The use of a hybridisation-based NGS enrichment panel for the confident identification of a broad range of low-frequency variants from as little as 50 ng of challenging clinical research FFPE samples

Graham Speight[1], Ephrem Chin[1], Jacqueline Chan[1], Robert Zeillinger[2], Nicole Concin[3], David Cook[1]

[1]Oxford Gene Technology, Oxford, UK; [2]Medical University of Vienna, Dept. of Obstetrics and Gynaecology, Vienna, Austria and [3]Medical University, Dept. of Gynaecology and Obstetrics, Innsbruck, Austria

Introduction

One of the challenges in the treatment of cancer is the high level of genetic complexity and tumour heterogeneity. Detailed information about the genetic profile of each individual tumour can help guide treatment strategies[1]. Epithelial ovarian cancer (EOC) is the most lethal gynaecological malignancy[2], and the type II tumours accounting for approximately 75% of all EOCs, are nearly always detected in advanced stages. These highly aggressive tumours are characterised by their morphological and molecular homogeneity and often (>80% of cases) contain *TP53* mutations.

The GANNET53 (Ganetespib in metastatic, p53 mutant, platinum-resistant ovarian cancer) trial started in October 2015 and aims to improve the prognosis and quality of life in platinum-resistant EOC patients (www.gannet53.eu). This European multi-centre clinical trial is currently in stage II during which biomaterials have been collected and analysed using a SureSeq™ hybridisation-based enrichment panel for targeted next-generation sequencing (NGS), to determine the *TP53* mutation status of the samples.

Enrichment methods

Multiplex PCR-based approaches are often used to analyse low input DNA from FFPE samples. However, they are not able to (without molecular barcodes) fully elucidate the true allele frequency and complexity, which is essential to fully evaluate highly heterogenic tumour samples. Hybridisation-based enrichment approaches typically demonstrate better uniformity, are more likely to preserve the complexity of the original sample, are more tolerant of both DNA quality and unknown variants in the capture region.[3]

	Advantages	Disadvantages
Multiplex PCR-based enrichment	• Simple and fast workflow – <1 day • Permits low DNA inputs • Low start-up costs	• Cannot remove PCR duplicates/bias – obscures true complexity • Allelic drop-out due to variants in priming sites • Poor uniformity of coverage
Hybridisation-based enrichment	• Highly uniform coverage • Tolerant of variants throughout target region • High sensitivity	• Requires greater DNA input • Multi-step workflow – 1-2 days

Table 1: Performance comparison of amplicon- and hybridisation-based capture methods

Hybridisation-based enrichment workflow

The SureSeq hybridisation-based workflow was used to determine the genetic profile of 32 ovarian tumours. The workflow of this approach is outlined in Figure 5.

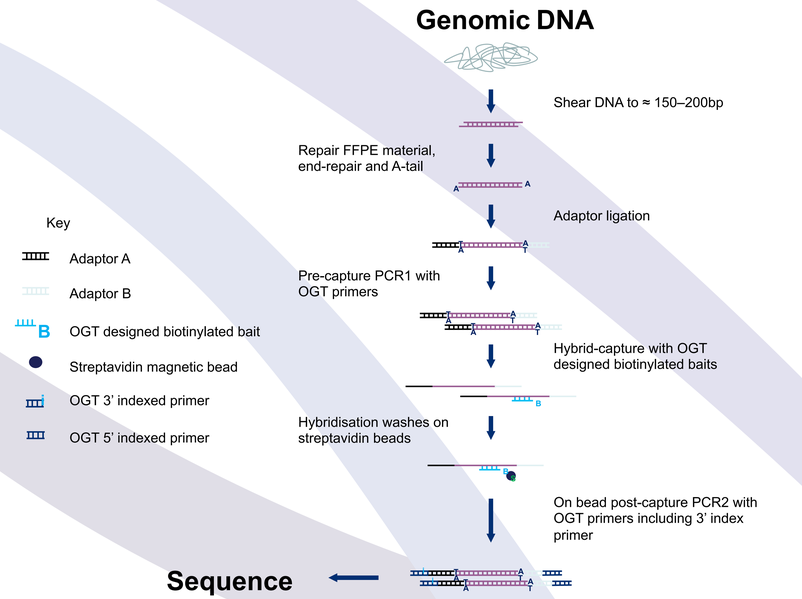

Figure 5: OGT SureSeq workflow. The SureSeq Ovarian Cancer panel targets seven key genes – ATM, ATR, BRCA1, BRCA2, NF1, PTEN and TP53

Formalin damage in DNA can be reduced using the SureSeq FFPE DNA Repair Mix

Genetic profiling of solid tumours is often problematic as tissue biopsies are typically archived as formalin-fixed, paraffin-embedded (FFPE) blocks, which preserve tissue morphology and permits long-term storage at room temperature. However, the methods used for fixation significantly damage and compromise the quality of nucleic acids in these samples. Formalin damage can fragment the DNA which can impair PCR (a required process in NGS library preparation). Consequently, library yields and the ability to obtain high-quality, meaningful sequence data are compromised, affecting the confident identification of variants.

We tested a range of FFPE derived DNA and found treatment with the SureSeq FFPE DNA Repair Mix significantly improved the mean target coverage, thereby increasing the confidence of the variant calls (Figure 1A). Use of the Repair Mix also enabled a reduction in the amount of DNA input down to 50 ng whilst maintaining good depth of coverage (Figure 1B).

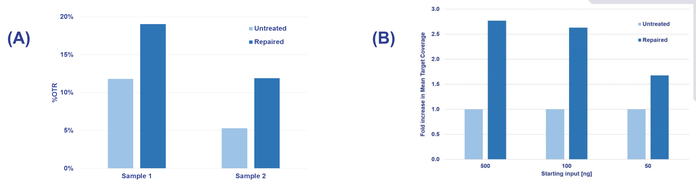

Figure 1: Example data obtained using FFPE DNA extracted from Ovarian tumour samples. Panel A shows that the SureSeq FFPE DNA Repair Mix improves on-target reads (OTR); Panel B demonstrates the use of lower DNA inputs whilst maintaining depth of coverage.

Use of the SureSeq Ovarian Cancer Panel with Type II EOC samples identifies variants in other DNA repair genes in addition to *TP53*

All 32 samples presented here were provided by the GANNET53 trial and contained ≥40% tumour content. DNA was extracted from tissue curls using standard methods. The resultant DNA was analysed using the hybridisation-based SureSeq Ovarian Cancer Panel and subsequently sequenced on an Illumina MiSeq®. Each sample received between 1/10th to 1/16th of a lane. The average depth of coverage (after removal of PCR duplicates) over the seven target genes (*ATM, ATR, BRCA1, BRCA2, NF1, PTEN* and *TP53*) was 666. We confidently detected one or more deleterious *TP53* variants in all 32 samples with the minor allele frequencies (MAF) ranging from 1.1 – 79.6%.

In addition to the mutations in *TP53*, 22 of the samples were found to have additional variants in *BRCA1* and *BRCA2,* (Integrated Genome Viewer [IGV[4]] images of examples are shown in Figures 2 & 3), of which 8 were likely germline (defined as having a MAF between 45-55% or >95%). The remaining putative somatic variants had MAFs ranging from 2.3 to 71.3%. Variants were also found in *ATM* in 5 of the mutant *TP53* tumour samples (example shown in Figure 4).

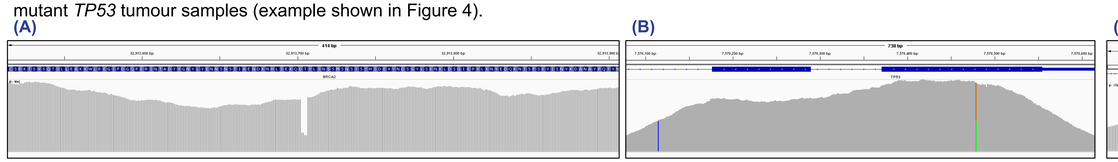

Figure 2: *BRCA2* exon 11 (panel A) and *TP53* exon 5 (panel B). This sample contains a 46% Pro151Ser SNV in *TP53* as well as a four nucleotide deletion with a 70% allele frequency in *BRCA2*.

Figure 3: Example coverage of *BRCA1* exon 10. The whole exon (3425 bp) is covered uniformly allowing confident detection of nine variants, each of 60% allele frequency. This sample also had a 34% Arf273His mutation in *TP53* (data not shown).

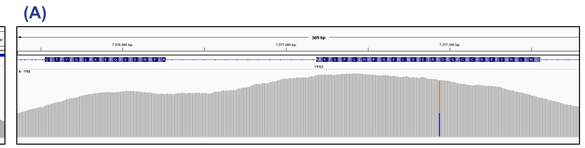

Figure 4: *TP53* exons 9 and 10 (panel A) and *ATM* exon 41 (panel B). This sample contains a 68% nonsense mutation in *TP53* and 53% Gly2023Arg SNV in *ATM*.

Conclusions

- We have demonstrated that it is possible to gain useful genetic information from as little as 50 ng of DNA derived from FFPE material when using the SureSeq FFPE DNA Repair Mix in combination with the SureSeq Ovarian Panel.
- Using this hybridisation-based capture approach we were able to identify a range of germline and somatic mutations in *TP53* from ovarian tumour tissue collected as part of the GANNET53 trial. Some of these mutations are likely to be the oncogenic drivers in the pathogenesis of these tumours.
- We also identified numerous *BRCA*1 and BRCA2 mutations in 71% of the samples confirming independent observations that these genes along with ATM are important in ovarian carcinomas.

References

1. Ross, J.S. and Cronin, M., 2011. Whole cancer genome sequencing by next-generation methods. *American journal of clinical pathology*, *136*(4), pp.527-539.
2. Kurman, R.J. and Ie-Ming, S., 2010. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *The American journal of surgical pathology*, *34*(3), pp.433-443.
3. Samorodnitsky, E., *et al.*, 2015. Evaluation of Hybridization Capture Versus Amplicon-Based Methods for Whole-Exome Sequencing. *Human mutation*, *36*(9), pp.903-914.
4. Thorvaldsdóttir, H., Robinson, J.T. and Mesirov, J.P., 2013. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in bioinformatics*, *14*(2), pp.178-192.

Oxford Gene Technology, Begbroke Science Park, Woodstock Road, Begbroke, Oxfordshire, OX5 1PF, UK
T: +44(0)1865 856826 (US: 914-467-5285) E: contact@ogt.com W: www.ogt.com