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A Multi-Center Assessment of a Next-Generation Sequencing Assay for Detection of Germline and Somatic *BRCA1* and *BRCA2* Gene Variants from Formalin-Fixed, Paraffin-Embedded Samples

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ABSTRACT

We designed a new, highly multiplexed amplification-based assay which allows detection of *BRCA1* and *BRCA2* gene variants in somatic and germline samples through semiconductor-based DNA sequencing. The current study summarizes the findings of over 22 research centers situated in 12 countries that were given early access to this new assay for analyses of their samples. The purpose was to evaluate the performance of the assay on a variety of sample types, including formalin-fixed, paraffin-embedded (FFPE) samples, under realistic conditions.

BRCA gene target regions, including all coding exons, were enriched via highly multiplexed amplification reactions. Libraries were prepared by using manual and automated preparation methods, followed by semiconductor-based sequencing on multiple instrument platforms. Single-nucleotide and insertion or deletion (indel) variants were identified using available software solutions. Participants reported the concordance between the variants detected by the new assay and expected variants, identified by orthologous techniques.

A high degree of coverage uniformity, 98%, was achieved on both germline and somatic samples. Sensitivity of variant detection was 97.8%, with positive predicted value of 94.3%. Performance of the new *BRCA* sequencing assay in this collaborative, early-access study indicates that the panel, planned for official release soon, will help advance *BRCA* gene research.

Figure 1. The Ion Torrent™ Oncomine™ *BRCA* Research Assay workflow leverages the proven performance of Ion AmpliSeq™ chemistry to enable detection of germline and somatic variants from blood and FFPE DNA samples. We demonstrated high sensitivity and specificity for variants at frequency ≥5% with only 10–20 ng input DNA.

RESULTS

Early-access data

We distributed the early-access version of the new *BRCA* research assay to 20 laboratories across 12 European countries. Twenty participants provided significant feedback, including access to data and variant calling results on a diversity of samples, using multiple platforms and multiple realistic experimental designs. Self-reporting of concordance between expected and observed variant calls was provided to field service representatives and collected into the results summarized below.

Table 1. Sample and variant summary from 22 participating sites.

Participating laboratories	Samples	Variants	City, country
Institute of Pathology, Medical University of Graz	8	7	Graz, Austria
OncoDNA IPG/BIO	16	15	Gosselies, Belgium
Department of Pathology, Herlev Hospital	NA	NA	Copenhagen, Denmark
Génétique Moléculaire, HUPC COCHIN, AP-HP	30	6	Paris, France
Institute of Pathology, MHH	15	15	Hannover, Germany
Molekularpathologie Trier (MPT)	8	17	Trier, Germany
Institute of Pathology, Charité Universitaetsmedizin	47	286	Berlin, Germany
GENOPATH	16	10	Bonn, Germany
GeneKor Medical S.A	94	426	Gerakas, Greece
Laboratorio di Anatomia Patologica Frederico II	6	25	Napoli, Italy
Instituto Europeo di Oncologia, Laboratorio di Anatomia Patologica	25	NA	Milan, Italy
Department of Internal Medicine, IRCCS Azienda Ospedaliera Universitaria San Martino - IST	32	NA	Genova, Italy
IRCCS Istituto Tumori "Giovanni Paolo II", Bari-Molecular Genetics Laboratory	8	NA	Bari, Italy
LUMC Department of Pathology	12	6	Leiden, Netherlands
Erasmus MC Pathology	14	8	Rotterdam, Netherlands
UMC Utrecht Pathology	24	NA	Utrecht, Netherlands
Ipatimup	8	NA	Porto, Portugal
Laboratorio de Dianas Terapéuticas, Hospital Universitario HM Sanchinarro	8	4	Madrid, Spain
Fundación Pública Galega de Medicina Xenómica	7	96	Santiago de Compostela, Spain
Pathology Department Hospital del Mar	16	NA	Barcelona, Spain
Sahlgrenska	NA	NA	Gothenburg, Sweden
Genoma SA	15	202	Geneva, Switzerland

Table 2. Summary of reported results of early-access *BRCA* research assay.

Site	Total variants	True positives	False negatives	Sensitivity (%)
Totals (15 sites)	1,131	1,107	24	97.8

Table 3. Sensitivity and specificity with full gene-sequencing data to establish known facts.

Site	Total variants	True positives	False positives	Sensitivity (%)	Positive predictive value (PPV), (%)
Totals (4 sites)	1,013	1,007	61	99.2	94.3

Assay design

As shown by the performance of the *BRCA* research assay at early-access sites, the initial prototype versions had good performance in detecting relevant single nucleotide variants (SNVs) and indels in the *BRCA* genes. The invaluable feedback from the early-access sites allowed us to further refine both the Ion AmpliSeq™ assay design and the Ion Reporter™ version 5.0 software algorithms for analysis. The new *BRCA* research assay covers 100% of the coding sequences of *BRCA1* and *BRCA2* genes, including all coding splice site and acceptor sites, with an average of 64 bp extension into adjoining introns. The assay is a 2-pool Ion AmpliSeq™ panel design containing 265 amplicons and is compatible with DNA samples extracted from FFPE as well as blood samples, and also automated and manual library preparation methods. Due to the enhanced assay performance on FFPE samples, variant calling was optimized for ≥5% allele frequency.

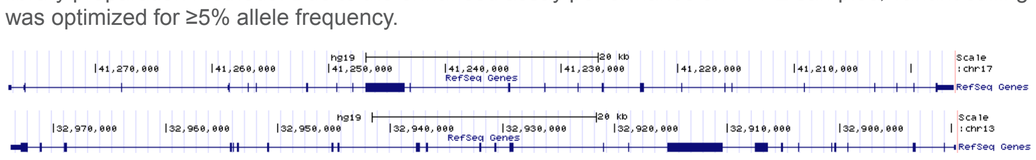


Figure 2. Genomic maps of *BRCA1/2*. All coding sequences were 100% covered by Ion AmpliSeq™ amplicons.

Verification data

Implementing the refinements based on the early-access data, we generated the new Oncomine *BRCA* Research Assay and verified its performance on blood, different cell lines, and FFPE samples.

1. High uniformity of base and amplicon coverage was demonstrated on FFPE tumor samples (Figure 3).
2. Mixtures of cell line DNAs containing known variants were used to measure variant calling of SNVs and indels present at 5% allele frequency. Both cell line and blood samples were used to measure germline SNVs and indels present at 50% and 100% allele frequency (Table 4).
3. Examples of detection of relevant indels in FFPE samples (Figure 4).

Oncomine *BRCA* assay performance

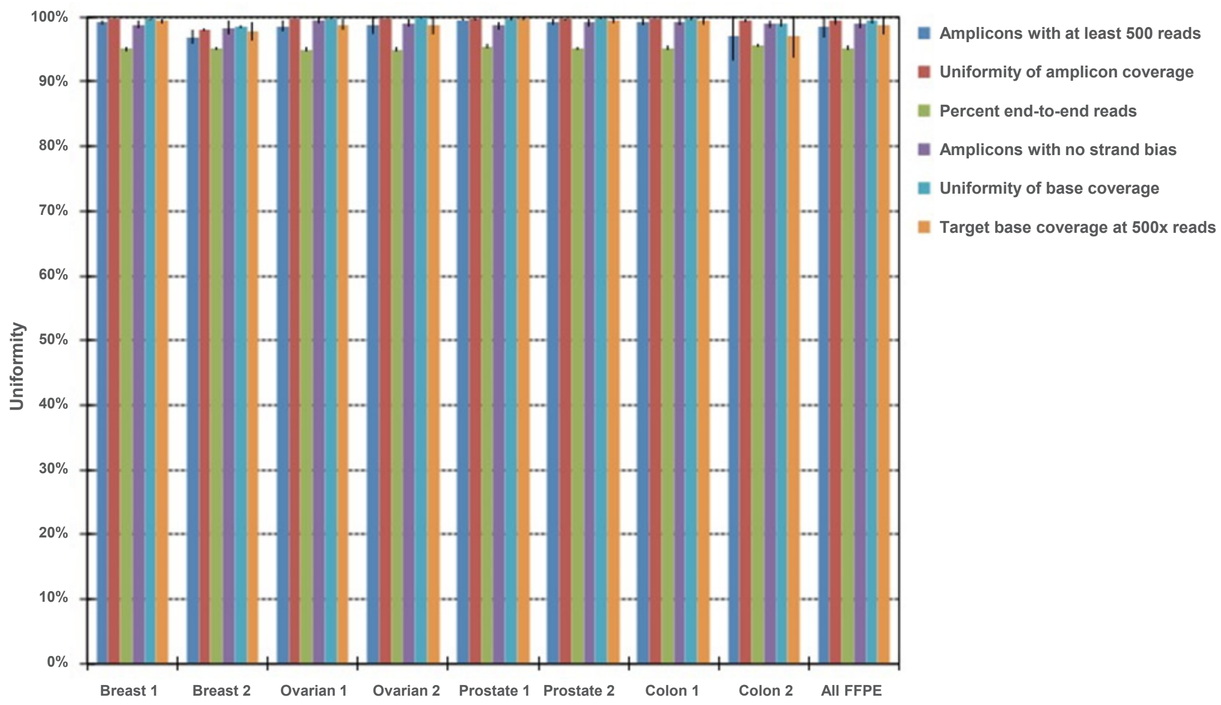
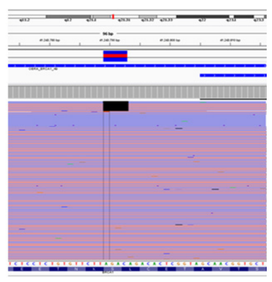


Figure 3. Summary of assay uniformity obtained with FFPE tumor specimens. Template and automated libraries (64 libraries) were prepared on the Ion Chef System, and sequenced on Ion PGM Hi-Q™ and Ion S5™ Systems with the Ion 530™ Chip Kit.

Table 4. Summary of assay verification results using the Oncomine *BRCA* Research Assay. At 5% allele frequency, >1,000 SNV and >600 indel variants measured. At 50% and 100% allele frequency, >4,000 SNV and >200 indel variants measured.

gDNA variants	Platform	Library	Template kit	SNV		Indel	
				Sensitivity (%)	PPV (%)	Sensitivity (%)	PPV (%)
5% allele frequency	PGM 318	Manual	OT2	100	100	99	99
		Chef	Chef	100	99	99	98
	S5 530	Manual	Chef	100	98	98	92
		Chef	Chef	100	92	99	99
50% and 100% allele frequency	PGM 318	Manual	OT2	100	100	100	100
		Chef	Chef	100	100	100	99
	S5 530	Manual	Chef	100	100	100	100
		Chef	Chef	100	100	100	100

***BRCA1* chr17:41243378_DelGTCT**



***BRCA2* chr13:32913838_DelA**

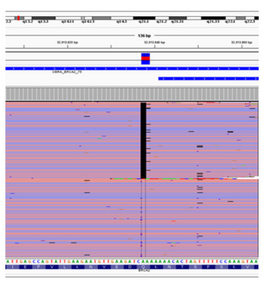


Figure 4. Examples of detection of relevant indel variants using the Oncomine *BRCA* Research Assay.

CONCLUSIONS

The excellent measured performance of the early-access version of the Oncomine *BRCA* Research Assay, revealed in this multi-center evaluation, allowed us to further verify the utility of this assay on a variety of sample types and other parameters of variants. This Research Use Only product is scheduled for commercial release in October 2016, and its combination of flexibility of use and accuracy of performance promises to significantly advance *BRCA* gene research.

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