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 50ng of challenging clinical research FFPE samples

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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 is the most lethal gynaecological malignancy[2], and the type II tumours, which account for approx., 75% of all EOC's, are nearly always detected in advanced stage. 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 – coordinator Dr. N. Concin) trial started in October 2015 and aims to improve the prognosis and quality of life in platinum-resistant EOC patients - http://www.gannet53.eu/. This Europe-wide multi-centre clinical trial is currently in stage II during which biomaterials have been collected and analysed using the SureSeq™ hybridisation-based enrichment for targeted next-generation sequencing, to determine the p53 status of patients.

Enrichment methods

A multiplex PCR-based approach tends to be quick, easily integrated into existing laboratory workflows, and performs well with low DNA inputs. However as all fragments have the same start and end point it is difficult to determine and remove PCR duplicates which obscure the minor alleles present within the sample. A hybridisation-based approach typically demonstrates better uniformity, is more likely to preserve the complexity of the original sample, and is tolerant of both DNA quality and variants in baits sequences.[3]

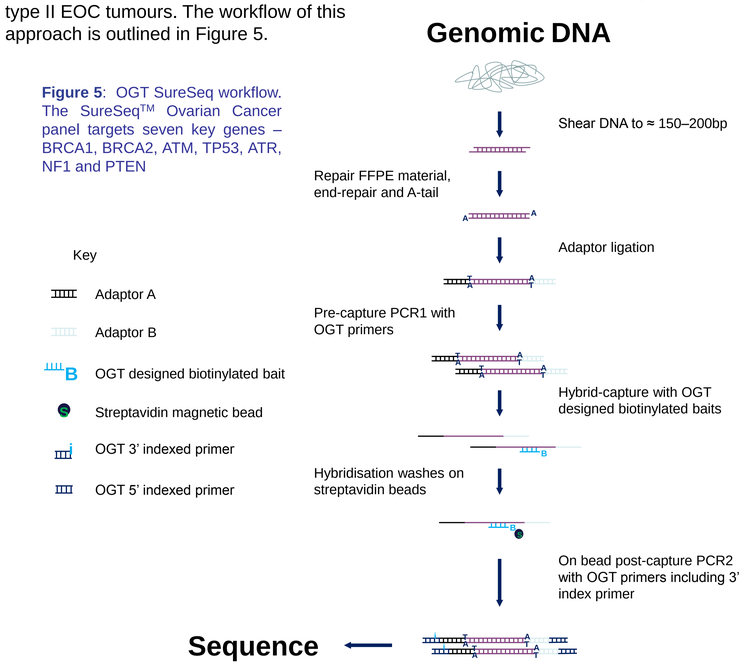
Table 1: Performance comparison of amplicon-based and hybridisation capture-based methods

	Advantages	Disadvantages
Multiplex PCR-based enrichment	• Simple and fast workflow - <1 day • Performs well with low DNA inputs • Low start-up costs	• Cannot remove PCR duplicates – 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

Hybridisation-based enrichment workflow

The SureSeq™ hybridisation-based approached was used to determine the genetic profile of 37 type II EOC 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 – BRCA1, BRCA2, ATM, TP53, ATR, NF1 and PTEN

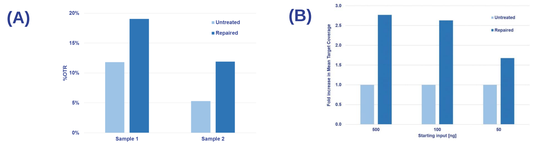


Formalin-damage in DNA can be reduced through use of repair mix

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 allows long-term storage at room temperature. However, the methods used for fixation significantly damage and compromise the quality of nucleic acids from these samples. Formalin damage can fragment the DNA which can impair PCR, a required process in NGS library preparation. Consequently, library yields and high quality, meaningful sequence data are compromised affecting the confident identification of variants.

We tested a range of FFPE DNA extracts and found treatment with the SureSeq FFPE DNA Repair Mix significantly improves mean target coverage thereby increasing the sensitivity of the assay (Figure 1a). Use of the Repair mix also allows enables users to reduce 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 cancer samples. Panel A show that the SureSeq FFPE DNA Repair Mix improves on-target rate; Panel B demonstrates the mix permits the use of lower DNA inputs whilst maintaining depth of coverage.



Type II EOC samples often contain variants in other DNA-repair genes in addition to p53

All samples (FFPE) contained ≥40% tumour cells (as determined by pathology); DNA extracted from curls were subsequently hybridised with the SureSeq™ Ovarian Panel and sequenced on an Illumina MiSeq with each sample receiving between 1/10th to 1/16th of a lane. The average depth of coverage (after removal of PCR duplicates) over the seven target genes for all 37 patient samples was 629. We confidently detected one or more deleterious *TP53* variants in 32/37 of the samples (three samples were severely degraded and 2 samples had a low target coverage) with the minor allele frequencies (MAF) ranging from 1.1 – 79.6%.

In addition to the mutations in *TP53*, twenty-three 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 13 were likely germline (defined as having a MAF between 40-60% of >88%). The remaining putative somatic variants had MAFs ranging 2.3 – 71.3%. Variants were also found in ATM in four of the mutp53 tumour samples (example shown in Figure 4).

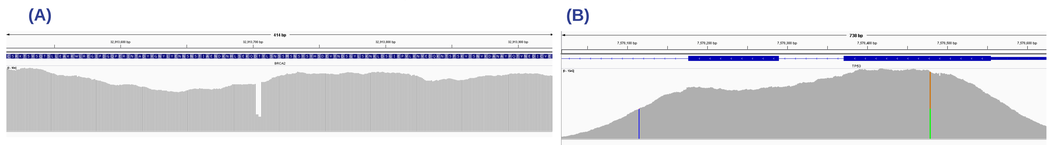


Figure 2: *Tp53* exon 5 (panel A) and BRCA2 exon 11 (panel B). This sample contains a 46% Pro151Ser SNV in p53 and a four nt deletion of 70% allele frequency in *BRCA2*.

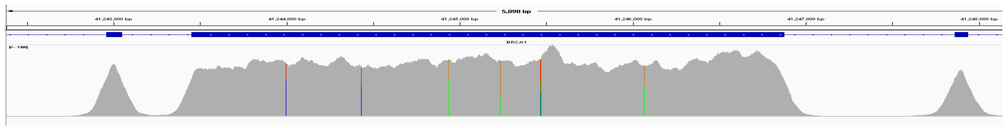


Figure 3: Example coverage of *BRCA1* exon 10. The whole exon (3425 bp) is covered uniformly allowing confident detection of nine variants, each of 60% allele frequency. This sample also had a 34% Arf273His mutation in *Tp53* (data not shown).

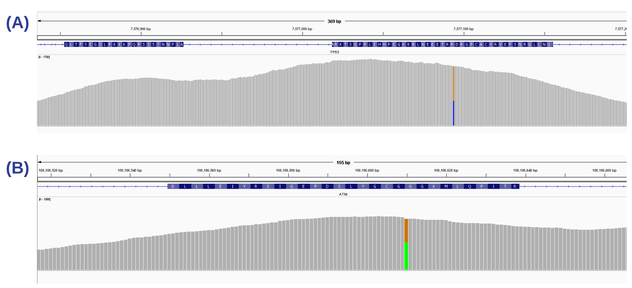


Figure 4: *Tp53* exons 9 and 10 (panel A) and *ATM* exon 41 (panel B) This sample contains a 68% nonsense mutation in p53 and 53% Gly2023Arg SNV in *ATM*.

Conclusions

- We have demonstrated it is possible to gain clinically useful genetic information from as little as 50 ng of formalin-compromised DNA.
- Using a hybridization-based capture approach we were able to identify a range of germline and somatic mutations in *Tp53* from type II EOC tissue gathered as part of the GANNET53 trial, which are the likely oncogenic driver in the pathogenesis of the tumours.
- We also identified *BRCA* mutations in 71% of the sample set confirming the observations that these typically breast cancer-associated genes also account for a large proportion of ovarian carcinoma cases.

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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., Jewell, B.M., Hagopian, R., Miya, J., Wing, M.R., Lyon, E., Damodaran, S., Bhatt, D., Reeser, J.W., Datta, J. and Roychowdhury, S., 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.

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