SureSeq

A Sysmex Group Company

NGS Custom Ovarian Cancer panel

Features

Contains the latest evidence-based genes involved in ovarian cancer research

- Gain insight into homologous repair deficiencies and cell cycle dysregulation

Validated for research on FFPE and whole blood

- Detect germline mutations in DNA derived from blood as well as both germline and somatic mutations in DNA derived from FFPE tissue

Fast and easy workflow

- Streamlined library preparation, short 4-hour hybridisation and intuitive software

Excellent uniformity of coverage across the whole panel

- Detect low-frequency variants with confidence, even in heterogeneous cancer samples

SureSeq

NGS Custom Ovarian Cancer panel

Introduction

A hybridisation-based NGS enrichment panel with complimentary Interpret NGS Analysis Software that delivers accurate and easy identification of variants. Validated for research use on FFPE samples and whole blood, this panel covers all coding exons of seven key genes and allows the analysis of variants associated with ovarian cancer and research into therapeutic response.

Contains the latest evidence-based genes involved in ovarian cancer research

Ovarian cancer is the leading cause of death from gynaecological cancers in the Western world[1]. Next generation Sequencing (NGS) is quickly becoming a commonly used tool for analysis of mutations — both single nucleotide variants (SNVs) and indels — in genes associated with ovarian cancer.

The SureSeq™ Ovarian Cancer Panel has been developed with leading cancer experts and covers all coding exons of seven genes (Table 1). The panel allows detection of known and novel variants in tumour suppressor genes as well as genes involved in homologous repair to advance research into ovarian cancer treatment and for use in clinical trials to help the development of new targeted therapies.

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

Table 1: The SureSeq Ovarian Cancer Panel targets seven genes implicated in ovarian cancer.

Validated on FFPE and whole blood

Mutations in certain genes can predispose an individual to develop cancer at some point during their lifetime. Screening for germline mutations in such genes allows research into familial risk of developing breast and ovarian cancer. On the other hand, assessment of somatic mutations in tumour samples can help research into drug response and the development of new therapies.

Various new generation drugs are being trialled to replace classical chemotherapy with the promise of improved efficacy and reduced side effects. One approach to select the right patients for clinical trials may be to test for somatic mutations within the tumour.

The SureSeq Ovarian Cancer Panel has been validated on DNA derived from FFPE tissue and whole blood to allow investigation of both germline and somatic mutations in ovarian cancer research.

Hybridisation-based enrichment

Heterogeneous cancer samples pose significant challenges as alleles are likely to be present at a lower fraction than would be expected for standard germline variants. Samples typically contain a mixture of cancer and normal cells, moreover cancer can consist of several molecularly distinct clones. In order to detect alleles that contribute only a small percentage to the reads at any locus, a highly uniform and sensitive enrichment is required. Utilising hybridisationbased enrichment, the SureSeq Ovarian Cancer Panel delivers excellent run-to-run consistency and extremely uniform coverage across the whole region of interest to allow sensitive detection of variants present even at low variant allele fraction (VAF) (Table 2).

Gene	Variant detected	Type of variant	Mean target coverage	% VAF detected
BRCA1	c.3424G>C (p.Ala1142Pro)	SNV	881	11.92%
BRCA2	c.556G>C (p.Ala186Pro)	SNV	728	1.92%
TP53	c.1129A>C (p.Thr377Pro)	SNV	1024	3.03%
ATR	c.7274G>A (p.Arg2425Gln)	SNV	1579	38.63%
NF1	c.8137_8138insG (p.Phe2714ValfsTer16)	Insertion	683	1.45%
NF1	c.3354delT (p.Ser1118ArgfsTer24)	Deletion	621	1.13%
ATR	c. 4154delC (p.Thr1385MetfsTer3)	Deletion	506	1.61%

Table 2: Example mutations detected in FFPE clinical research samples using the SureSeq Ovarian Cancer Panel. The ability to detect VAFs as low as 1.13% gives added confidence in the variants being called and facilitates the exploration of tumour heterogeneity. Rows 1–4: low-frequency SNVs; rows 5–7: low-frequency indels. Samples kindly provided by Biopathology Department of Gustave Roussy, Villejuif, France.

Fast and easy workflow

Hybridisation-based enrichment is now well recognised as providing superior results over amplicon-based enrichment technology. To date, the protocol has required more DNA and the library preparation protocol has been longer and more complex. In combination with the OGT SureSeq Library Preparation Kit, these issues have been addressed. There are fewer hands-on steps, turnaround times have been significantly improved and the panel has been optimised to work with as little as 500 ng of DNA derived from FFPE* or whole blood.

Interpret — OGT's powerful, standalone NGS data analysis package — is complimentary with the SureSeq Ovarian Cancer Panel and allows the conversion of FASTQ NGS files into an intuitive interactive report. The user friendly report allows for easy filtering of the variants without the need for additional in-house bioinformatics resource.

*For samples passing QC citeria.

Gene	Chr	Start	End	Alt	Ref	HGVSc (Gene Symbol)	Zygosity	Total Depth	Ref Depth	Alt Depth	Allele Frequency	Genotype	Type
ATR	3	142178144	142178144	C	T	BRCA1:c.1067A>G	Heterozygous	530	273	275	48.49%	0/1	snp
ATR	11	108183167	108183167	A	G	BRCA1:c.1067A>G	Heterozygous	546	0	546	100%	1/1	snp
BRCA2	13	32907420	32907421	GA	G	BRCA1:c.1067A>G	Heterozygous	502	269	229	45.98%	0/1	del
BRCA2	13	32911888	32911888	A	G	BRCA1:c.1067A>G	Heterozygous	588	261	327	55.61%	0/1	snp
BRCA2	13	32913055	32913055	A	G	BRCA1:c.1067A>G	Heterozygous	574	1	572	100%	1/1	snp
BRCA2	13	32915005	32915005	G	C	BRCA1:c.1067A>G	Heterozygous	593	0	593	100%	1/1	snp
BRCA2	13	32929387	32929387	T	C	BRCA1:c.1067A>G	Heterozygous	616	599	17	2.76%	0/0	snp
BRCA2	13	41246481	41246481	T	C	BRCA1:c.1067A>G	Heterozygous	567	0	565	100%	1/1	snp
TP53	17	7579472	7579472	G	C	BRCA1:c.1067A>G	Heterozygous	514	0	514	100%	1/1	snp
NF1	17	29705947	29705947	T	C	BRCA1:c.1067A>G	Heterozygous	534	19	515	96.44%	1/1	snp
BRCA1	17	41246481	41246481	T	C	BRCA1:c.1067A>G	Heterozygous	542	287	254	46.95%	0/1	snp

Figure 1: An example batch analysis report in Interpret, enabling simple and rapid identification of result. Let OGT customise your report to meet your exact requirements.

Excellent uniformity of coverage across the whole panel

Enrichment assay optimisation is a crucial step in ensuring accuracy and sensitivity of targeted sequencing. Where regions are poorly enriched, they will generate fewer sequencing reads. If a variant falls into a region not covered at all, or covered by only a few reads, that variant is likely to be missed. OGT's expert bait design ensures efficient and more uniform capture of all targeted regions, so that all variants present can be called with maximum confidence (Figure 2). Uniform enrichment also allows proportionately lower sequencing depth to be used to identify lowfrequency variants, potentially lowering sequencing costs and increasing sample throughput.

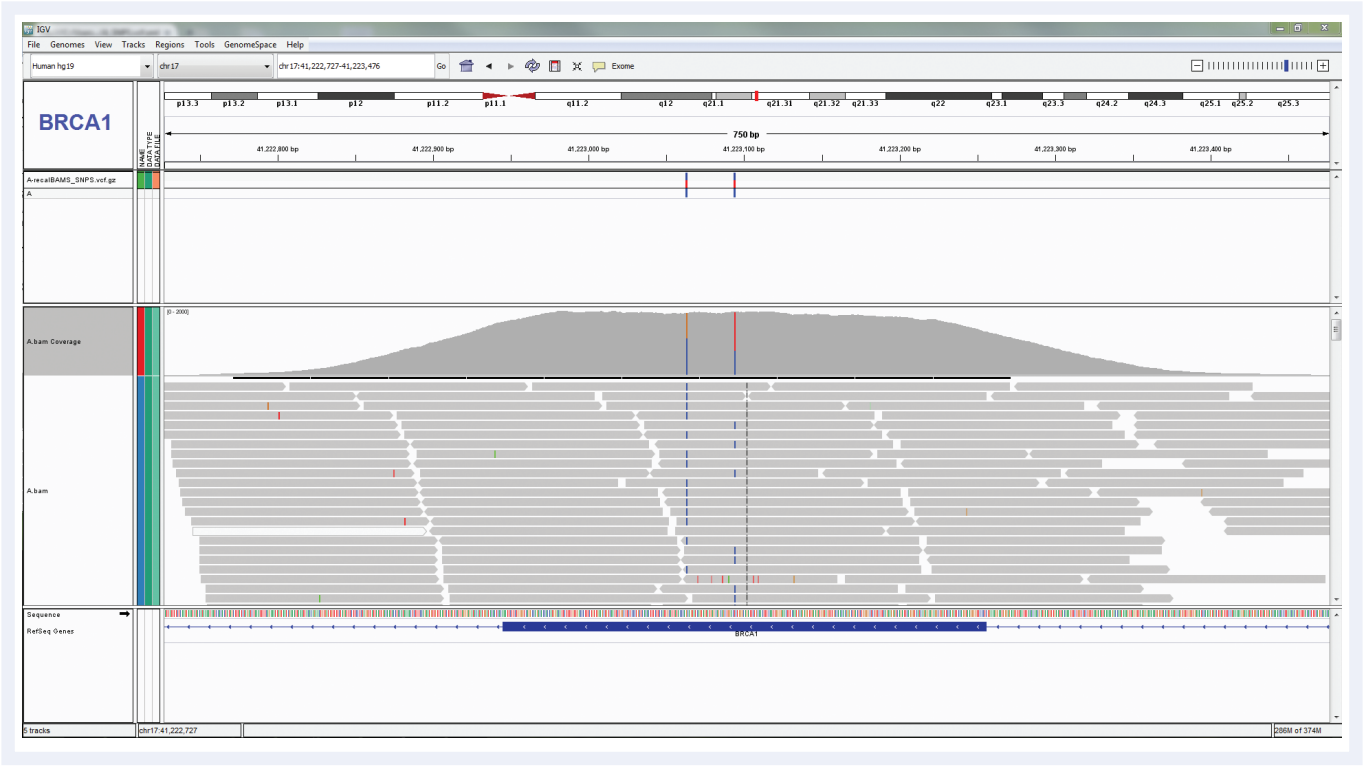

Figure 2: *BRCA1* Exon 15 (NM_007299) visualised in IGV from the Broad Institute. The whole region is uniformly covered ensuring detection of all — even rare variants — with confidence. The mean target coverage for the exon is 1744x.

Ordering information

UK +44 (0) 1865 856800

US +1 914 467 5285

contact@ogt.com

ogt.com

Product	Contents	Cat. No.
SureSeq Ovarian Cancer Panel (16 reactions)	Enrichment baits sufficient for 16 samples; Interpret Software	600073
SureSeq Ovarian Cancer Panel (96 reactions)	Enrichment baits sufficient for 96 samples; Interpret Software	600074
SureSeq NGS Library Preparation Complete Solution (16)	Bundle of 1x SureSeq NGS Library Preparation Kit (16), containing adaptors, PCR primers and enzymes, 1x SureSeq NGS Index Kit – Collection A, 1x SureSeq NGS Hyb & Wash Kit (16), 1x Dynabeads M270 Streptavidin (2ml) and 1x AMPure XP beads (10ml). Sufficient for 16 samples	500084
SureSeq NGS Library Preparation Complete Solution (48)	Bundle of 1x SureSeq NGS Library Preparation Kit (48), containing adaptors, PCR primers and enzymes, 1x SureSeq NGS Index Kit – Collection B, 3x SureSeq NGS Hyb & Wash Kit (16), 3x Dynabeads M270 Streptavidin (2ml) and 3x AMPure XP beads (10ml). Sufficient for 48 samples	500085

References

1. Helleman J. *et al.*, (2006) Molecular profiling of platinum resistant ovarian cancer. Int J Cancer 118(8):1963-71.

A Sysmex Group Company

What binds us, makes us.

Oxford Gene Technology Ltd., Begbroke Science Park, Woodstock Road, Begbroke, Oxfordshire, OX5 1PF, UK