The application of a hybridisation-based next-generation sequencing enrichment panel for the analysis of somatic variants in tumour samples and reference standards

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Introduction

• Breast and Ovarian cancers are some of the most common cancers in women.
• Next-generation sequencing (NGS) has enabled the simultaneous study of mutations in high-penetrance breast cancer predisposition genes.
• These include BRCA1, BRCA2, TP53, PTEN, and PIK3CA, as well as more moderate risk genes such as PALB2, ATM, and MSH6.

Using OGT’s extensive background in assay design, we have developed a range of fully tested and optimised baits targeting all coding exons of a range of key cancer-related genes (Table 1).

SureSeq hybridisation workflow

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

Use of target-capture allows the removal of PCR duplicates which can obscure the minor alleles present within a sample.

Hybridisation-based enrichment generates highly uniform coverage of key targets

To confidently call low level variants, NGS read-depths 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, the uniformity of coverage from captured-based approaches such as SureSeq consistently outperforms those enriched using an amplicon method (Figure 2).

Furthermore, in our sample set, we found the high levels of uniformity are maintained when starting with ~250 ng DNA (light blue bars). The uniformity of 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 confidently assessed.

Uniformity of coverage using amplicon and hybridisation capture-based approaches

The superior uniformity of coverage of key exons in BRCA1 and BRCA2 from Sample 6 with SureSeq compared to an amplicon-based panel is shown in Figure 3. Regions of low complexity such as repetitive sequence and high/low GC content often appear skewed in NGS data. However, as a hybridisation capture-based approach will also provide data from the flanking regions adjacent to the target the coverage profile is “smoother” than seen in panel B. This enables reliable identification of somatic single nucleotide variants (SNVs) and indels in solid tumour samples.

Accurate detection of variants from reference standards

The high mean target coverage and uniformity of coverage was maintained across the two sample types and different starting amounts of DNA (Table 2) which ranged from 1000 to 45 ng of DNA.

References