Development of a target-capture NGS assay for use in molecular-based research of myeloid Measurable Residual Disease

Rebecca Biloune[1]
Natalie Milner[1], Giulia Poloni[1], Venu Pullabhatla[1], Lyudmila Georgieva[1], James Reid[1] and Graham Speight[1]
[1] OGT

Background & objectives

Introduction: Molecular methods are increasingly supporting traditional immunophenotypic multiparameter flow cytometry (MFC) in detection of measurable residual disease (MRD), including acute myeloid leukaemia (AML). Recent advances in next-generation sequencing (NGS) have reduced cost and improved accuracy, facilitating its use in MRD. We have developed a target-capture NGS approach to support researchers in studies of molecular-based MRD monitoring in AML.

Aim: We developed a focussed SureSeq™ Myeloid MRD panel for use with our existing library preparation kit, OGT™ Universal NGS Complete Workflow Solution. In this study we aimed to evaluate the panel and workflow for suitability with the deep sequencing and rare variant detection requirements of MRD.

Methods

We tested the OGT Universal NGS Workflow Solution in conjunction with the SureSeq Myeloid MRD panel for suitability of detecting variants present at low frequency. The panel covers 43 hotspot exons in 13 genes (11.3 kb baited, 8 kb targeted) relevant to AML. The workflow and panel were tested using a Myeloid Reference Standard (Horizon Discovery) at 200 ng, 250 ng, 500 ng input DNA. This is a well characterised control material containing a range of variant types (SNVs, Indels and ITDs) suitable for use in a monitoring assay. For lower variant frequencies, we diluted the reference DNA to create frequencies in the range of 1.0 - 0.05% variant allele frequency (VAF). Sequencing was conducted using NextSeq™ (Illumina). Data was analysed using OGT's Interpret software.

Results

Libraries captured with the Myeloid MRD panel achieved a coverage of 20,000x when sequenced with 20 million reads per sample. We observed that increasing reads above this level only marginally increased the depth and substantially increased the duplication rate and support for UMI family size filtering. We detected a 300 bp *FLT3* ITD down to 0.1% and achieved 100% detection of SNVs and Indels at 0.05% in target regions, including *NPM1*.

Conclusion

Detecting very low frequency alleles requires highly uniform and sensitive target enrichment. We used the SureSeq Myeloid MRD assay to demonstrate reliable and accurate detection of variants down to 0.05% VAF. This assay provides researchers with the capability to use capture based NGS technology for Myeloid MRD monitoring.