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The Oncomine™ BRCA Expanded next-generation sequencing assay: Development and analytical validation of a new panel for detection of SNV, insertions, deletions and copy number variants in a panel of 15 genes involved in homologous recombination repair of double-strand break DNA damage

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INTRODUCTION

Breast cancer is the second most commonly diagnosed cancer (11.6% of total cases) world wide[1]. Many tumors display phenotypic similarity across tissue types, characterized by defects in biological pathways leading to susceptibility to particular therapies[2]. Deficiencies in one such pathway, homologous recombination DNA damage repair (HR DDR), affect the response of breast and ovarian cancers to agents that inhibit PARP function.

The Oncomine™ BRCA Expanded panel targets 15 genes (BRCA1 and 2, ATM, BARD1, BRIP1, CDK12, CHEK2, FANCD2, MRE11, NBN, PALB2, PPP2R2A, RAD51B, RAD54L, and TP53) that play various roles in the HR DDR pathway. The assay includes 1011 amplicons covering both hotspot variants and coding regions of these genes. An additional set of 255 pre-qualified genes is available for assay customization, including a recommended set of 25 genes of particular relevance to HR DDR pathway.

Small variants (SNV and INDEL) and large rearrangements (LRs) such as copy number variations (CNV) in these genes can be detected with next-generation sequencing (NGS). This panel is designed for FFPE samples and can also be used with samples having non-degraded DNA. Germline and somatic mutations, such as SNPs, Indels, Copy number gains, can be detected for all genes, and exon deletions can be detected for *BRCA1* and *BRCA2*.

MATERIALS AND METHODS

The panel uses Ion AmpliSeq technology and is run on the Ion S5 Gene Studio™ instrument. Automated variant calling workflow is provided within Ion Reporter™. AcroMetrix Oncology Hotspot Control (AOHC) samples are used for verification of small variants calling. Samples with known gene copy number gains are used for CNV calling verification.

RESULTS

Genes in Oncomine™ BRCA Expanded panel

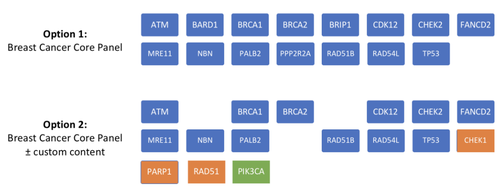
Table 1. 15 gene panel

Gene	Num_Amps	Coverage
ATM	194	99.6
BARD1	42	100
BRCA1	113	100
BRCA2	152	100
BRIP1	72	99.9
CDK12	67	100
CHEK2	29	99.3
FANCD2	81	98.4
MRE11	42	100
NBN	38	100
PALB2	60	100
PPP2R2A	26	100
RAD51B	23	100
RAD54L	39	100
TP53	23	100

Table 2. 25 expanded genes

Gene	Num_Amps	Coverage
CHEK1	26	100
EPCAM	27	96.6
FANCB	35	100
FANCC	34	100
FANCE	26	99.2
FANCF	11	100
FANCG	30	100
FANCI	72	100
FANCL	27	100
FANCM	84	100
GEN1	37	99.4
MLH3	56	100
PARP1	54	100
PMS1	37	98.7
PRKDC	204	99.6
RAD50	63	99.5
RAD51	20	93.8
RAD51C	23	100
RAD51D	20	100
RAD52	26	100
RNASEH2A	14	100
RNASEH2B	22	100
RNASEH2C	10	98.9
TP53BP1	84	99.9
XRCC2	11	100

Figure 1. Customized panel can include any of 270 genes including expanded genes, and can remove genes in the core panel



General performance evaluation

Figure 2. Amplicon design and sequencing results

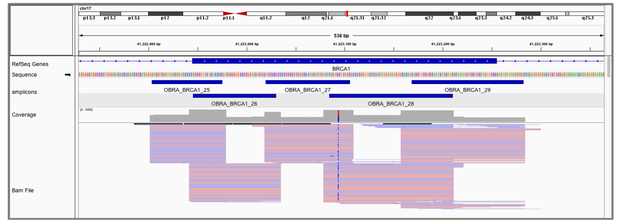
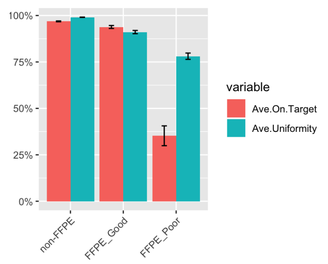
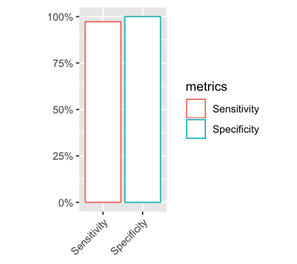


Figure 3. On target and uniformity



Average percent readds on target and uniformity of amplicon coverage of Oncomine™ BRCA Expanded panel on non-FFPE, good-quality FFPE and poor-quality FFPE samples.

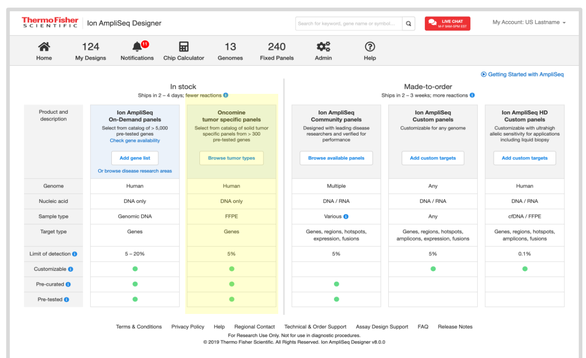
Figure 4. Sensitivity and specificity in variants detection



Sensitivity and specificity of Oncomine™ BRCA Expanded panel based on 144 variants detection results using AcroMetrix Oncology Hotspot Control (AOHC) samples.

Panel Selection on AmpliSeq.com

Figure 5. Oncomine™ tumor specific panels on Ampliseq.com



Variant calling examples

Figure 6. A gene with known copy number gains is being detected

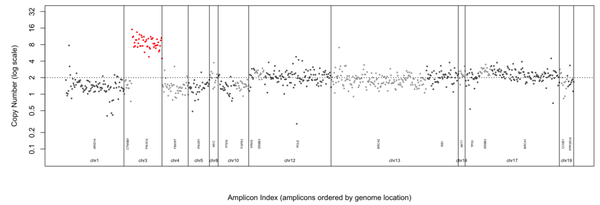
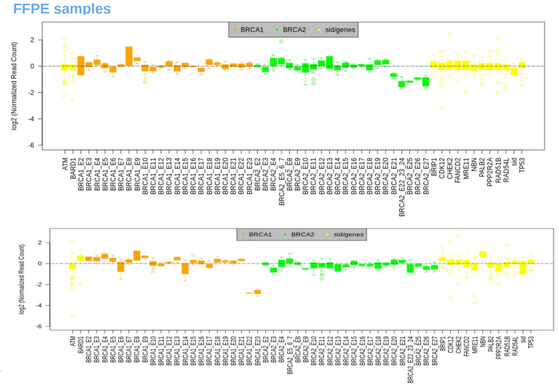


Table 3. 32 out of 33 SNVs in TP53 are being detected using AOHC samples

Locus	COSMIC ID	Genotype	Ref	Function	Protein Change	Coverage	Alt Allele Frequency	Detected
chr17:7572986	COSM307348	G/A	G	nonsense	p.Gln375Ter	1498	9.95	Yes
chr17:7573010	COSM1191161	T/C	T	unknown	NA	1492	10.19	Yes
chr17:7574003	COSM11073	G/A	G	nonsense	p.Arg342Ter	2000	3.2	No
chr17:7574012	COSM11286	C/A	C	nonsense	p.Glu339Ter	1999	6.25	Yes
chr17:7574013	COSM11071	G/A	G	missense	p.Arg337Cys	1999	6.35	Yes
chr17:7574026	COSM11514	C/A	C	missense	p.Gly334Val	1998	6.31	Yes
chr17:7576855	COSM11354	G/A	G	nonsense	p.Gln331Ter	1997	6.31	Yes
chr17:7576865	COSM44823	A/C	A	nonsense	p.Tyr327Ter	2000	6.5	Yes
chr17:7576883	COSM46088	T/C	T	synonymous	p.Lys321=	2000	6.4	Yes
chr17:7576897	COSM10786	G/A	G	nonsense	p.Gln317Ter	1996	6.71	Yes
chr17:7577022	COSM10663	G/A	G	nonsense	p.Arg306Ter	1717	6.99	Yes
chr17:7577046	COSM10710	C/A	C	nonsense	p.Glu298Ter	1720	6.86	Yes
chr17:7577105	COSM10863	G/A	G	missense	p.Pro278Leu	2000	6.55	Yes
chr17:7577120	COSM10660	C/T	C	missense	p.Arg273His	2000	5.95	Yes
chr17:7577538	COSM10662	C/T	C	missense	p.Arg248Gln	2000	5.25	Yes
chr17:7577548	COSM6932	C/T	C	missense	p.Gly245Ser	2000	7.4	Yes
chr17:7577559	COSM10812	G/A	G	missense	p.Ser241Phe	2000	6.9	Yes
chr17:7577580	COSM10725	T/C	T	missense	p.Tyr234Cys	2000	6.6	Yes
chr17:7578190	COSM10758	T/C	T	missense	p.Tyr220Cys	2000	6.2	Yes
chr17:7578196	COSM44317	A/T	A	missense	p.Val218Glu	1997	6.11	Yes
chr17:7578203	COSM10667	C/T	C	missense	p.Val216Met	2000	6.25	Yes
chr17:7578235	COSM43947	T/C	T	missense	p.Tyr205Cys	1998	6.06	Yes
chr17:7578388	COSM10738	C/T	T	missense	p.Arg181His	1942	7.21	Yes
chr17:7579295	COSM44985	C/T	C	unknown	NA	2000	5.65	Yes
chr17:7579312	COSM43904	C/T	C	synonymous	p.Thr125=	2000	5.4	Yes
chr17:7579358	COSM10716	C/A	C	missense	p.Arg110Leu	2000	6.05	Yes
chr17:7579368	COSM46103	A/C	A	missense	p.Tyr107Asp	1999	6.15	Yes
chr17:7579472	COSM250061	G/C	G	missense	p.Pro72Arg	1999	94.2	Yes
chr17:7579521	COSM12168	C/A	C	nonsense	p.Glu56Ter	2000	5.95	Yes
chr17:7579536	COSM44907	C/A	C	nonsense	p.Glu51Ter	1998	6.01	Yes
chr17:7579553	COSM43664	A/G	A	missense	p.Leu45Pro	1999	5.7	Yes
chr17:7579575	COSM46286	G/A	G	nonsense	p.Gln38Ter	1996	5.96	Yes
chr17:7579801	NA	G/C	G	unknown	NA	1991	88.5	Yes

Figure 7. Oncomine™ tumor specific panel exon deletions detected in germline and FFPE samples



Additional Oncomine™ tumor-specific panels

Figure 8. List of additional Additional Oncomine™ tumor-specific panels

- Ion Torrent™ Oncomine™ Bladder Panel
- Ion Torrent™ Oncomine™ Kidney Panel
- Ion Torrent™ Oncomine™ *BRCA* Expanded Panel
- Ion Torrent™ Oncomine™ Liver Panel
- Ion Torrent™ Oncomine™ Colorectal and Pancreatic Panel
- Ion Torrent™ Oncomine™ Lymphoma Panel
- Ion Torrent™ Oncomine™ Gastric and Esophageal Panel
- Ion Torrent™ Oncomine™ Melanoma Panel
- Ion Torrent™ Oncomine™ Gynecological Panel
- Ion Torrent™ Oncomine™ Prostate Panel

HIGHLIGHTS

- Customizable panel with verified performance for clinical research
- Low sample input requirement (20 ng input DNA)
- Works with FFPE tissue samples
- End-to-end workflow including bioinformatics and reporting solutions
- Quick turnaround time (2 days from DNA to data)

CONCLUSIONS

An NGS assay with a comprehensive data analysis approach was developed that is capable of detecting both small mutations and LRs simultaneously in FFPE samples with high sensitivity. This is an important assay for *BRCA1/2* and HR DDR translational research.

REFERENCES

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2. Heeke, A. L., Pishvaian, M. J., Lynce, F., Xiu, J., Brody, J. R., Chen, W. J., … Isaacs, C. (2018). Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types. JCO precision oncology, 2018, 10.1200/PO.17.00286. doi:10.1200/PO.17.00286

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