SureSeq

The assessment by next-generation sequencing of FFPE derived tumour DNA using an ovarian cancer and a custom solid tumour hybridisation-based enrichment panel approach

Jacqueline Chan, Juliette Forster, William Wright, Graham Speight [1]Oxford Gene Technology (OGT).

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What binds us, makes us.

Introduction

- One of the challenges in cancer research is the high level of genetic complexity and tumour heterogeneity.
- Research that generates detailed information about the genetic profile of each individual tumour will further our understanding and may be used in the future to guide treatment strategies[2].
- Next Generation Sequencing has enabled the simultaneous study of multiple mutations in high-penetrance 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.

To overcome these issues, we have used the SureSeq™ 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.

ATM	*ATR*	*BRCA1*	*BRCA2*	*NF1*	*PTEN*	*TP53*

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

To evaluate the application of a hybridisation-based approach we:

- Compared the uniformity of coverage between PCR-based and hybridisation-based enrichment approaches for the analysis of *BRCA1* and *BRCA2* in solid tumour samples[a].
- Identified important somatic variants in *TP53* from DNA extracted from FFPE blocks of type II epithelial ovarian cancer (EOC) samples[b].
- Assessed the performance of a 4.5 kb custom panel from the SureSeq myPanel™ NGS Custom Cancer Panel range using the formalin-compromised Quantitative Multiplex Reference Standard from Horizon Diagnostics.

Hybridisation-based enrichment generates highly uniform coverage of key targets

To confidently call low level variants, NGS reads need to be evenly distributed across all regions of interest. Uniformity of coverage is a useful value with which to compare this distribution and can be expressed as the percentage of target bases that have greater than 20% of the mean coverage. As reported extensively in the literature[2-4], the uniformity of coverage from capture-based approaches such as SureSeq consistently outperform those enriched using amplicon-based methods (Figure 2). Furthermore, in our sample set we found the high levels of uniformity are maintained when starting with ~250 ng DNA (brown bars).

The uniformity of the coverage for most samples is greater than 99% of bases covered at 20% of the mean, ensuring that all bases within the panel can be assessed.

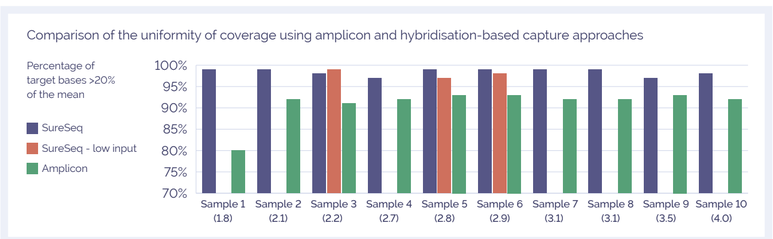


Figure 2: Assessment of the uniformity of sequencing coverage from FFPE-derived DNA using an amplicon and the SureSeq hybridisation capture-based approaches. Enrichment by SureSeq sequence capture (dark blue bars) demonstrates better uniformity than that of an amplicon-based approach (green bars). The level of uniformity is maintained when starting with ~250 ng DNA (brown bars). Samples are ordered by increasing DNA Integrity Number (DIN) determined by Agilent 2200 TapeStation - value in brackets.

Somatic variants can be confidently detected in Type II Ovarian Cancer Research samples

The superior uniformity of coverage enables reliable identification of somatic single nucleotide variants (SNVs) and indels in solid tumour samples. Figure 3 illustrates some examples of somatic deletions (panel A) and SNVs (panel B) that have been found in exon 6 of *TP53* from FFPE blocks of type II EOC samples.

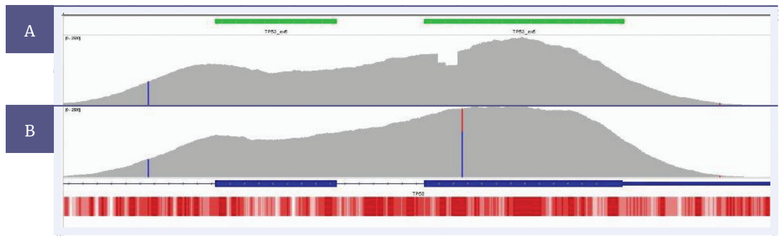


Figure 3: Sequence coverage of *TP53* exons 5 and 6 from type II EOC FFPE-derived DNA. The SureSeq hybridisation-based capture approach achieved a high depth of coverage over the GC rich exon 5 of *TP53*. This has enabled a 18 bp deletion - panel A, and a C->T SNV (R175H) - panel B to be detected at a minor allele frequency of 23.5% and 33.3%, respectively. Targeted region - green; depth of coverage per base - grey; gene coding region as defined by RefSeq - blue; GC percentage- red; visualised using Integrated Genomics Viewer[5].

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

We found pre-treatment with the SureSeq FFPE DNA Repair Mix improves the mean target coverage of formalin comprised samples (Horizon Diagnostics), thereby increasing the flexibility of the assay (Figure 4). Use of the repair mix also enables a reduced DNA input down to 50 ng whilst maintaining a good depth of coverage (Figure 5).

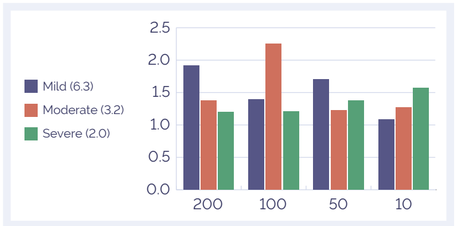
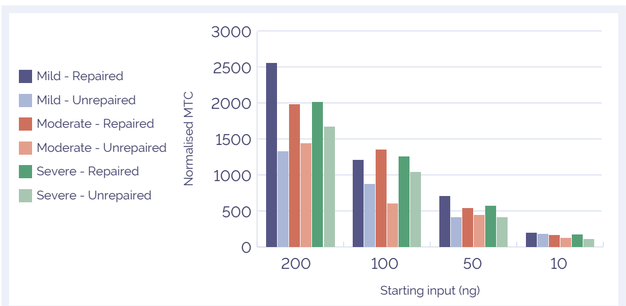


Figure 4: Improvement in mean target coverage through use of the FFPE repair mix. All three standards showed an improvement in coverage of the target bases when treated with FFPE repair mix. The mean fold increase across all sample types and input amounts was 1.5x. Samples are ordered by DNA Integrity Number (DIN) - value in brackets.

Figure 5: Effect of reduced amount of DNA input on mean target coverage. The mean target coverage decreases with reduced amount of input DNA but the FFPE repair mix treatment helps maintain a good depth of coverage.



Accurate detection of variants from reference standards

All samples had 100% concordance for 20 reported variants with 97.5% having allele frequencies within 5% of the expected value (Table 2).

Gene	Variant	Expected coverage	Mild			Moderate			Severe		
			200 ng	100 ng	50 ng	200 ng	100 ng	50 ng	200 ng	100 ng	50 ng
EGFR	T790M	1.0%	1.5%	1.0%	0.5%	0.8%	1.2%	0.5%	0.9%	1.1%	1.4%
EGFR	ΔE746-A750	2.0%	1.6%	1.3%	1.8%	1.2%	2.7%	0.6%	1.8%	1.7%	2.0%
EGFR	L858R	3.0%	3.5%	3.9%	4.0%	3.2%	3.5%	3.1%	3.0%	3.2%	3.7%
KRAS	G12D	6.0%	5.8%	6.4%	6.3%	5.3%	4.1%	6.4%	6.9%	5.3%	6.5%
MET	V237fs	6.5%	5.4%	6.2%	6.7%	6.9%	6.7%	4.4%	5.4%	6.1%	5.4%
PI3KCA	E545K	9.0%	9.9%	9.8%	9.5%	9.2%	8.2%	9.4%	8.0%	6.9%	9.6%
cKIT	D816V	10.0%	8.8%	11.4%	10.6%	9.1%	9.4%	9.7%	9.1%	7.7%	9.5%
IDH1	S261L	10.0%	7.3%	8.4%	7.6%	7.5%	9.6%	8.5%	8.1%	7.6%	8.3%
BRAF	V600E	10.5%	12.0%	12.9%	11.3%	11.0%	11.1%	8.2%	11.7%	12.3%	9.9%
FLT3	S985fs	10.5%	7.1%	9.0%	8.1%	7.8%	8.0%	8.4%	7.7%	7.7%	7.9%
FLT3	V197A	11.5%	8.5%	7.8%	7.1%	8.9%	8.4%	9.4%	7.8%	7.7%	8.5%
NRAS	Q61K	12.5%	13.6%	15.8%	14.7%	11.2%	12.6%	14.5%	13.2%	13.2%	14.1%
KRAS	G13D	15.0%	14.5%	14.1%	16.4%	14.7%	14.4%	12.9%	12.8%	13.2%	13.7%
PI3KCA	H1047R	17.5%	16.6%	16.1%	17.1%	18.7%	17.8%	21.0%	18.9%	17.5%	16.4%
EGFR	G719S	24.5%	26.3%	25.9%	26.0%	24.2%	26.1%	25.8%	24.9%	24.9%	26.4%
NOTCH 1	P668S	31.5%	28.5%	28.8%	25.8%	25.6%	28.9%	26.8%	28.3%	30.0%	27.0%
ALK	P1543S	33.0%	31.7%	30.9%	30.3%	29.1%	29.1%	31.6%	30.3%	32.8%	32.7%
APC	R2714C	33.0%	32.6%	30.7%	31.3%	31.2%	30.2%	30.6%	30.3%	30.0%	26.8%
BRCA2	A1689fs	33.0%	33.0%	31.4%	34.3%	31.0%	29.6%	26.7%	33.9%	31.3%	34.9%
FBXW7	G667fs	33.5%	26.5%	28.4%	28.5%	29.4%	29.1%	28.9%	29.8%	29.9%	30.6%

Table 2: Difference between the expected and observed allele frequency in a characterised sample. The variants - 15 SNVs and 5 deletions, ranging from 1% to 33.5% minor allele frequency, were determined using OGT's Interpret software.

*The SureSeq FFPE DNA Repair Mix can only be purchased in conjunction with SureSeq NGS panels, not as a standalone product.

Conclusions

We have shown that the use of SureSeq hybridisation based panels with the SureSeq FFPE repair mix provides:

- Superior uniformity of coverage than a PCR enrichment approach and these levels of uniformity are maintained across a range of starting DNA (FFPE derived) input amounts.
- Enhanced sequencing metrics (mean target coverage) allowing greater confidence in calling low allele frequency variants.
- Very high concordance (100%) for variant detection in formalin compromised samples.
- Accurate detection of low allele frequency variants (< 5% MAF) when using small amounts of DNA (50ng).
- Robust and accurate detection of somatic variants in FFPE derived samples.

References

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Further information **contact@ogt.com | ogt.com | Oxford Gene Technology Ltd., Begbroke Science Park, Woodstock Road, Begbroke, Oxfordshire, OX5 1PF, UK. | SureSeq**: For Research Use Only; Not for Diagnostic Procedures.

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