The application of a hybridisation-based next-generation sequencing (NGS) enrichment panel for the analysis of key genes involved in ovarian and breast tumours using DNA from FFPE samples

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Introduction
One of the challenges in cancer research is the high level of genetic complexity and tumour heterogeneity. Detailed information about the genetic profile of each individual tumour may help guide treatment strategies.1,2

NGS has enabled the simultaneous study of multiple mutations in high-attention cancer predisposition genes. However, tissue biopsies are typically archived as formalin-fixed, paraffin embedded (FFPE) blocks which can significantly compromise the quality and amount of nucleic acids available for genomics research. Overcome these issues, we have used the SureSeq3 FFPE DNA Repair Mix, in combination with a hybridisation-based NGS custom enrichment panel, the SureSeq Ovarian Cancer Panel (Table 1) to identify somatic variation in key DNA repair genes associated with ovarian cancer.

SureSeq hybridisation workflow
The SureSeq hybridisation-based enrichment was used throughout this study; the workflow of this is outlined below in Figure 1.

To evaluate the application of a hybridisation-based approach we:
• Compared the uniformity of coverage between a PCR-based and a hybridisation-based enrichment approach for the analysis of BRCAl and BRCAl2 in solid tumour samples4.
• Identified potentially damaging variants in TP53, BRCAl and BRCAl2 genes from DNA extracted from FFPE blocks of type II epithelial ovarian cancer (EOC) samples.

Confident detection of germline and somatic variants in key cancer-related genes
We tested 32 EOC samples determined by pathology to contain ≥40% tumour cells and identified one or more deleterious somatic variants in key cancer-related genes. However, tissue biopsies are typically archived as formalin-fixed, paraffin embedded (FFPE) blocks which can significantly compromise the quality and amount of nucleic acids available for genomics research.

Formaldehyde cross-linking is a prerequisite: without pre-treatment, enrichment of FFPE DNA is not feasible.3

To overcome these issues, we have used the SureSeq4 FFPE DNA Repair Mix, in combination with a hybridisation-based NGS custom enrichment panel, the SureSeq Ovarian Cancer Panel (Table 1) to identify somatic variation in key DNA repair genes associated with ovarian cancer.

Table 1: Key ovarian cancer-related genes in the SureSeq Ovarian Cancer Panel

<table>
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<tr>
<th>Gene</th>
<th>Symbol</th>
<th>Chromosome</th>
<th>Exon</th>
<th>Start (bp)</th>
<th>End (bp)</th>
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<td>BRCAl</td>
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<td>2079190</td>
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<tr>
<td>BRCAl2</td>
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<td>17p13.1</td>
<td>2</td>
<td>145018</td>
<td>1762591</td>
</tr>
</tbody>
</table>

Figure 1: OGT SureSeq workflow. The SureSeq workflow allows users to go from extracted DNA to sequence in 1.5 days with minimal handling time.

Figure 2: Data obtained using FFPE DNA extracted from ovarian cancer research samples. Panel A shows that the SureSeq FFPE DNA Repair Mix improves the depth of coverage per base, green horizontal bars the targeted region, and the red heatmap - the GC content.

Figure 3: Assessment of the uniformity of sequencing coverage from FFPE-derived DNA using an amplicon and the SureSeq hybridisation-based capture approach. Enrichment by SureSeq (dark blue bars) demonstrates better uniformity than that of an amplicon-based approach (green bars). The level of uniformity is maintained with SureSeq when starting with ~250 ng DNA (light blue bars). Samples are ordered by increasing DNA Integrity Number (DIN) determined by Agilent 2200 TapeStation – value in brackets.

Figure 4: This sample (DIN score 2.5) was found to contain a 2 bp deletion found in exon 10 of TP53 (transcript NM_000546).

Figure 5: We tested a range of FFPE-derived DNA and found pre-treatment with the SureSeq FFPE DNA Repair Mix significantly improves the mean target coverage, thereby increasing the flexibility of the assay (Figure 2A). Use of the Repair mix also enables a reduced DNA input down to 50 ng to be used (if necessary) whilst maintaining a good depth of coverage (Figure 2B).

Figure 6: This sample (DIN score 3.0) was found to have two potentially damaging variants in exon 8 of TP53 – a germline SNV (rs268345076) and a single base deletion present at 3%.

Figure 7: Shows uniformity of coverage in BRCAl exon 11 (transcript NM_007214) – panel A. The evenness of coverage enables confident detection of seven variants, each of 60% allele frequency. This sample (DIN score 3.2) also had a 34% Arg273His mutation in TP53 (rs268345076) – panel B.

Conclusions
• It is possible to obtain important sequence information from as little as 50 ng of formalin-compromised DNA.
• Superior uniformity of coverage was demonstrated using a hybridisation-based enrichment approach.
• High levels of uniformity were maintained across a range of starting DNA input amounts in formalin-compromised DNA.
• The SureSeq hybridisation-based approach is a robust method for the identification of germline and somatic mutations in TP53, BRCAl and BRCAl2 from type II EOC tissue samples.

References

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