SureSeq

A Sysmex Group Company

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 NGS Complete Workflow combines the rapid Universal NGS Workflow 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	*KIT*	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	*WT1*	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		

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).

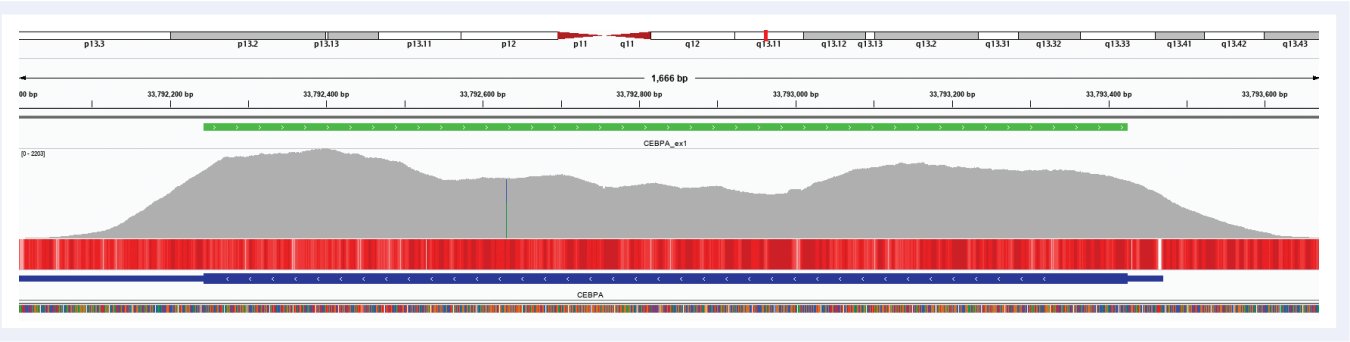

Figure 1: Illustration of the excellent coverage uniformity of the CEBPA gene. Depth of coverage per base (grey). Targeted region (green). Gene coding region as defined by RefSeq (blue). GC percentage (red).

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).

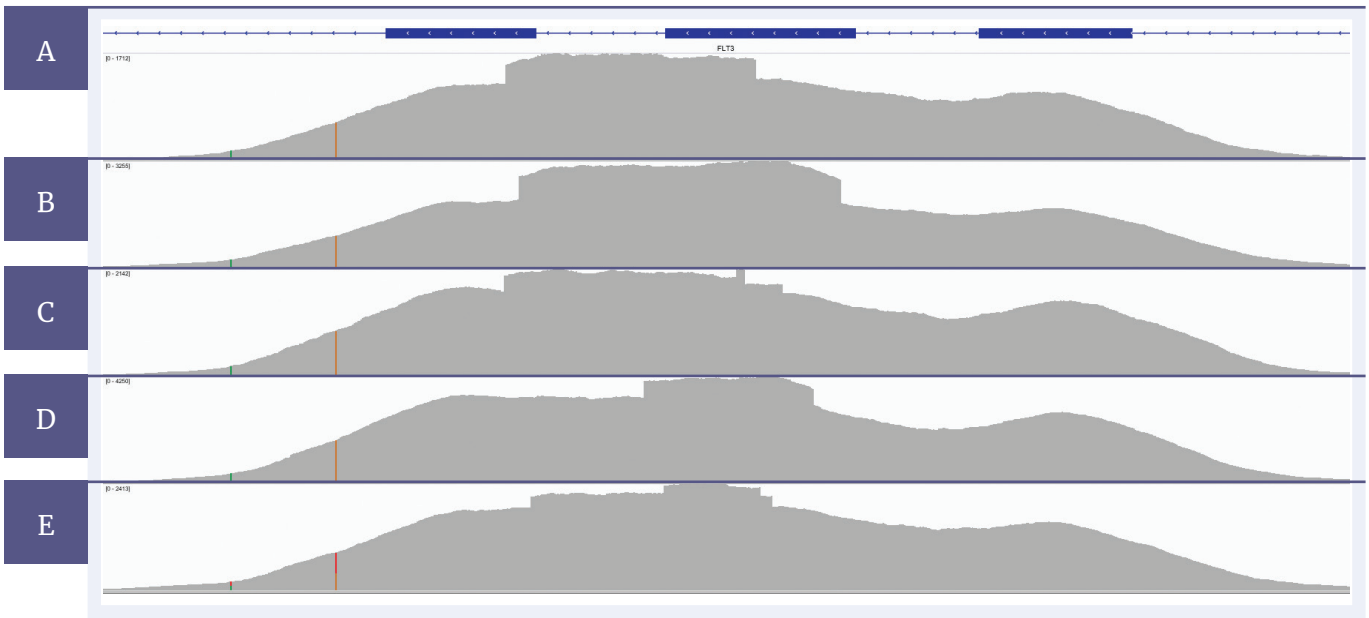

Figure 2: *FLT3*-ITDs of various sizes and even regions containing multiple ITDs can be confidently detected. ITD sizes are A 174 bp, B 225 bp, C 195 bp with additional 6 bp, D 120 bp and E 168 bp with additional 69 bp.

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 recently launched OGT Universal Library Preparation Kit delivers a straightforward and robust workflow with fewer hands-on steps, and improved turnaround times. For increased convenience and flexibility, the Universal NGS Complete 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. Universal Hyb & Wash buffer simplifies this key step while offering excellent coverage uniformity and reproducibility.

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	92.3 kb
Mean target coverage	>700x
Coverage uniformity	>99% of bases at >20% of mean target coverage
DNA input recommended	>500ng high quality DNA
Limit of detection	SNVs/ indels: 2.5 - 5% VAF
Samples per MiSeq® v2 run	16 samples / run

Ordering information

UK +44 (0) 1865 856800

US +1 914 467 5285

contact@ogt.com

ogt.com

Product	Contents	Cat. No.
SureSeq Myeloid Plus Complete 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 (24). 1 x Universal Bead Kit (24). 1 x Universal index adapter kit (24). Interpret Software	780002-24
SureSeq Myeloid Plus Complete 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 (96). 1 x Universal Bead Kit (96). 1 x Universal index adapter kit (96). Interpret Software.	780002-96
SureSeq Myeloid Plus Panel (24)	Enrichment baits sufficient for 3 x 8-sample pools	770002-24
SureSeq Myeloid Plus Panel (96)	Enrichment baits sufficient for 12 x 8-sample pools	770002-96
Universal NGS Workflow Solution (24)	Bundle of 1 x Universal Library Preparation Kit (24) containing, PCR primers and enzymes. 1 x Universal Hybridisation & Wash Kit (24). 1 x Universal index adapter kit (24).	770500-24
Universal NGS Workflow Solution (96)	Bundle of 1 x Universal Library Preparation Kit (96) containing PCR primers and enzymes. 1 x Universal Hybridisation & Wash Kit (96). 1 x Universal index adapter kit (96).)	770500-96

References

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

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What binds us, makes us.

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