease-associated genes, for MRD assessment.

Together, we can really make an impact in understanding, exploring and analyzing the risk of relapse in AML.

**Request a quote at [www.ogt.com](http://www.ogt.com) or contact one of our experts at [contact@ogt.com](mailto:contact@ogt.com).**

### SureSeq Myeloid MRD Panel: technical information

Feature	Specification	
Number of targets	45 hotspot exons from 13 genes	
Panel size	11.2 kb	
Mean target coverage	Up to 20,000x	
Workflow	Ultra Low	Standard
Limit of detection SNVs, indels, ITDs	0.01%	0.1%
DNA input recommended	2 x 200 ng	200 ng
Samples per run		
NextSeq 500 High Output	16	24
NextSeq 2000 P3	48	72
NextSeq 2000 P4	72	108
NovaSeq® SP	32	48
NovaSeq S1	64	96

### Ordering information

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Product	Contents	Cat. No.
SureSeq Myeloid MRD Complete NGS Workflow Solution V2 (48)	Enrichment baits sufficient for 12 hybridization reactions. Bundle of 1 x Universal Library Preparation Kit (96) containing PCR primers and enzymes. 1 x Universal Hybridization & Wash Kit V2 (96). 1 x Pre-PCR Universal Bead Kit (96). 1 x Post-PCR Universal Bead Kit (96). 1 x Universal Index Adapter Kit (96). Interpret NGS Analysis Software	780126-48
SureSeq Myeloid MRD Panel (48)	Enrichment baits sufficient for 12 hybridization reactions. Interpret NGS Analysis Software	770026-48
Universal NGS Workflow Solution V2 (96)	Bundle of 1 x Universal Library Preparation Kit (96) containing PCR primers and enzymes, 1 x Universal Hybridization & Wash Kit V2 (96). 1 x Pre-PCR Universal Bead Kit (96). 1 x Post-PCR Universal Bead Kit (96). 1 x Universal Index Adapter Kit (96)	770510-96

### References

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**What binds us,  
makes us.**

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