SureSeq

Target capture next-generation sequencing (NGS) for use in molecular-based research of myeloid measurable residual disease (MRD)

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What binds us, makes us.

Introduction

Improved methods of disease status monitoring with detection of low-level variants are essential for early therapeutic interventions and to potentially prevent disease recurrence.

At present, two different types of methods are used for detection of measurable residual disease (MRD): immunophenotypic based on multiparameter flow cytometry (MFC), and molecular methods which include real-time quantitative polymerase chain reaction (RQ-PCR), digital droplet PCR (ddPCR) or next-generation sequencing (NGS). Each of these methods differ in their applicability, specificity and sensitivity to detect MRD.

NGS provides a solution for evaluation of multiple genes in a single assay. Together with significant reduction in sequencing cost and improved accuracy NGS can now be used in monitoring.

In this study we aimed to evaluate OGT's SureSeq™ Myeloid MRD Complete NGS Workflow Solution V2 for suitability with deep sequencing and rare variant detection required for investigation of MRD monitoring.

Methods

Workflow

Libraries were generated using OGT's Universal NGS Workflow Solution V2 (Figure 1). The workflow is ideally suited for low frequency variant detection through incorporation of Unique Dual Indexes (UDIs) and Unique Molecular Identifiers (UMIs).

Panel

The SureSeq Myeloid MRD Panel has been designed in collaboration with leading cancer experts to incorporate 13 key genes relevant to AML research. Utilising OGT's intelligent panel design capabilities, the SureSeq Myeloid MRD Panel in combination with OGT's Universal NGS Workflow Solution V2 offers accurate detection of a range of variants: single nucleotide variants (SNVs), insertion-deletions (indels) and internal tandem duplications (ITDs) in *FLT3* as low as 0.01% Variant Allele Frequency (VAF).

Gene	Exons	Gene	Exons
CSF3R	Exons 13-17	*FLT3*	Exons 13, 14 and 20
MPL	Exon 10	*IDH2*	Exons 4 and 5
SF3B1	Exons 13-16	*TP53*	Exons 2-11
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, indels and *FLT3*-ITDs in 46 hotspot exons across 13 genes relevant to AML research.

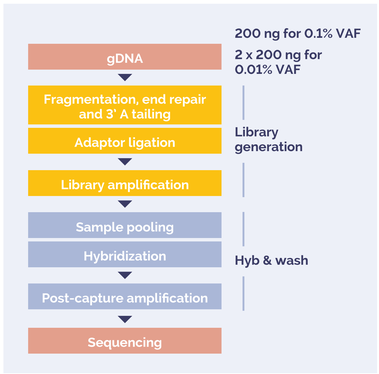


Figure 1: DNA to sequencer in 1.5 days with minimal handling time.

Samples

The workflow and panel capabilities were demonstrated by using 36 research samples orthogonally validated and shown to carry variants within panel targeted regions. Twenty-nine samples had at least one variant and seven had no variants. In addition, the workflow and the panel were tested using Myeloid Reference DNA Standard (Horizon Discovery), with 6 SNVs, 2 indels and a 300 bp *FLT3*-ITD. DNA samples were diluted to create a range of frequencies down to 0.05% variant allele frequency (VAF).

Sequencing

Sequencing was conducted using 2 x 150 bp reads on an Illumina NextSeq 550 High output® V2 300.

Bioinformatic Analysis

Sequencing data analysis was performed using OGT's proprietary cloud-based Interpret NGS Analysis Software which includes a specific MRD hotspot analysis pipeline. This pipeline has been fine-tuned to the SureSeq Myeloid MRD Complete NGS Workflow Solution V2 to achieve optimal sensitivity and specificity by adding extra modules to OGT's standard somatic pipeline. These additions include: improved base error correction (UMI processing), UMI-based QC metrics, global and local error models for SNV calling aimed at reducing false-positives, improvements to our ITD detection algorithm, and sample specific monitoring plots in the user interface.

Results I

Highly uniform UMI coverage across targeted regions

High depth and uniformity of coverage was achieved for all targeted genes and genomic regions allowing for confident detection of low frequency gene specific variants.

Uniform coverage is essential to enable reliable variant detection across all targets. High uniformity is demonstrated (Figure 2) – including difficult to target: *NPM1* ex 11.

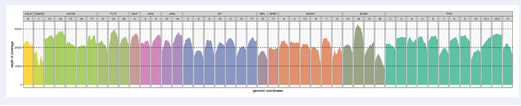


Figure 2: IGV plot showing coverage profile of target regions in the Myeloid MRD panel.

Results II

Detection of SNVs and indels in the critical regions

Data presented here are from 29 research samples that were processed using the SureSeq Myeloid MRD Complete NGS Workflow Solution V2 in combination with OGT's Interpret NGS Analysis Software.

Table 2 lists the range of SNVs detected in the 29 research samples. These include SNVs in key genes *CSF3R*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *RUNX1*, *SF3B1*, *TP53* that range from 0.85 - 0.039% VAF (Table 2).

Gene	HGVSc	HGVSp	Position (hg38)	Exon #	Total read depth	Reference allele	Alternative allele	% VAF	Pval	Rank
CSF3R	c.2047G>A	p.Gly683Arg	chr1:36466902	17/17	18859	C	T	0.085	0.0022	100.00
FLT3	c.2503G>T	p.Asp835Tyr	chr13:28018505	20/24	18097	C	A	0.072	< 1e-16	100.00
IDH1	c.395G>A	p.Arg132His	chr2:208248388	4/10	12347	C	T	0.146	0.0005	99.33
IDH2	c.419G>A	p.Arg140Gln	chr15:90088702	4/11	14948	C	T	0.040	0.0147	99.33
IDH2	c.419G>A	p.Arg140Gln	chr15:90088702	4/11	15961	C	T	0.038	0.0212	98.66
IDH2	c.429G>C	p.Leu143=	chr15:90088692	4/11	18894	C	G	0.085	< 1e-16	100.00
JAK2	c.1849G>T	p.Val617Phe	chr9:5073770	14/25	22111	G	T	0.045	< 1e-16	100.00
JAK2	c.1849G>T	p.Val617Phe	chr9:5073770	14/25	20462	G	T	0.039	< 1e-16	100.00
JAK2	c.1849G>T	p.Val617Phe	chr9:5073770	14/25	16146	G	T	0.056	< 1e-16	100.00
JAK2	c.1849G>T	p.Val617Phe	chr9:5073770	14/25	22038	G	T	0.023	< 1e-16	97.32
KIT	c.2447A>T	p.Asp816Val	chr4:54733155	17/21	17901	A	T	0.045	< 1e-16	99.33
RUNX1	c.486G>T	p.Arg162Ser	chr21:34880579	5/9	14968	C	A	0.114	< 1e-16	99.33
RUNX1	c.593A>T	p.Asp198Val	chr21:34859494	6/9	13320	T	A	0.030	< 1e-16	97.99
RUNX1	c.1389C>G	p.Pro463=	chr21:34792189	9/9	11805	G	C	0.051	< 1e-16	98.66
SF3B1	c.2098A>G	p.Lys700Glu	chr2:197402110	15/25	14196	T	C	0.303	< 1e-16	100.00
SF3B1	c.2098A>G	p.Lys700Glu	chr2:197402110	15/25	16019	T	C	0.050	< 1e-16	100.00
SF3B1	c.1997A>C	p.Lys666Thr	chr2:197402636	14/25	22929	T	G	0.214	< 1e-16	100.00
TP53	c.638G>T	p.Arg213Leu	chr17:7674893	6/11	22935	C	A	0.161	< 1e-16	100.00
TP53	c.742C>T	p.Arg248Trp	chr17:7674221	7/11	12459	G	A	0.120	0.015	100.00
TP53	c.742C>T	p.Arg248Trp	chr17:7674221	7/11	11713	G	A	0.145	0.006	100.00
TP53	c.646G>A	p.Val216Met	chr17:7674885	6/11	15908	C	T	0.025	4.00E-06	97.99
TP53	c.509C>T	p.Thr170Met	chr17:7675103	5/11	29214	G	A	0.065	0.004	100.00
TP53	c.108G>A	p.Pro36=	chr17:7676261	4/11	18552	C	T	0.075	0.0015	96.64
TP53	c.637C>T	p.Arg213Ter	chr17:7674894	6/11	24779	G	A	0.165	0.0011	100.00
TP53	c.375G>A	p.Thr125=	chr17:7675994	4/11	18692	C	T	0.144	0.0007	100.00
TP53	c.108G>A	p.Pro36=	chr17:7676261	4/11	16731	C	T	0.155	< 1e-16	97.99

Table 2: SNV detection: Data generated using the SureSeq Myeloid MRD Panel in combination with the OGT's Universal NGS Workflow Solution V2 and OGT's Interpret NGS Analysis Software. Rank and Pval relate to local and global background error.

Table 3 lists the range of Indels detected in the 29 research samples. These include 6 *NPM1* type A insertion and other genes *TP53*, *CEBPA*, *RUNX1* (Table 3).

Gene	HGVSc	HGVSp	Position (hg38)	Exon #	Total read depth	Reference allele	Alternative allele	% VAF
NPM1	c.860_863dup	p.Trp288CysfsTer12	chr5:171410539	11/11	23029	C	TCTG	0.035
NPM1	c.860_863dup	p.Trp288CysfsTer12	chr5:171410539	11/11	19603	C	TCTG	0.020
NPM1	c.860_863dup	p.Trp288CysfsTer12	chr5:171410539	11/11	14517	C	TCTG	0.028
NPM1	c.860_863dup	p.Trp288CysfsTer12	chr5:171410539	11/11	16778	C	TCTG	0.018
NPM1	c.860_863dup	p.Trp288CysfsTer12	chr5:171410539	11/11	16629	C	TCTG	0.036
NPM1	c.860_863dup	p.Trp288CysfsTer12	chr5:171410540	11/11	21049	T	TCTG	0.100
TP53	c.578del	p.His193LeufsTer54	chr17:7674952	6/11	20923	AT	A	0.038
CEBPA	c.200_201insAGA	p.Tyr67delinsTer	chr19:33302214	1/1	12018	G	TCT	0.092
RUNX1	c.720_733del	p.His242AlafsTer14	chr21:34834481	7/9	23943	GGGGCTGGGTGGTGT	G	0.058

Table 3: Indel detection: Data generated using the SureSeq Myeloid MRD Panel in combination with the OGT's Universal NGS Workflow Solution V2 and OGT's Interpret NGS Analysis Software.

Results III

Detection of SNVs, indels and *FLT3*-ITD using Myeloid Reference DNA Standard

SureSeq Myeloid MRD Complete NGS Workflow Solution V2 in combination with OGT's Interpret NGS Analysis Software confidently detected all anticipated variants within the Myeloid Reference standard including an *NPM1* insertion and a 300 bp *FLT3*-ITD.

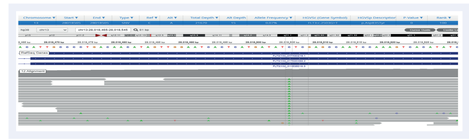


Figure 3: Example of a SNV detection *FLT3* c.2503 G>T (p.Asp835Tyr).

Gene	HGVSc	HGVSp	Position (hg38)	Reference allele	Alternative allele	Myeloid Reference Standard				Negative control			
						Total read depth	% VAF	Pval	Rank	Total read depth	% VAF	Pval	Rank
SF3B1	c.2219G>A	p.Gly740Glu	chr2:197401989	C	T	34347	0.10	0	100	26186	0	1	0
IDH1	c.394C>T	p.Arg132Cys	chr2:208248389	G	A	34639	0.08	0.046	99.3	27327	0.02	0.53	94.6*
JAK2	c.1849G>T	p.Val617Phe	chr9:5073770	G	T	41990	0.06	0	100	33162	0	1	0
FLT3	c.2503G>T	p.Asp835Tyr	chr13:28018505	C	A	40292	0.08	0	100	31161	0	1	0
IDH2	c.515G>A	p.Arg172Lys	chr15:90088606	C	T	32967	0.03	0	97.3	25426	0	1	0
TP53	c.722C>T	p.Ser241Phe	chr17:7674241	G	A	29041	0.07	0	100	21565	0	1	0
NPM1	c.860_863dup	p.Trp288CysfsTer12	chr5:171410540	C	CTCTG	33952	0.05	-	-	27365	0	-	-
JAK2	c.1611_1616del	p.Phe537_Lys539delinsLeu	chr9:5070022	TTCACAA	T	43032	0.01	-	-	34705	0	-	-
FLT3	c.1919_1920ins	ITD 300	chr13:28033909	C	-	12265	>0.05	-	-	10473	0	-	-

Table 4: Detected SNVs, indels and ITD in Myeloid Reference DNA Standard (Horizon Discovery).
*P-value and Rank metrics allow for improved variant filtering at sites with high background, as only variants with a P-value < 0.05 and a Rank > 95% will be considered.

Results IV

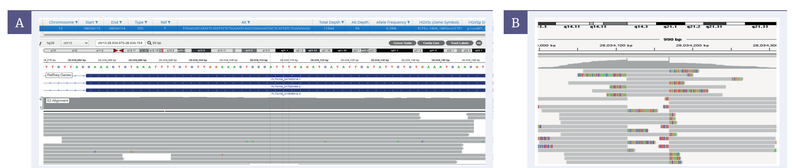
Detection of a *FLT3* internal tandem duplications (*FLT3*-ITDs)

FLT3-ITDs are challenging to target, and subsequently detect due to their inherent repeat content and length (up to 300 bp).

The unique detection algorithms incorporated into Interpret NGS Analysis Software enable accurate detection and quantification of *FLT3*-ITDs, including multiple and large ITDs (Figure 2).

Gene	HGVSc	Length	Position (hg38)	Exon #	Total read depth	% VAF
FLT3	NM_004119.3:c.1770_1793ins	24	chr13:28034125	14/24	11340	0.81
FLT3	NM_004119.3:c.1804_1805ins	57	chr13:28034114	14/24	11844	0.71
FLT3	NM_004119.3:c.1814_1815ins	21	chr13:28034104	14/24	23831	0.03

Table 5: *FLT3*-ITD detection: Data generated using the SureSeq Myeloid MRD Panel in combination with the OGT's Universal NGS Workflow Solution V2 and OGT's Interpret NGS Analysis Software.



A: panel A shows the output from OGT's Interpret NGS Analysis Software for a low-frequency 57bp *FLT3*-ITD at 0.78%;
B: panel B shows a subset of the supporting clipped reads.

Conclusions

- High depth and uniformity of coverage was achieved for all targeted genes and genomic regions allowing for confident detection of low frequency variants
- We have demonstrated that the SureSeq Myeloid MRD Panel in combination with the OGT's Universal NGS Workflow Solution V2 and OGT's Interpret NGS Analysis Software can be reliably used to detect AML relevant gene specific variants SNVs, indels and *FLT3*-ITDs down to a possible 0.01% VAF
- Our approach provides researchers with the capability to use capture-based NGS technology to simultaneously characterize AML gene variants in MRD monitoring.

Learn more about the MRD panel

Further information contact@ogt.com | ogt.com | Oxford Gene Technology Ltd., Unit 5, Oxford Technology Park, 4A Technology Drive, Kidlington, Oxfordshire, OX5 1GN, UK

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