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A Next-Generation Sequencing Study to Detect Large Rearrangements in *BRCA1* and *BRCA2* with High Sensitivity in Blood and FFPE Samples

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ABSTRACT

Introduction: Identifying germline and somatic mutations in the *BRCA1* and *BRCA2* genes is important to support translational cancer research, as these genes are implicated in inherited risk and response to certain therapies. Although small variants such as single-nucleotide variants (SNVs) and indels in these genes are commonly detected, large rearrangements (LRs) such as exon-level copy number variations are difficult to detect using traditional sequencing approaches and often require an additional test such as multiplex ligation-dependent probe amplification (MLPA).

Methods: A total of 219 samples were screened for exon-level copy number variations using the Ion Torrent™ Oncomine™ *BRCA* Research Assay and MLPA. This next-generation sequencing (NGS) assay covers 100% of all exons of *BRCA1* and *BRCA2* with 265 amplicons (targeted regions). A comprehensive bioinformatics algorithm was developed to detect both small variants and exon-level copy number variations in *BRCA1* and *BRCA2*.

Results: LRs were detected at high sensitivity from 219 blood and formalin-fixed, paraffin-embedded (FFPE) research samples, even in the absence of control sample(s). Of the 23 samples that were positive for LRs using MLPA, 22 (95.7%) were also detected by the NGS-based assay. A range of LRs were detected, including heterozygous whole-gene deletions, single- and multiple-exon heterozygous deletions, single- and multiple-exon duplications, and homozygous multiple-exon deletions.

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, and is an important advancement in *BRCA1* and *BRCA2* translational 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 and specificity for large indels, and exon or gene deletions or duplications with only 10–20 ng input DNA.

RESULTS

Early access: We distributed the early-access version of the new Oncomine *BRCA* Research Assay to 20 laboratories across 12 European countries. Samples from four participants were tested for a total of 19 exon or gene deletions or duplications confirmed by MLPA. The early-access assay data were supplemented with data from two additional collaborator sites, including 4 known exon or gene deletion or duplication variants sequenced with the released Oncomine *BRCA* Research Assay. We used this combined set of 11 runs to evaluate our candidate algorithms, and the results are presented in Table 1.

Table 1. Detecting large indels, and exon or gene deletions or duplications in *BRCA1* and *BRCA2.*

Bioinformatics						
Algorithm	**Runs**	**Samples**	**Truth**	**TP**	**FN**	**Sensitivity**
HMM	11	219	23	11	12	48%
VCIB	11	219	23	22	1	96%

VERIFICATION DATA

Upon selecting the principal components analysis-based VCIB algorithm, we developed a software pipeline for accurate detection of NGS data for large indels, and exon or gene deletions or duplications in *BRCA1* and *BRCA2*. After refining the pipeline, we integrated it into the Ion Reporter Software 5.4 and tested performance on an expanded set of 375 samples sequenced with the commercially released Oncomine *BRCA* Research Assay. The samples were from a combination of FFPE, blood, and cell-line sources, 19 of which had known exon or gene deletions or duplications. Results of this verification study are presented in Table 2.

Table 2. Detecting large indels, and exon or gene deletions or duplications in *BRCA1* and *BRCA2.* The extra FN results from one multi-exon deletion variant, the full extent of which is not fully called by our method.

Runs	Samples	Positives	TP	FN	FP	Sensitivity	Specificity
22	375	19	18	2	27	90.0%	94.9%

ANALYSIS PIPELINE DESIGN

The new Oncomine *BRCA* Research Assay covers 100% of the coding sequences of *BRCA1* and *BRCA2,* including all coding splice sites and acceptor sites, with an average of 64 bp extending into adjoining introns. The assay is a 2-pool Ion AmpliSeq design containing 265 amplicons, compatible with DNA samples extracted from FFPE as well as blood samples, and also with automated and manual library preparation methods.

We took advantage of the multiple-overlapping amplicon coverage at each exon to develop an analysis pipeline detecting these important variants in single samples. By comparison of amplicon coverage data against a bioinformatics baseline built from 200 normal samples, we employed two parallel paths to identify different classes of *BRCA1* and *BRCA2* large rearrangements.

The core track utilizes the VCIB noise-reduction algorithm to call copy number changes in individual exons. These exon-level variants are merged into single calls of contiguous blocks of exons if the data warrant doing so. The second analysis stream detects whole-gene deletions in either gene via a statistical comparison of the distribution of normalized amplicon coverage values across the entire set of *BRCA1* and *BRCA2* coding exons. This sensitive test can detect somatic gene loss events that frequently are present in relevant tumor samples, as well as germline whole-gene deletions.

EXAMPLES OF DETECTED VARIANTS

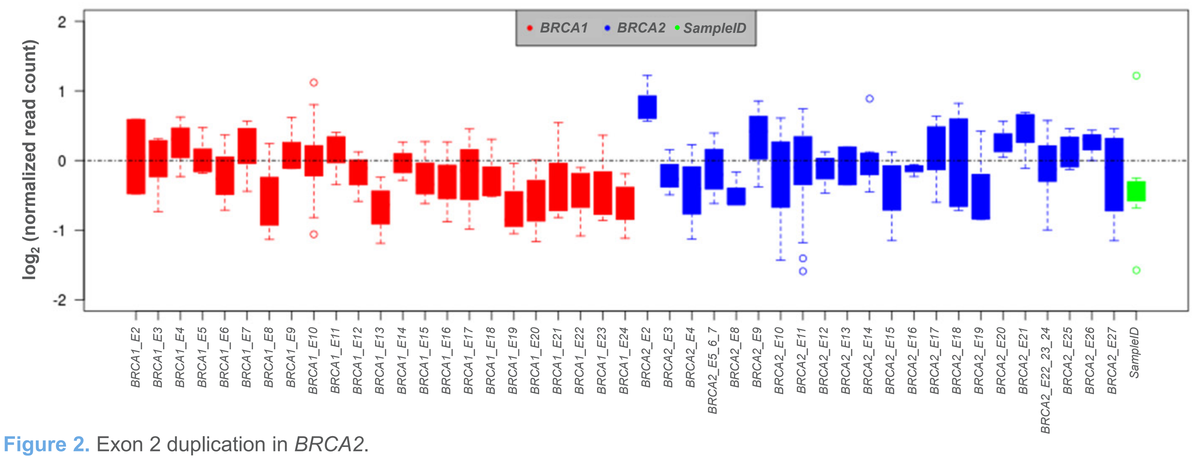

Figure 2. Exon 2 duplication in *BRCA2*.

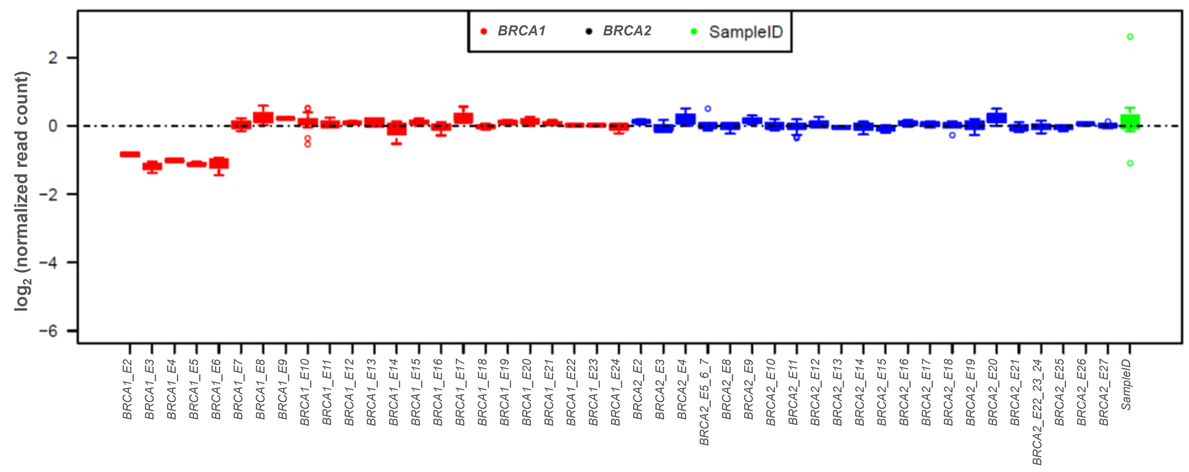

Figure 3. Exon 2–6 deletion in *BRCA1.*

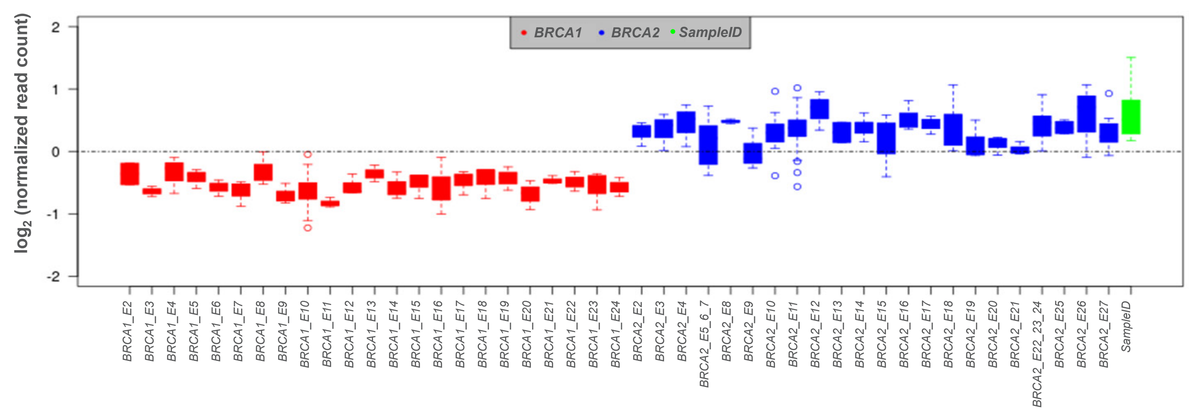

Figure 4. Whole-gene deletion of *BRCA1.*

CONCLUSIONS

The excellent measured performance of the Oncomine *BRCA* Research Assay, revealed in this multicenter evaluation, allowed us to further develop the exon or gene deletion or duplication detection pipeline for inclusion in Ion Reporter Software 5.4. This Research Use Only software enhancement is scheduled for commercial release in June 2017, and the assay's combination of flexibility of use and accuracy of performance promises to significantly advance *BRCA1* and *BRCA2* gene research.

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