SureSeq

A Sysmex Group Company

Myeloid MRD Panel

Coming soon

Features

Designed in collaboration with leading cancer experts

- Detect SNVs and indels in 45 hotspot exons across 13 genes associated with accurate MRD detection in AML samples — including MDS and MPN implicated genes

Class-leading sensitivity

- Designed to detect low-frequency variants down to 0.05% VAF with confidence, even in challenging genes such as *NPM1*, *CEBPA* and *FLT3-ITD*

Rapid, cost-efficient and customisable

- Simultaneously assay multiple targets with a single, customisable NGS panel

Complimentary data analysis software

- Powerful and intuitive Interpret NGS Analysis Software for confident variant calling, including longitudinal monitoring

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Introduction

Measurable residual disease (MRD) — the presence of cancerous cells below the threshold of detection of conventional morphologic methods — is routinely investigated post treatment to research the risk of relapse and to explore potential therapeutic strategies.

Several methodologies are currently used to assess MRD in acute myeloid leukaemia (AML), including immunophenotypic multiparameter flow cytometry (MFC), real-time quantitative polymerase chain reaction (RT-PCR), digital droplet PCR (ddPCR) and next-generation sequencing (NGS).

MFC provides valuable information about antigen expression implicated in MRD but does not assess genetic biomarkers, while PCR-based techniques are highly sensitive but are limited by the number of targets that can be detected simultaneously.

In contrast next-generation sequencing (NGS) offers highly sensitive and specific analysis of multiple AML-associated genes, allowing comprehensive MRD analysis of a wide-range of AML subtypes, including the identification of sample-specific mutations.

Expert-led, evidence-based content

The SureSeq™ Myeloid MRD Panel has been designed in collaboration with leading cancer experts and in accordance with the European LeukemiaNet (ELN) recommendations[1] to offer a single, cost-effective NGS assay to investigate MRD in AML samples. The panel content incorporates key genes for assessing AML, including genes implicated in MPN and MDS to investigate secondary AML progression. In addition, genes related to research on potential drug response are included, for truly comprehensive and informative sample analysis.

Utilising OGT's intelligent panel design capabilities, the SureSeq Myeloid MRD Panel surpasses the typical sensitivities offered by alternative NGS panels to accurately detect SNVs, indels and internal tandem duplications (ITDs) down to 0.05% VAF.

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

Table 1: The SureSeq Myeloid MRD Panel targets SNVs and indels in 45 hotspot exons across 13 genes associated with accurate MRD detection in AML samples, including MDS and MPN implicated genes to investigate AML progression. The assay also accurately detects *FLT3*-ITDs.

Unparalleled uniformity and depth of coverage

NPM1 is the most commonly mutated gene in adult AML, present in approximately 25–35% of cases[2], making it an essential marker for MRD monitoring. *NPM1* mutations occur almost exclusively in exon 11; a difficult region to sequence due to multiple perfectly matched copies replicated across the genome. The sophisticated bait design used in the SureSeq Myeloid MRD Panel overcomes this issue to deliver exceptional coverage uniformity, enabling reliable detection of all target regions (Figure 1).

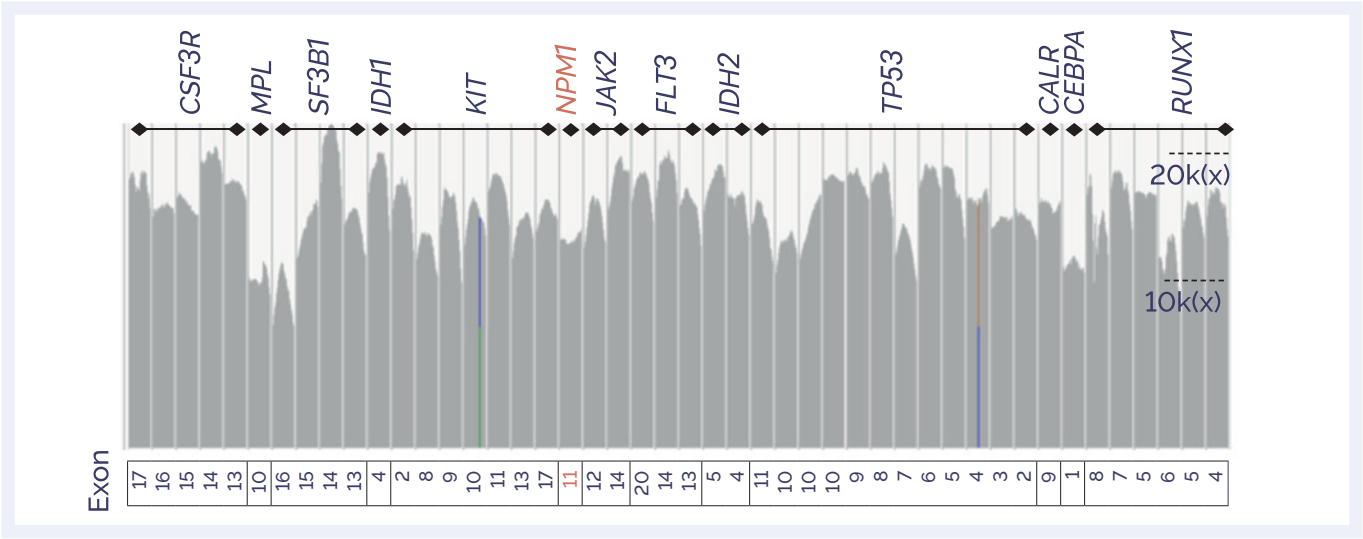

Figure 1: IGV plot showing high uniformity of coverage of all target regions in the panel, including *NPM1* exon 11.

FLT3 internal tandem duplications (ITDs) are present in approximately 25% of AML cases and are an important negative prognostic marker[3]; however, their inherent repeat content and length (up to 300 bp[4]) make them challenging to target, and subsequently detect. As a result, they are masked in many gene panels, necessitating additional techniques to characterise these important mutations. The unique detection algorithms incorporated into the complimentary Interpret NGS Analysis Software enable accurate detection and quantification of *FLT3*-ITDs, including multiple and large ITDs (Figure 2).

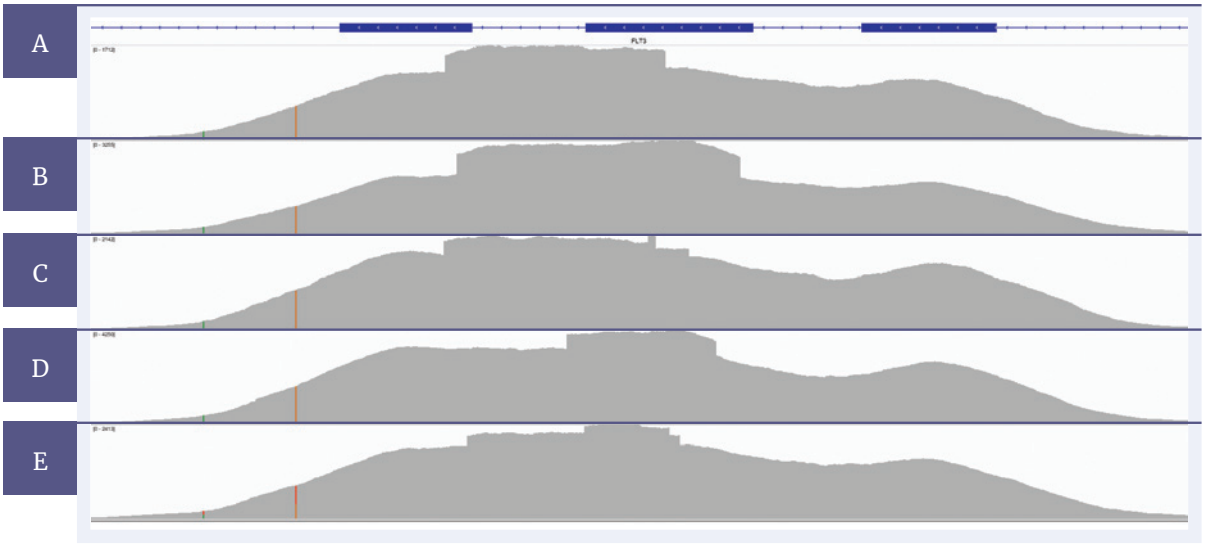

Figure 2: *FLT3*-ITDs of various sizes and even regions containing multiple ITDs can be confidently detected. ITD sizes are A 174 bp, B 225 bp, C 195 bp with additional 6 bp, D 120 bp and E 168 bp with additional 69 bp.

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Combining coverage uniformity with a focused, expert-led panel design allows greater sequencing depth, which further enhances sensitivity at lower sequencing cost. SNVs, indels and ITDs across all targeted genes and regions can be detected down to 0.05% VAF (Table 2).

	Gene	Variant	Coordinate	0.1% VAF		0.05% VAF		Neg. ctrl	
				Read depth	Observed VAF	Read depth	Observed VAF	Read depth	Observed VAF
SNV	*SF3B1*	c.2219G>A	chr2:197401989	7426	0.13	14282	0.03	15076	0
	IDH1	c.394C>T	chr2:208248389	6743	0.09	15572	0.06	15734	0
	JAK2	c.1849G>T	chr9:5073770	10408	0.12	22348	0.08	22065	0
	FLT3	c.2503G>T	chr13:28018505	9747	0.11	24098	0.04	23942	0
	IDH2	c.515G>A	chr15:90088606	7294	0.08	16554	0.04	18875	0
	TP53	c.722C>T	chr17:7674241	5166	0.12	12609	0.03	13769	0
Indel	*NPM1*	c.860_863dup	chr5:171410539	8164	0.11	18630	0.07	17630	0
	JAK2	c.1611_1616del	chr9:5070021	12270	0.03	28197	0.02	25411	0
ITD	*FLT3*	ITD300	chr13:28033909	6045	≥ 0.1	12265	≥ 0.05	10473	0

Table 2: Detection of SNVs, indels and an ITD, with expected frequency ranges of 0.1%-0.05%. SNVs are filtered to remove unique molecular identifiers (UMI) with a read family size of one. The SureSeq Myeloid MRD Panel was used on a Myeloid Reference DNA Standard (Horizon Discovery) according to the standard protocol, with sequencing on a NextSeq® 500 (Illumina).

Rapid, streamlined workflow

All SureSeq NGS panels combine the superior performance of hybridisation-based enrichment with the streamlined and automatable Universal NGS Complete Workflow Solution to deliver unparalleled results with minimal hands-on time (Figure 3). The incorporation of Unique Molecular Identifiers (UMIs) and Unique Dual Indexes (UDIs) prior to sample amplification, allows true variants to be distinguished from PCR artefacts, for highly sensitive and reliable results.

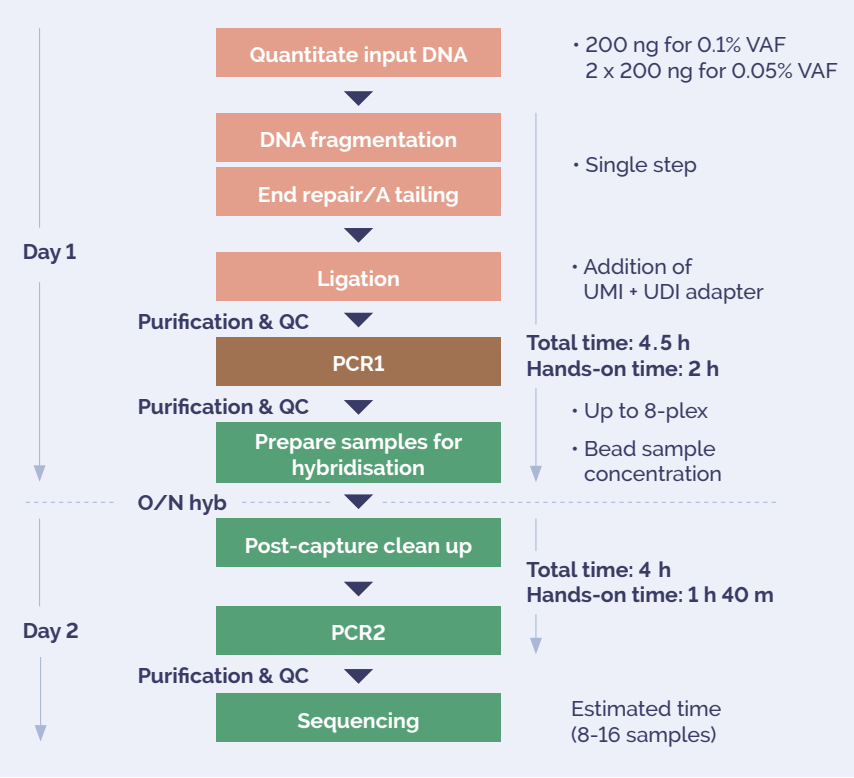

Figure 3: The streamlined SureSeq Myeloid MRD Panel workflow.

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Complimentary data analysis software

OGT's powerful and easy-to-use Interpret NGS Analysis Software facilitates analysis and visualisation of a wide range of somatic variants including structural aberrations. Designed to work seamlessly with all SureSeq NGS panels, the software delivers fast and accurate detection of all SNVs, indels and ITDs. Following detection, all variants are displayed in the user-friendly variant browser, for effortless translation of all your myeloid MRD data into meaningful results. The reporting tool also enables visualisation of changing MRD dynamics over time (Figure 4). The software can be deployed locally or in the cloud to suit your analysis infrastructure.

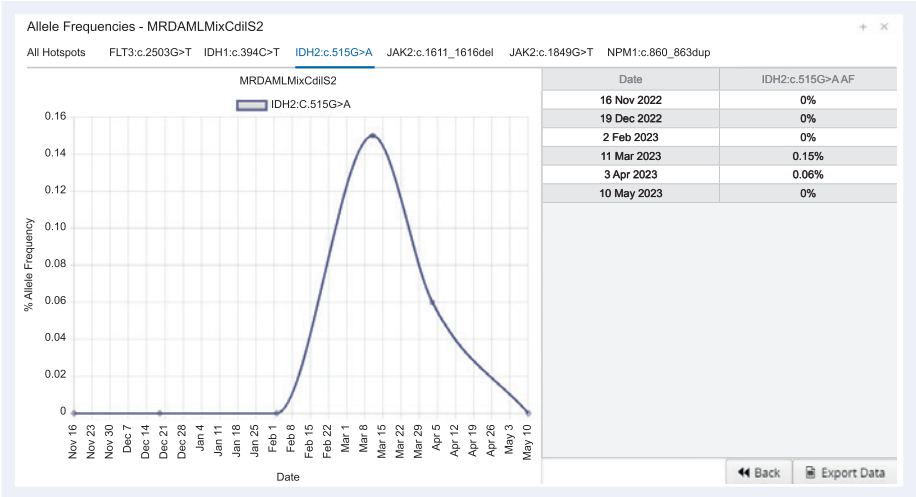

Figure 4: Interpret NGS Analysis Software can load and display multiple data sets, enabling easy visualisation of genetic changes in longitudinal MRD sample studies.

Customisable, future-proof content

Science doesn't stand still, so we've made it easy for you to benefit from the latest scientific discoveries and guidance by customising the SureSeq Myeloid MRD Panel to meet your precise requirements. Choose from our regularly updated, expert-curated library of pre-optimised cancer content to create your ideal custom SureSeq myPanel™ assay. Alternatively, browse our full range of myeloid panels, including the focused three-gene SureSeq Core MPN Panel and the SureSeq Pan-Myeloid Panel, incorporating key variants in 70 genes implicated in a wide range of myeloid disorders. In addition, the RNA-based SureSeq Myeloid Fusion Panel enables detection of 30 common fusions and novel fusion partners for key myeloid cancer genes.

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	
Limit of detection SNVs, indels, ITDs	0.05%	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
NovaSeq® SP	32	48
NovaSeq S1	64	96

Ordering information

UK +44 (0) 1865 856800

US +1 914 467 5285

contact@ogt.com

ogt.com

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Product	Contents	Cat. No.
SureSeq Myeloid MRD Complete NGS Workflow Solution V2 (48)	Enrichment baits sufficient for 12 hybridisation reactions. Bundle of 1 x Universal Library Preparation Kit (96) containing PCR primers and enzymes. 1 x Universal Hybridisation & 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 hybridisation 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 Hybridisation & 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

Request a quote at www.ogt.com or contact one of our experts at contact@ogt.com.

References

1. European LeukaemiaNET. Available at: www.leukemia-net.org.
2. Hindley *et al.*, Int J Mol Sci 2021; 22(18):10040.
3. Daver *et al.*, Leukemia 2019; 33: 299–312.
4. Spencer *et al.*, J Mol Diagn 2013; 15(1):81–93.

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What binds us, makes us.

Oxford Gene Technology Ltd., Begbroke Science Park, Woodstock Road, Begbroke, Oxfordshire, OX5 1PF, UK