

Myeloid Plus Workflow

Features

Designed in collaboration with recognised cancer experts

 Detect SNVs and indels in 49 genes implicated in a variety of Myeloid malignancies, together with 44 SNPs as ID markers and 4 sex chromosome genes

Unparalleled uniformity and high depth of coverage

 Detect low-frequency variants down to 2.5% VAF with confidence

Robust detection of *FLT3*-ITDs and *KMT2A*-PTDs

 Replace multiple single gene assays with one comprehensive panel

Fast and easy workflow

 Streamlined library preparation, rapid hybridisation and intuitive software allowing easy variant analysis



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Introduction

Myeloid malignancies are a heterogenous group of diseases, associated with a wide variety of variants ranging from mutations to structural variations. The SureSeq™ Myeloid Plus Complete NGS Workflow Solution combines the rapid Universal NGS Workflow Solution V2 hybridisation–based target enrichment method together with OGT's expert bait design to detect 49 key genes implicated in myeloid disorders.

Designed in collaboration with recognised cancer experts, SureSeq Myeloid Plus detects variants in key genes implicated in acute myeloid leukaemia (AML), myeloproliferative neoplasms (MPN), and myelodysplastic syndrome (MDS), providing researchers with a single NGS workflow delivering a comprehensive picture of the genetic make-up of each myeloid sample.

The SureSeq Myeloid Plus Panel accurately detects SNVs and indels in genes such as *CEBPA*, *JAK2*, *CALR* and *MPL*, as well as structural variants including *FLT3*-ITDs and *KMT2A*-PTDs, and is able to consistently detect clinically relevant variants down to 2.5 – 5% variant allele frequency (VAF).

Gene	Exons	Gene	Exons	Gene	Exons
ASXL1	Exon 12	GNAS	Exons 8-10	PTPN11	Exons 3 and 13
BCOR	All coding exons	IDH1	Exon 4	RAD21	All coding exons
BCORL1	All coding exons	IDH2	Exons 4 and 5	RUNX1	All coding exons
BRAF	Exon 15	IKZF1	All coding exons	SETBP1	Exon 4
CALR	Exon 9	IRF1	All coding exons	SF3B1	Exons 13-16
CBL	Exons 8 and 9	JAK1	All coding exons	SH2B3 (LNK)	All coding exons
CEBPA	All coding exons	JAK2	Exons 12 and 14	SRSF2	Exon 1
CSF3R	Exons 14-17	КІТ	Exons 2, 8-11, 13, and 17	STAG2	All coding exons
CTNNA1	All coding exons	KMT2A (MLL)	All coding exons	TET2	Exons 3-11
CUX1	All coding exons	KRAS	Exons 2 and 3	TP53	All coding exons (transcript NM_000546)
DDX41	All coding exons	MPL	Exon 10	U2AF1 (U2AF35)	Exons 2 and 6
DNMT3A	All coding exons	MYD88	Exons 3 and 5	WTI	Exons 7 and 9
ETNK1	All coding exons	NOTCH1	Exons 26-28, 34 and partial 3' UTR	ZRSR2	All coding exons
ETV6	All coding exons	NPM1	Exon 12	AMELY	All coding exons
EZH2	All coding exons	NRAS	Exons 2 and 3	AMELX	All coding exons
FBXW7	Exons 9-11	PAX5	All coding exons	TGIF2LY	All coding exons
FLT3	Exons 13, 14, 15, 20	PHF6	All coding exons	TGIF2LX	All coding exons
GATA2	Exons 2-6	PPM1D	All coding exons		

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Robust detection of CEBPA variants, FLT3-ITDs and KMT2A-PTDs

Mutations in the CEBPA gene are among the most common molecular alterations in AML, which itself is the most common type of acute leukaemia in adults^{1,2}. Sequencing of CEBPA is challenging due to the presence of repeat regions and the high GC-content of the gene, leading to poor coverage across these regions and potentially missed variants. OGT's expert bait design overcomes these issues and provides exceptional coverage uniformity, enabling reliable detection of variants and eliminating the requirement for supplementary fill-in with Sanger sequencing (Figure 1).

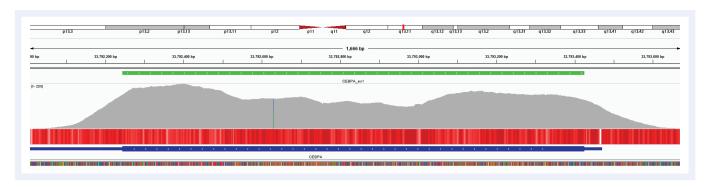


Figure 1: Illustration of the excellent coverage uniformity of the CEBPA gene. Depth of coverage per base (grey). Targeted region (green). Gene coding region as defined by RefSeq (blue). GC percentage (red).

FLT3 internal tandem duplications (ITDs) are challenging to target, and subsequently detect, because they are by nature repetitive and can be very long. As a result, FLT3-ITDs are generally masked in most panel designs, necessitating additional techniques to obtain a comprehensive genetic picture. OGT employs sophisticated bait designs to generate uniform coverage across, as well as upstream and downstream of the repetitive region. In combination with our complimentary NGS analysis software Interpret, this allows easy detection of FLT3-ITDs ranging from a handful of base pairs to >200 bp (Figure 2).

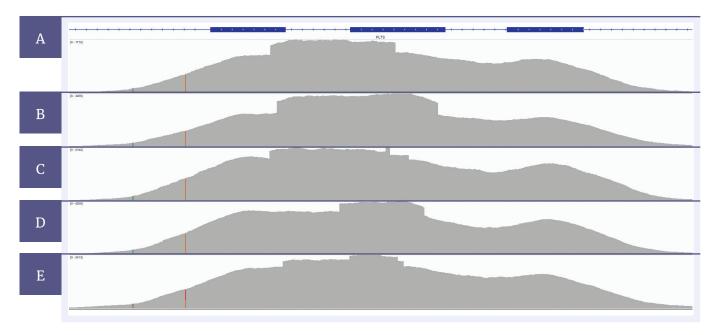


Figure 2: FLT3-ITDs of various sizes and even regions containing multiple ITDs can be confidently detected. ITD sizes are A 174 bp, B 225 bp, C 195 bp with additional 6 bp, D 120 bp and E 168 bp with additional 69 bp.

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Other tandem duplications frequently observed in AML are partial tandem duplications (PTDs) in *KMT2A* (MLL). Similar to ITDs, *KMT2A*-PTDs are notoriously difficult to detect due to their size, with duplications spanning multiple exons. With OGT's expertise in hybridisation-based panel design, SureSeq offers robust detection of all sizes of *KMT2A*-PTDs, alleviating the burden of running multiple assays (Figure 3).



Figure 3: PTD detected spanning exons 2-8 of KMT2A by OGT's Interpret NGS analysis software.

Fast and easy workflow

Hybridisation-based enrichment is now well recognised as providing superior results over amplicon-based enrichment technology. The OGT Universal NGS Workflow Solution delivers a straightforward and robust workflow with fewer hands-on steps, and improved turnaround times. For increased convenience and flexibility, the Universal NGS workflow is performed with a combined enzymatic fragmentation, end repair and A tailing step, and convenient bead concentration steps, whilst still delivering libraries of the highest quality. The inclusion of unique Dual Index Adapters increases multiplexing efficiency and confidence, whilst enhancing capabilities to include sensitive applications. The included Universal Hybridisation & Wash Kit simplifies this key step while offering excellent coverage uniformity and reproducibility.

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Complimentary Interpret NGS analysis software

Interpret is OGT's powerful and easy-to-use NGS analysis solution, facilitating analysis and visualisation of a wide range of somatic variants and structural aberrations. Designed to work seamlessly with all SureSeq panels, Interpret perfectly complements the SureSeq Myeloid Plus Panel, delivering fast and accurate detection of all SNVs, indels, ITDs and PTDs covered by the panel. Following detection, all variants can be easily visualised in the user-friendly variant browser, for an effortless translation of all your myeloid data into meaningful results.

The OGT Partnership

The OGT partnership approach is key to providing the highest level of service, working closely with you to understand your unique challenges, customising our approach to meet your exact needs.

Bespoke panel content

You never have to sequence genes you're not interested in and can always modify each panel to what's relevant to your research. If the SureSeq Myeloid Plus Panel doesn't meet your exact requirements, you can choose from our regularly updated, expert-curated library of pre-optimised cancer content to create your ideal custom SureSeq myPanel™ Myeloid Panel. Alternatively, have a look at the other myeloid panels we have available, including our focused 3-gene SureSeq Core MPN Panel and the SureSeq pan-Myeloid Panel, incortporating key variants in 70 genes implicated in a wide range of myeloid disorders, or our disease-specific content, such as our SureSeq myPanel NGS Custom AML panels.

Feature	Specification
Number of genes	49 (+4)
Panel size	132 kb
DNA input recommended	200-500ng high quality DNA
Limit of detection	SNVs/ indels: 2.5 - 5% VAF

Myeloid Plus Workflow

Ordering information

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Product	Contents	Cat. No.
SureSeq Myeloid Plus Complete NGS Workflow Solution (24)	Enrichment baits sufficient for 3 x 8-sample pools. Bundle of 1 x Universal Library Preparation Kit (24) containing PCR primers and enzymes. 1 x Universal Hybridisation & Wash Kit V2 (24). 1 x Pre-PCR Universal Bead Kit (24). 1 x Post-PCR Universal Bead Kit (24). 1 x Universal Index Adapter Kit (24). Interpret NGS Analysis Software	780102-24
SureSeq Myeloid Plus Complete NGS Workflow Solution (96)	Enrichment baits sufficient for 12 x 8-sample pools. 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	780102-96
SureSeq Myeloid Plus Panel (24)	Enrichment baits sufficient for 3 x 8-sample pools. Interpret NGS Analysis Software	770002-24
SureSeq Myeloid Plus Panel (96)	Enrichment baits sufficient for 12 x 8-sample pools. Interpret NGS Analysis Software	770002-96
Universal NGS Workflow Solution V2 (24)	Bundle of 1 x Universal Library Preparation Kit (24) containing PCR primers and enzymes. 1 x Universal Hybridisation & Wash Kit V2 (24). 1 x Pre-PCR Universal Bead Kit (24). 1 x Post-PCR Universal Bead Kit (24). 1 x Universal Index Adapter Kit (24)	770510-24
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

References

- 1. Siegel et al., CA Cancer J Clin 2015; 65(1):5-29.
- 2. Pabst et al., Nat Genet 2001;27:263-270.



A Sysmex Group Company

What binds us, makes us.

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