# SureSeq

# Development of a target-capture NGS assay for use in molecular-based research of myeloid measurable residual disease (MRD)

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What binds us, makes us.

## Introduction

Molecular technologies incorporating Next-Generation sequencing (NGS) are increasingly utilised to support traditional immunophenotypic multiparameter flow cytometry in MRD detection, including acute myeloid leukemia (AML) disease monitoring.

Real-time qPCR (RT-qPCR) and digital droplet PCR (ddPCR) are highly sensitive technologies but are limited by the number of targets that can be detected in one assay.

NGS offers the opportunity to evaluate many genes in a single assay. Improved accuracy together with falling costs are facilitating the use of NGS in MRD.

We have developed a target-capture NGS approach to support researchers in studies of molecular-based MRD monitoring in myeloid malignancies.

This method provides the opportunity to evaluate many genes and variant types in a single assay.

## Materials and Methods

### Workflow

Libraries were generated using OGT's Universal NGS Workflow (Fig. 1). The workflow is ideally suited to low frequency variant detection through the inclusion of Unique Dual Indexing (UDIs) and Unique Molecular Identifiers (UMIs).

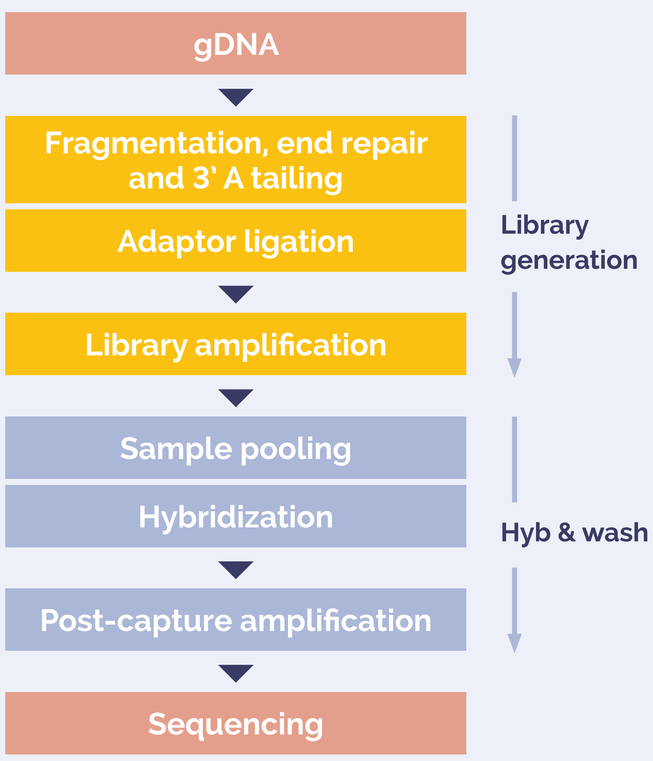


Figure 1: DNA to sequencer in 1.5 days with minimal handling time.

### Samples

Myeloid Reference DNA Standard (Horizon Discovery), with 6 SNVs, 2 indels and a 300 bp *FLT3* ITD diluted with normal DNA to generate a range of variant allele frequencies (VAFs) between 0.02x - 0.1%.

### Panel

SureSeq™ Myeloid MRD Panel targeting 43 exons in 13 genes (8 kb target; 11 kb baited).

### Sequencing

2 x 150 PE reads, Illumina NextSeq® 500 High Output.

### Bioinformatic Analysis

Sequencing data analysis was performed using OGT's proprietary Interpret software, including read mapping, error correction, coverage calculation and variant calling.

### Assay Development

A variant at 0.1% VAF requires a depth of 10,000x to be detected with 10 supporting reads.

A variant at 0.05% VAF requires a depth of 20,000x to be detected with 10 supporting reads.

We increased reads/sample and monitored the depths through:

- Depth of coverage distribution.
- Uniformity of coverage across targets.

We used the UMIs for error correction and determined the effect of increased reads on:

- Distribution of UMI family size (FS).
- Percentage of data removed in FS2 filtering.

## Results

### UMI coverage supports detection at 0.05% VAF

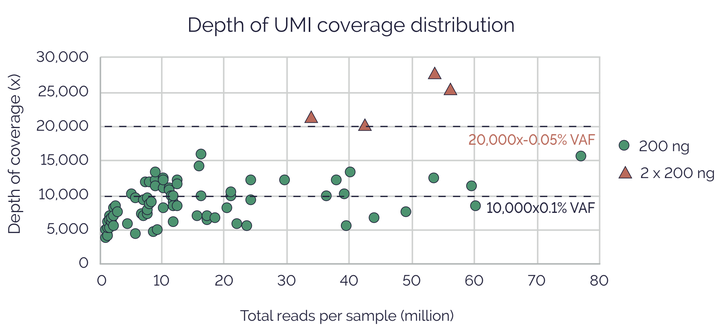


Figure 2: Relationship between sequencing and unique depth of coverage

- Average depth of coverage increases proportionally up to 10 million total reads (green circles).
- At higher total reads, unique coverage is increased by increasing the input DNA (brown triangles).
- >20,000x coverage is achieved with 40 million reads/sample.

### Highly uniform UMI coverage across target regions

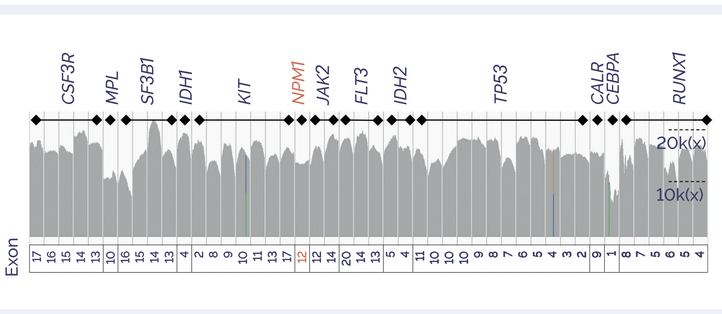


Figure 3: IGV plot showing coverage profile of target regions in the Panel

- Uniform coverage is essential to maintain equal sensitivity across all targets.
- High uniformity is demonstrated (Fig. 3)-including difficult target: *NPM1* exon 12.

### Average UMI family size increases with total reads

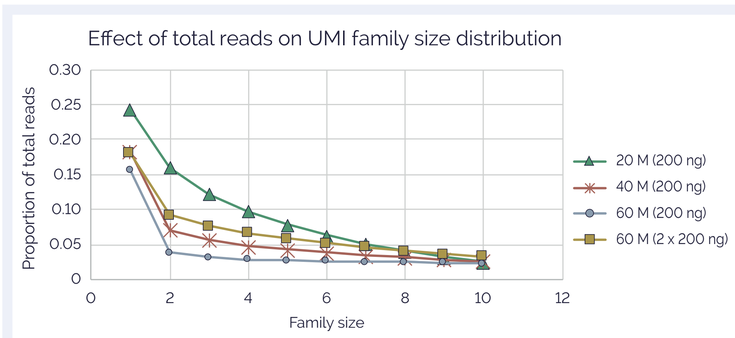


Figure 4: UMI family size (FS) distribution showing increase with increasing reads/sample

### Increasing total reads reduces error correction data loss

- Removing reads with a family size of 1 reduces background for SNVs and increases the accuracy of the call.
- Increasing the reads per sample increases the family size (Fig. 4).
- Increasing the DNA input caused a small decrease in the family size with 60 M reads/sample (gold squares).
- An increase in family size corresponds to a reduction in FS1 reads removed in error correction (Table 1).

| Reads/sample (million) | Mean UMI coverage | Mean Reads in FS1 |
|---|---|---|
| 10 M-20 M | 8,930x | 24% |
| 30 M-40 M | 9,193x | 18% |
| 50 M-60 M | 11,460x | 16% |
| 35 - 60 M (2 x 200ng) | 23,224x | 18% |

Table 1: Percentage of reads removed in error correction reduces as depth increases.

### 100% detection of variants at ≥0.04% VAF (40 M reads/sample)

| Gene | Variant | Expected frequency 0.04% | | Expected frequency 0.05% | | Negative control | |
|---|---|---|---|---|---|---|---|
| | HGVSc | Read depth | Observed VAF | Read depth | Observed VAF | Read depth | Observed VAF |
| *SF3B1* | c.2219G>A | 15,289 | 0.07% | 14,312 | 0.03% | 15,086 | 0.00% |
| *JAK2* | c.1849G>T | 24,520 | 0.02% | 22,375 | 0.08% | 22,017 | 0.00% |
| *FLT3* | c.2503G>T | 26,556 | 0.04% | 24,127 | 0.04% | 23,991 | 0.00% |
| *IDH2* | c.515G>A | 18,950 | 0.02% | 16,565 | 0.04% | 18,404 | 0.00% |
| *TP53* | c.722C>T | 14,199 | 0.07% | 12,618 | 0.03% | 13,838 | 0.00% |
| *NPM1* | c.860_863dup | 16,536 | 0.05% | 11,536 | 0.09% | 14,350 | 0.00% |
| *JAK2* | c.1611_1616del | 26,267 | 0.03% | 25,683 | 0.03% | 22,017 | 0.00% |
| *FLT3* | ITD300 | 13,119 | 0.05% | 12,208 | 0.04% | 21,686 | 0.00% |

Table 2: Detection of SNVs, Indels and an ITD, with expected frequency ranges of 0.04%-0.05%. SNVs are filtered to remove FS1 reads.

- OGT's Universal NGS Workflow Solution with the SureSeq Myeloid MRD Panel confidently detected all anticipated variants ≥0.04% including *NPM1* insertion and a 300 bp *FLT3* ITD (Table 2).
- No supporting reads are observed in the negative control.

## Conclusions

- The OGT Universal NGS Workflow is suitable for use in MRD monitoring providing high uniformity across all target regions including *NPM1* exon 12.
- Increasing input DNA and reads per sample improves variant detection and accuracy through greater coverage and improved error correction.
- Our approach achieves coverage depth required to detect somatic mutations ≥ 0.04% VAF.
- 100% detection of variants ≥ 0.04% VAF: SNVs, indels (including *NPM1*) and a 300 bp *FLT3* ITD.
- This assay provides researchers with the capability to use capture-based NGS technology to simultaneously detect a number of variants in MRD monitoring.

Further information **contact@ogt.com | ogt.com | Oxford Gene Technology Ltd., Begbroke Science Park, Woodstock Road, Begbroke, Oxfordshire, OX5 1PF, UK.**


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