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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 20 research centers situated in 12 countries that were given early access to the 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 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 the panel, planned for official release soon, will help advance BRCA gene research.

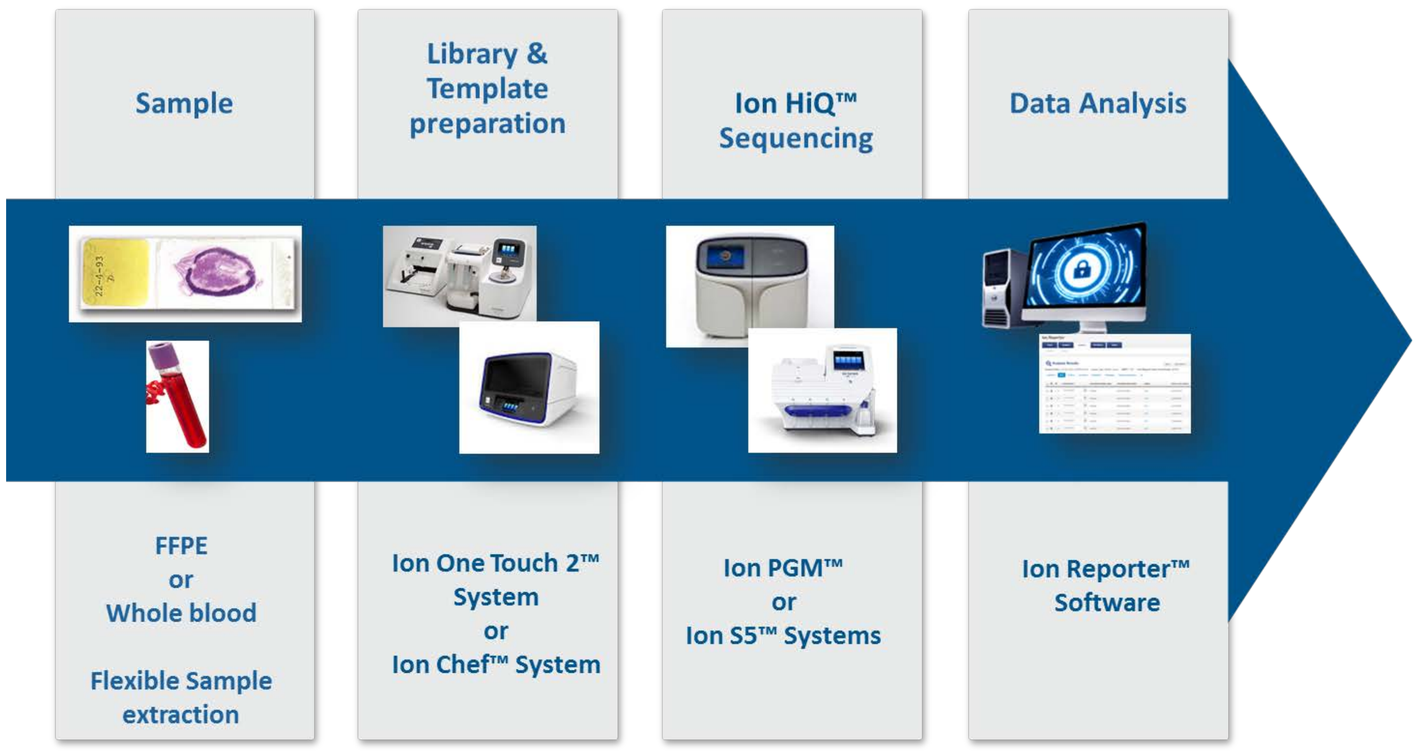


Figure 1. The Oncomine® BRCA Research Assay workflow leverages the proven performance of Ion AmpliSeq™ chemistry to enable detection of germline and somatic variants from blood and FFPE DNA samples. We demonstrated high sensitivity/specificity for variants at frequency ≥ 5% with only 10-20 ng input DNA.

RESULTS

Early Access Data

We distributed the early access version of the new BRCA research assay to 22 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.

Participating Laboratories	Samples	Variants	City, Country
University Medical School	8	7	Graz, Austria
OncoDNA IPG/BIO	16	15	Gosselies, Belgium
Herlev	na	na	Copenhagen, Denmark
Hospital Cochin	30	6	Paris, France
Institute of Pathology, MHH	15	15	Hannover, Germany
Molekularpathologie	8	17	Trier, Germany
Institut of Pathology, Charite Universitaetsmedizin	47	286	Berlin, Germany
GENOPATH	16	10	Bonn, Germany
Genekor	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
IRCCS Azienda Ospedaliero Universitaria	32	na	Genova, Italy
San Martino – Departimento di Medicina, Interna e Specialita Mediche, IRCCS	8	na	Bari, Italy
LUMC	12	6	Leiden, Netherlands
Erasmus MC Pathology	14	8	Rotterdam, Netherlands
Pathology UMC	24	na	Utrecht, Netherlands
Ipatimup	8	na	Porto, Portugal
Laboratorio de Dianas Terapeuticas	8	4	Madrid, Spain
Fundacion Xenomica de Santiago	7	96	Santiago de Compostela, Spain
Hospital del Mar	16	na	Barcelona, Spain
Sahlgrenska	na	na	Gothenburg, Sweden
Genoma SA	15	202	Geneva, Switzerland
Total			

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

Site	Total Variants	True Positives	False Negatives	Sensitivity
Totals (15 Sites)	1131	1107	24	97.8

Table 2. Summary of reported results of Early Access BRCA Assay.

Site	Total Variants	True Positives	False Positives	Sensitivity	Positive Predictive Value
Totals (4 Sites)	1013	1007	61	99.2	94.3

Table 3. Assay Sensitivity and Specificity from 4 Early Access Sites with full gene sequencing data to establish known truth.

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 and insertions/deletions 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 Oncomine® BRCA Research Assay covers 100% of the coding sequences of BRCA1 and BRCA2 including all coding splice site and acceptor sites with an average of 64bp extension into adjoining introns. The assay is a 2 pool Ion AmpliSeq™ 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.

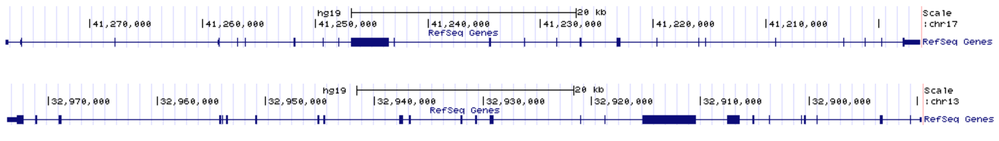
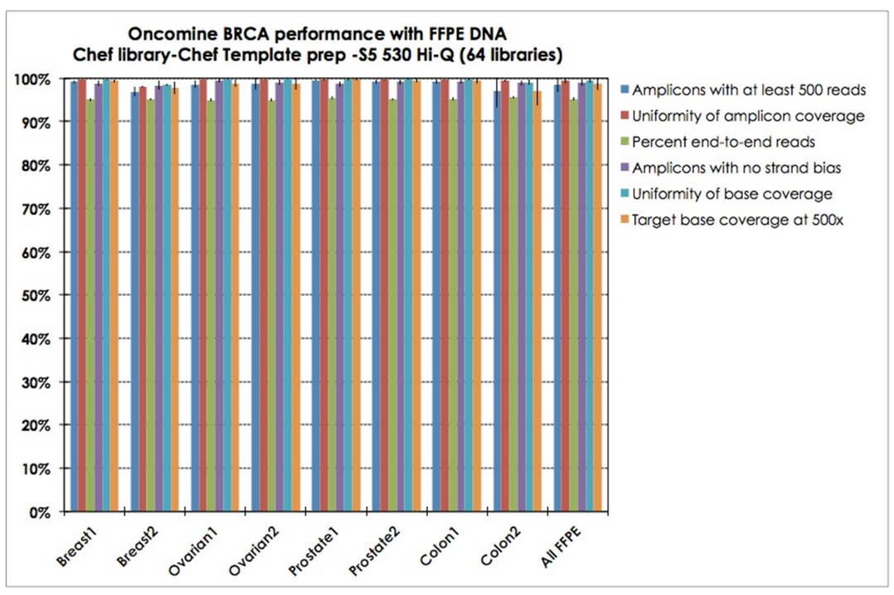


Figure 2. Genomic maps of BRCA1/2. All coding sequences were 100% covered by Ion AmpliSeq amplicons.

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, cell line 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)

Figure 3. Summary of assay uniformity obtained with FFPE tumor specimens and using automated library and template preparation on Ion Chef™ and sequencing on Ion S5™.



gDNA variants	Platform	Library	Templating	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%, 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

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%, 100% allele frequency, > 4,000 SNV and > 200 indel variants measured.

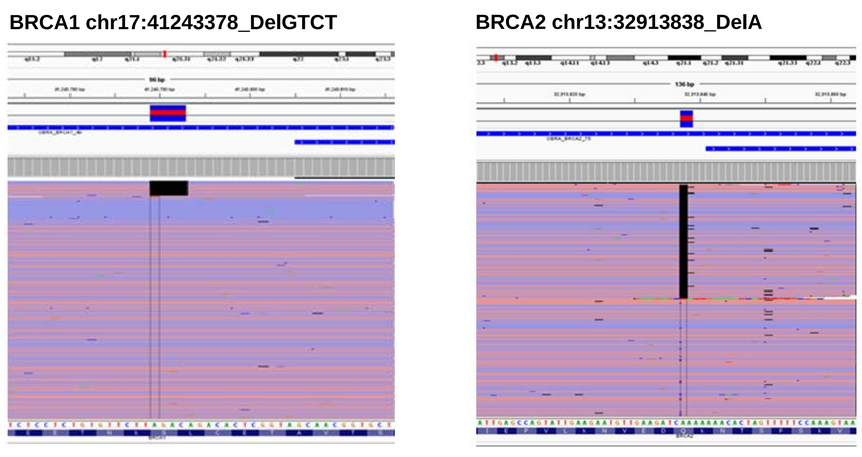


Figure 4. Examples of detection of relevant Indel variants using the Oncomine® BRCA Research Assay.

CONCLUSIONS

The excellent measured performance of the Early Access version of the BRCA Research Assay, revealed in this multi-center evaluation, allowed us to further enhance the assay to produce the final Oncomine® BRCA Research Assay. This Research Use Only product is scheduled for commercial release next month, and its combination of flexibility of use and accuracy of performance promises to significantly advance BRCA gene research.

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