Evaluation of the Oncomine *BRCA* Research Assay for variant detection by next-generation sequencing

Summary

- *BRCA1* and *BRCA2* (*BRCA1/2*) are human genes that play an important role in DNA repair.
- Inherited mutations in *BRCA1/2* are associated with an increased risk of certain cancers, including breast and ovarian cancer. Somatic mutations in *BRCA1/2* are common in several cancer types.
- Platinum-based chemotherapies as well as inhibitors of poly (ADP-ribose) polymerase 1 (PARP) exploit the defect in DNA repair present in *BRCA1/2* mutants.
- Ion AmpliSeq™ multiplex PCR technology combined with Ion Torrent™ next-generation sequencing (NGS) was used to develop the Ion Torrent™ Oncomine™ *BRCA* Research Assay, which detects variants within the entire coding sequence and flanking intronic regions of *BRCA1/2*.
- The recommended input of DNA is 20 ng, which may be extracted from blood, cell lines, or formalin-fixed, paraffin-embedded (FFPE) samples.
- Sample processing can be automated to reduce hands-on time, and the sample-to-report turnaround time is 3 days.
- Single-nucleotide variants (SNVs), multi-nucleotide variants (MNVs), short insertions or deletions (indels), and whole-exon, multiple-exon, or entire-gene aberrations are detected with high sensitivity and specificity.
- Automated informatics analysis within Ion Reporter™ Software provides a full set of basic variant annotations, including the ability to cross-reference the ClinVar database.
- Ion Torrent™ Oncomine™ Reporter provides relevant annotations to support variant interpretation.

Introduction

Breast cancer early onset gene 1 (*BRCA1*) and 2 (*BRCA2*) were initially discovered as major hereditary breast cancer susceptibility genes [1-5]. Inherited deleterious mutations in these genes also confer an increased risk in women for ovarian cancer [6] and in men for breast cancer and prostate cancer [7,8]. Both men and women who inherit deleterious *BRCA1* or *BRCA2* mutations are at increased risk for pancreatic cancer [9,10].

It is now understood that the *BRCA1/2* genes encode important tumor suppressor proteins that function in DNA repair [11-14]. Specifically, BRCA1/2 are required for the homologous recombination (HR) repair of DNA double-strand breaks (DSBs) [15]. DSBs are highly unstable and threaten the integrity of the genome. If not repaired, DSBs may cause other genetic alterations or they may induce cell senescence or apoptosis. HR defects also confer sensitivity to certain DNA-damaging agents. For example, the sensitivity of epithelial ovarian cancers to platinum chemotherapy is related to an underlying defect in HR-mediated DNA repair [16,17]. In addition, small-molecule inhibitors of PARP induce synthetic lethality in cells with defective HR, including aberrant *BRCA1/2* [18,19]. The PARP inhibitor olaparib was approved by the US FDA for use in women with deleterious germline *BRCA*-mutated (gBRCAm) advanced ovarian cancer and with deleterious gBRCAm HER2-negative metastatic breast cancer. The PARP inhibitor rucaparib was approved by the US FDA for use in women with a deleterious *BRCA* mutation (germline and/or somatic) and advanced ovarian cancer. Deleterious mutations in *BRCA1/2* are therefore important biomarkers for approved cancer therapies. Several additional PARP inhibitors are in late-phase clinical trials [20].

Furthermore, relevant variant types in *BRCA1/2* include small SNV and indel mutations, and also large intragenic rearrangements that represent approximately 10% of deleterious *BRCA1/2* variants [21]. A robust assay to identify deleterious *BRCA1/2* mutations, including large rearrangements, is therefore critical for informing research in this rapidly expanding field.

The Oncomine *BRCA* Research Assay covers 100% of the coding sequences of *BRCA1/2*, including all splice and acceptor sites, with an average extension of 63 bp into adjoining introns. The assay leverages Ion AmpliSeq multiplex PCR technology and is optimized for detection of low-frequency variants from compromised FFPE samples. The recommended input is 20 ng of DNA extracted from either FFPE or blood samples.

The assay was developed for ease of use and accommodates manual and automated laboratory workflows. Ion Reporter Software provides full variant annotation and enables easy cross-reference to ClinVar, a leading archive of human genetic variant interpretations. Files in Variant Call Format (VCF) are generated and may be used with Oncomine Reporter software, which contains relevant annotations that are expertly curated from approved drug labels, US and European clinical guidelines, and global clinical trial registries.

Materials and methods

Sequencing workflow

The Oncomine *BRCA* Research Assay for manual library preparation (Cat. No. A32840) or automated library preparation using the Ion Chef™ System (Cat. No. A32841) was used with genomic DNA (gDNA) from cell line, FFPE, or blood samples. The Invitrogen™ RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Cat. No. AM1975) or Ion AmpliSeq™ Direct FFPE DNA Kit (Cat. No. A31133) was used to prepare DNA from FFPE samples. Table 1 summarizes the various library preparation, template preparation, and sequencing workflows used to evaluate the assay.

Table 1. Library preparation, template preparation, and sequencing workflows.

Sample type	Library preparation	Library multiplexing	Template preparation and sequencing	Template multiplexing
Somatic	Ion Chef System	8	Ion Chef System, Ion PGM System, Ion 318 Chip	One 8-plex library per chip
			Ion Chef System, Ion S5 System, Ion 530 Chip	Four 8-plex libraries per chip
Somatic	Manual	8	Ion OneTouch 2 System, Ion PGM System, Ion 318 Chip	One 8-plex library per chip
			Ion Chef System, Ion S5 System, Ion 530 Chip	Four 8-plex libraries per chip
Germline	Ion Chef System	8	Ion Chef System, Ion PGM System, Ion 318 Chip	Four 8-plex libraries per chip
			Ion Chef System, Ion S5 System, Ion 530 Chip	
Germline	Manual	32	Ion OneTouch 2 System, Ion PGM System, Ion 318 Chip	One 32-plex library per chip
			Ion Chef System, Ion S5 System, Ion 530 Chip	

Cell line gDNA samples: somatic mutation allele frequencies

Libraries were made from Coriell cell line gDNA samples with characterized SNVs and indels. Nine different gDNA samples with 11 known SNVs (Table 2) were combined to obtain variants at 5% frequency to simulate somatic allele frequencies present in tumor samples. All 5% SNV variants were derived from heterozygous variants in samples NA12877, NA12879, NA12881, NA12882, NA12884, NA12885, NA12886, NA12888, and NA12893.

Ten different gDNA samples with 10 known indels (Table 3) within *BRCA1/2* were combined to obtain indel variants at 5% frequency.

Table 2. *BRCA1* SNVs used to simulate somatic allele frequencies.

Gene	Mutation	ClinVar status
BRCA1	c.2082C>T, p.S694S	Benign
BRCA1	c.2311T>C, p.L771L	Benign
BRCA1	c.2612C>T, p.P871L	Benign
BRCA1	c.3113A>G, p.E1038G	Benign
BRCA1	c.3548A>G, p.K1183R	Benign
BRCA1	c.4308T>C, p.S1436S	Benign
BRCA1	c.4358-2885G>A	Benign
BRCA1	c.4837A>G, p.S1613G	Benign
BRCA1	c.4987-68A>G	Benign
BRCA1	c.4987-92A>G	Benign
BRCA1	c.5152+66G>A	Benign

Table 3. *BRCA1/2* indels used to simulate somatic allele frequencies.

Gene	Mutation	ClinVar status
BRCA1	c.68_69delAG, p.E23Vfs	Pathogenic
BRCA1	c.2071delA, p.R691Dfs	Pathogenic
BRCA1	c.2157dupA, p.E720Rfs	Pathogenic
BRCA1	c.3756_3759delGTCT, p.S1253Rfs	Pathogenic
BRCA1	c.5319dupC, p.N1774Qfs	Pathogenic
BRCA2	c.755_758delACAG, p.D252Vfs	Pathogenic
BRCA2	c.5722_5723delCT, p.L1908Rfs	Pathogenic
BRCA2	c.6174delT, p.F2058Lfs	Pathogenic
BRCA2	c.6198_6199delTT, p.S2067Hfs	Pathogenic
BRCA2	c.6275_6276delTT, p.L2092Pfs	Pathogenic

Cell line and blood gDNA samples: germline mutation allele frequencies

Coriell cell line gDNA samples (NA12878, NA24385) with 21 previously characterized SNVs (Table 4) were combined to obtain variants at 50% and 100% frequency to simulate germline allele frequencies.

Eight different blood gDNA samples with 8 known indels (Table 5) were combined to obtain indel variants at 50% and 100% frequency.

Table 4. *BRCA1/2* SNVs used to simulate germline allele frequencies.

Gene	Mutation	ClinVar status
BRCA1	c.2311T>C, p.L771L	Benign
BRCA1	c.2612C>T, p.P871L	Benign
BRCA1	c.3113A>G, p.E1038G	Benign
BRCA1	c.3548A>G, p.K1183R	Benign
BRCA1	c.4308T>C, p.S1436S	Benign
BRCA1	c.4358-2885G>A	Benign
BRCA1	c.4837A>G, p.S1613G	Benign
BRCA1	c.4987-68A>G	Benign
BRCA1	c.4987-92A>G	Benign
BRCA1	c.5152+66G>A	Benign
BRCA1	c.5744C>T, p.T1915M	Benign
BRCA1	c.8755-66T>C	Benign
BRCA2	c.-26G>A	Benign
BRCA2	c.681+56C>T	Benign
BRCA2	c.2082C>T, p.S694S	Benign
BRCA2	c.3396A>G, p.K1132K	Benign
BRCA2	c.4563A>G, p.L1521L	Benign
BRCA2	c.6513G>C, p.V2171V	Benign
BRCA2	c.7242A>G, p.S2414S	Benign
BRCA2	c.7397C>T, p.A2466V	Benign
BRCA2	c.7806-14T>C	Benign

Table 5. *BRCA1/2* indels in blood samples, used to simulate germline allele frequencies. NA: not available.

Gene	Mutation	ClinVar status
BRCA1	c.3531delT, p.F11177Lfs*33	Pathogenic
BRCA1	c.3700_3704delGTAAA, p.V1234Qfs	Pathogenic
BRCA1	c.5266dupC, p.Q1756Pfs	Pathogenic
BRCA2	c.6267_6269delGCAinsC, p.E2089_H2090delinsDfs*2	Pathogenic
BRCA2	c.7913_ 7917delTTCCT, p.F2638*	NA
BRCA2	c.9027delT, p.H3010Ifs	Pathogenic
BRCA2	c.9403delC, p.L3135Ffs*28	Pathogenic
BRCA2	c.10095delCinsGAATTATATC, p.S3366Nfs	NA

Challenging *BRCA1/2* indel variants from molecular standards

In addition to cell line, FFPE, and blood samples, we characterized *BRCA1/2* variants in commercial molecular standards (Horizon Discovery and SeraCare) to broaden the evaluation of the assay with challenging indel variants, including long indels and indels in homopolymer contexts. Eighty-four replicates of the Horizon Discovery control and 32 replicates of the SeraCare control were sequenced. Table 6 lists *BRCA1/2* indel variants from Horizon Discovery and SeraCare molecular standards.

Results

Germline and somatic variant distribution in *BRCA1/2*

To understand the distribution of *BRCA1/2* variants, we surveyed ClinVar for germline variants and cancer whole-exome sequencing (WES) data for somatic variants. ClinVar (**ncbi.nlm.nih.gov/clinvar/**) is a freely accessible, public archive of reports of the relationships among human genetic variations and interpretations, with supporting evidence [22]. Over 99% of the >10,000 *BRCA1/2* variants reported in ClinVar were of germline origin. SNVs represented 59% of all *BRCA1* variants and 53% of all *BRCA2* variants (Figure 1). Indels represented 41% of all *BRCA1* variants and 47% of all *BRCA2* variants.

To understand the distribution of somatic *BRCA1/2* variants in cancer, we analyzed cancer WES data from >10,000 clinical tumor samples obtained from The Cancer Genome Atlas (TCGA) and using a standardized variant calling pipeline [23]. From this data set that spanned 33 cancer types, we identified 1,003 somatic variants in *BRCA1/2*. Figure 1 summarizes the distribution of somatic variant types within *BRCA1/2* reported in the cancer WES data. In contrast to the germline variant distribution, 91–93% of the somatic variants were SNVs, and only 7–9% were indels. This analysis demonstrated that indels were far more prevalent in germline samples reported in ClinVar, relative to somatic samples reported in TCGA studies.

Table 6. Challenging *BRCA1/2* indel variants from Horizon Discovery and SeraCare molecular standards.

Sample	Gene	Mutation	ClinVar status
Horizon Discovery	*BRCA2*	c.5073delA, p.K1691Nfs	Pathogenic
Horizon Discovery	*BRCA2*	c.5351delA, p.N1784Tfs	Pathogenic
Horizon Discovery	*BRCA2*	c.8021dupA, p.I2675Dfs	Pathogenic
Horizon Discovery	*BRCA2*	c.9403delC, p.L3135Ffs	Pathogenic
SeraCare	*BRCA1*	c.68_69delAG, p.E23Vfs	Pathogenic
SeraCare	*BRCA1*	c.2834_2836delGTAinsC, p.S945Tfs	Pathogenic
SeraCare	*BRCA1*	c.3084_3094delTAATAACATTA, p.N1029Rfs	Pathogenic
SeraCare	*BRCA1*	c.3481_3491delGAAGATACTAG, p.E1161Ffs	Pathogenic
SeraCare	*BRCA1*	c.3756_3759delGTCT, p.S1253Rfs	Pathogenic
SeraCare	*BRCA1*	c.5177_5180delGAAA, p.R1726Kfs	Pathogenic
SeraCare	*BRCA1*	c.5266dupC, p.Q1756Pfs	Pathogenic
SeraCare	*BRCA2*	c.1310_1313delAAGA, p.K437Ifs	Pathogenic
SeraCare	*BRCA2*	c.8975_9100del126, p.P2992_T3033del	Likely pathogenic

Next, we analyzed the coding sequences of *BRCA1/2* to define the length distribution and number of homopolymer (HP) runs. As shown in Table 7, the longest HP sequences in *BRCA1/2* were 3 cases of 8A-mers (1 in *BRCA1* and 2 in *BRCA2*) and 9 cases of 7A-mers (1 in *BRCA1* and 8 in *BRCA2*). The number of HP runs increased at shorter HP lengths (19 at 6-mer and 85 at 5-mer). The *BRCA1* coding sequence contained 19 HP sequences of length ≥5, and 97.8% of the HP runs of 5–8 nucleotides in *BRCA2* were poly(A).

Finally, we analyzed the number of indel variants in the TCGA somatic mutation data associated with HP sequence contexts ≥5. As shown in Table 8, the frequency of HP-associated somatic indel variants was limited to 0.64% of all somatic *BRCA1* variants and 3.2% of all somatic *BRCA2* variants. Combined, just 2.4% of all somatic *BRCA1/2* variants were associated with HP sequence contexts.

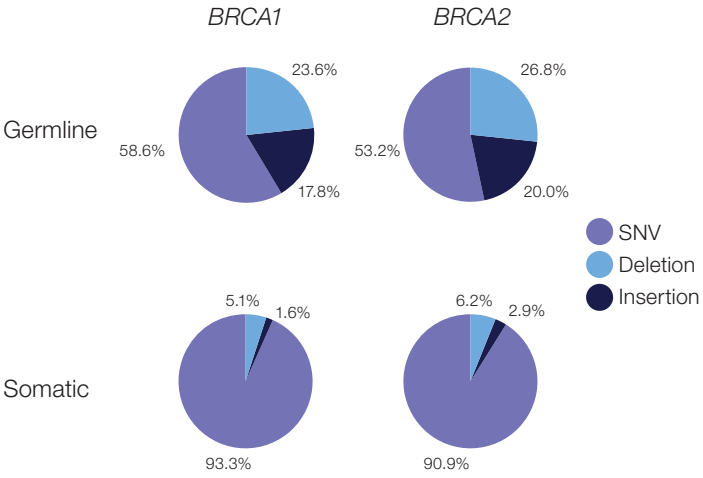

Figure 1. The distribution of variant types in *BRCA1/2* reported in ClinVar (germline) and in The Cancer Genome Atlas (somatic).

Targeted amplicon designs

Ion AmpliSeq primers and amplicons were designed to cover all coding exons and flanking noncoding regions of *BRCA1/2*. An example of the overlapping amplicon coverage for two regions, exons 5–6 in *BRCA1* and exons 22–24 in *BRCA2*, is shown in Figure 2. A similar design strategy was used across the entire coding sequence of *BRCA1/2*. The targeted amplicon design achieved 100% coding sequence coverage and an average of 63 bp of coverage of flanking intronic regions.

Table 7. Length distribution of HP runs in *BRCA1/2*. The consensus coding sequences of *BRCA1* (CCDS11453.1, 5,592 nt) and *BRCA2* (CCDS9344.1, 10,257 nt) were derived from the National Center for Biotechnology Information Consensus CDS project website.

	HP length	A	C	G	T
BRCA1	5	9	1	1	3
	6	3	0	0	0
	7	1	0	0	0
	8	1	0	0	0
BRCA2	5	56	0	0	15
	6	13	0	0	3
	7	8	0	0	0
	8	2	0	0	0

Table 8. Distribution of variant types, including indels associated with HP sequence ≥5, in TCGA somatic mutation data.

	BRCA1	***BRCA2***	***BRCA1/2* (combined)**
Positive samples	263	431	694
Positive variants	314	689	1,003
SNV	293 (93.3%)	626 (90.9%)	919 (91.6%)
Indel	21 (6.7%)	63 (9.1%)	84 (8.3%)
Indel with HP ≥5	2 (0.64%)	22 (3.2%)	24 (2.4%)

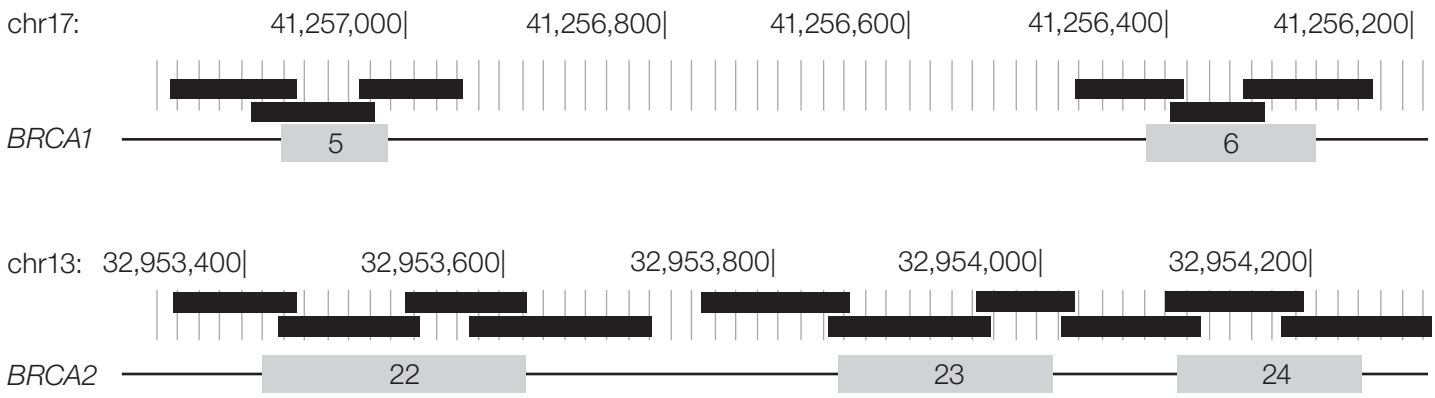

Figure 2. Multiple overlapping amplicons (black bars) provide complete coverage of exons 5–6 of *BRCA1* and exons 22–24 of *BRCA2*.

Uniformity, on-target rate, and depth of coverage

To characterize the performance of the Oncomine *BRCA* Research Assay, cell line, FFPE, and blood samples were tested using both manual and automated (Ion Chef System) library preparation on Ion PGM™ and Ion S5™ Systems.

The Oncomine *BRCA* Research Assay generated consistent and uniform coverage of *BRCA1/2* target regions:

- The uniformity of amplicon and base coverage (defined as the percentage of amplicons or bases with a read depth higher than 20% of the mean read depth) was 100% in all workflows for libraries with somatic mutations (Table 9). The uniformity of amplicon and base coverage was 96–100% in all workflows for libraries with germline mutations (Table 10).
- The target base coverage (defined as the percentage of bases included in the target regions covered with at least 500 reads) was 98–100% in all workflows for libraries with somatic mutations (Table 9). The target base coverage was 96–100% in all workflows for libraries with germline mutations (Table 10).
- With somatic samples, in all workflows, 97–100% of the amplicons had at least 500 reads (Table 9). With germline samples, across all workflows, 96–100% of the amplicons had at least 500 reads (Table 10).

Table 9. Uniformity, on-target rate, and depth of coverage for libraries with somatic mutations.

Sequencer, chip	Library preparation	Template preparation	Sample type	No. of samples	No. of libraries	Average reads per library	Uniformity of amplicon coverage (%)	Uniformity of base coverage (%)	Target base coverage at 500x (%)	Amplicons with at least 500 reads (%)
Ion PGM System, Ion 318 Chip	Ion Chef System	Ion Chef System	FFPE	16	72	449,543	100	100	100	100
Ion PGM System, Ion 318 Chip	Ion Chef System	Ion Chef System	gDNA	4	40	675,596	100	100	100	100
Ion PGM System, Ion 318 Chip	Manual	Ion OneTouch 2 System	FFPE	16	64	639,363	100	100	98	97
Ion PGM System, Ion 318 Chip	Manual	Ion OneTouch 2 System	gDNA	4	32	531,163	100	100	100	100
Ion S5 System, Ion 530 Chip	Manual	Ion OneTouch 2 System	FFPE	16	80	442,546	100	100	99	99
Ion S5 System, Ion 530 Chip	Manual	Ion OneTouch 2 System	gDNA	4	40	580,589	100	100	100	100
Ion S5 System, Ion 530 Chip	Ion Chef System	Ion Chef System	FFPE	16	64	487,867	100	100	100	100
Ion S5 System, Ion 530 Chip	Ion Chef System	Ion Chef System	gDNA	4	30	518,358	100	100	100	100

Table 10. Uniformity, on-target rate, and depth of coverage for libraries with germline mutations.

Sequencer, chip	Library preparation	Template preparation	Sample type	No. of samples	No. of libraries	Average reads per library	Uniformity of amplicon coverage (%)	Uniformity of base coverage (%)	Target base coverage at 500x (%)	Amplicons with at least 500 reads (%)
Ion PGM System, Ion 318 Chip	Ion Chef System	Ion Chef System	Blood, gDNA	8 blood gDNA, 2 gDNA controls	230	168,192	100	100	98	98
Ion PGM System, Ion 318 Chip	Manual	Ion OneTouch 2 System	Blood, gDNA	8 blood gDNA, 2 gDNA controls	160	130,565	96	96	96	96
Ion S5 System, Ion 530 Chip	Manual	Ion Chef System	Blood, gDNA	8 blood gDNA, 2 gDNA controls	128	548,948	97	97	100	100
Ion S5 System, Ion 530 Chip	Ion Chef System	Ion Chef System	Blood, gDNA	8 blood gDNA, 2 gDNA controls	115	531,192	100	100	100	100

Sensitivity and PPV of SNV and indel detection

We analyzed the variant calling results of hundreds of sequencing runs and summarized the results for SNVs and indels measured at somatic (5%) and germline (50%, 100%) allele frequencies. At 5% allele frequency, 11 SNVs (Table 2) and 10 indels (Table 3) were measured, resulting in >1,200 SNV and >600 indel variant sequencing replicates, respectively, across various workflows. At 50% and 100% allele frequencies, 21 SNVs (Table 4) and 8 indels (Table 5) were measured, resulting in >4,300 SNV and >200 indel variant sequencing replicates, respectively, across various workflows. To characterize performance, we calculated sensitivity and positive predictive value (PPV). The results are shown in Table 11 and summarized below.

SNV performance at somatic allele frequencies:

- The sensitivity of SNV detection at somatic allele frequencies was 100% across both manual and automated workflows on the Ion PGM and Ion S5 Systems.
- The PPV of SNV detection at somatic allele frequencies was 99–100% across both manual and automated workflows on the Ion PGM System, and 92–98% across both manual and automated workflows on the Ion S5 System.

SNV performance at germline allele frequencies:

- The sensitivity of SNV detection at germline allele frequencies was 100% across both manual and automated workflows on the Ion PGM and Ion S5 Systems.
- The PPV of SNV detection at germline allele frequencies was 100% across both manual and automated workflows on the Ion PGM and Ion S5 Systems.

Indel performance at somatic allele frequencies:

- The sensitivity of indel detection at somatic allele frequencies was 99% across both manual and automated workflows on the Ion PGM System, and 98–99% across both manual and automated workflows on the Ion S5 System.
- The PPV of indel detection at somatic allele frequencies was 98–99% across both manual and automated workflows on the Ion PGM System, and 92–99% across both manual and automated workflows on the Ion S5 System.

Indel performance at germline allele frequencies:

- The sensitivity of indel detection at germline allele frequencies was 100% across both manual and automated workflows on the Ion PGM and Ion S5 Systems.
- The PPV of indel detection at germline allele frequencies was 99–100% across both manual and automated workflows on the Ion PGM System, and 100% across both manual and automated workflows on the Ion S5 System.

Table 11. Sensitivity and PPV of SNV and indel detection at somatic and germline allele frequencies.

gDNA variant allele frequency	Variant class	Platform	Library preparation	Template preparation	Sensitivity	PPV	Sensitivity	PPV
					SNV		Indel	
5% allele frequency	Somatic	Ion PGM System, Ion 318 Chip	Manual	Ion OneTouch 2 System	100	100	99	99
			Ion Chef System	Ion Chef System	100	99	99	98
		Ion S5 System, Ion 530 Chip	Manual	Ion Chef System	100	98	98	92
			Ion Chef System	Ion Chef System	100	92	99	99
50%, 100% allele frequency	Germline	Ion PGM System, Ion 318 Chip	Manual	Ion OneTouch 2 System	100	100	100	100
			Ion Chef System	Ion Chef System	100	100	100	99
		Ion S5 System, Ion 530 Chip	Manual	Ion Chef System	100	100	100	100
			Ion Chef System	Ion Chef System	100	100	100	100

Detection of homopolymers

A subset of the indel variants used to characterize the performance of the assay from blood and cell line sources was considered to have challenging sequence contexts, including HP sequence contexts. Other challenging indel variants in HP sequence contexts were tested in molecular standards from Horizon Discovery and SeraCare. As shown in Table 12, indel variants associated with HP tracts of up to 5 bases were consistently detected with sensitivity of 94–100%. A Horizon Discovery variant with an HP run of 6 was infrequently detected, and a SeraCare variant with an HP run of 8 was not detected. In contrast, 2 variants in HP sequence contexts of 7 bases in length were consistently detected with sensitivity of 93–94%.

Detection of long indels

The ability of the assay to detect long indels was also characterized. The variants included 8 multi-nucleotide and large indels ranging from 2 to 126 bases in length. Five of these variants, including the largest indel of 126 bases, were consistently detected with 100% sensitivity (Table 13). Upon inspection, it was determined that the failure to consistently detect the 12-base indel was likely due to the variant's proximity to the end of the amplicon (4 bases).

Table 12. Sensitivity of indel detection in HP sequence contexts.

Sample	Gene	Mutation	ClinVar status	HP length of reference allele	Sensitivity
Horizon Discovery	*BRCA2*	c.9403delC, p.L3135Ffs	Pathogenic	2	100%
Coriell 5%	*BRCA1*	c.5319dupC, p.N1774Qfs	Pathogenic	3	100%
SeraCare	*BRCA1*	c.5266dupC, p.Q1756Pfs	Pathogenic	3	100%
Coriell 5%	*BRCA1*	c.2071delA, p.R691Dfs	Pathogenic	4	94.2%
Coriell 5%	*BRCA1*	c.2157dupA, p.E720Rfs	Pathogenic	4	100%
Blood	*BRCA1*	c.3531delT, p.F1177Lfs	Pathogenic	5	100%
Horizon Discovery	*BRCA2*	c.8021dupA, p.I2675Dfs	Pathogenic	6	14.3%
Horizon Discovery	*BRCA2*	c.5073delA, p.K1691Nfs	Pathogenic	7	94.1%
Horizon Discovery	*BRCA2*	c.5351delA, p.N1784Tfs	Pathogenic	7	92.9%
SeraCare	*BRCA2*	c.1813dupA, p.I605Nfs	Pathogenic	8	0%

Table 13. Sensitivity of long indel detection.

Sample	Gene	Mutation	ClinVar status	Indel variant length	Sensitivity
SeraCare	*BRCA1*	c.68_69delAG, p.E23Vfs	Pathogenic	2	100%
SeraCare	*BRCA1*	c.3756_3759delGTCT, p.S1253Rfs	Pathogenic	4	100%
SeraCare	*BRCA1*	c.5177_5180delGAAA, p.R1726Kfs	Pathogenic	5	63.0%
SeraCare	*BRCA2*	c.1310_1313delAAGA, p.K437Ifs	Pathogenic	5	91.0%
SeraCare	*BRCA1*	c.2834_2836delGTAinsC, p.S945Tfs	Pathogenic	6	100%
SeraCare	*BRCA1*	c.3084_3094delTAATAACATTA, p.N1029Rfs	Pathogenic	11	100%
SeraCare	*BRCA1*	c.3481_3491delGAAGATACTAG, p.E1161Ffs	Pathogenic	12	13.0%
SeraCare	*BRCA2*	c.8975_9100del126, p.P2992_T3033del	Likely pathogenic	126	100%

Detection of large (whole exon, multiple exon, or entire gene) aberrations in FFPE and blood samples

Large intragenic rearrangements represent approximately 10% of deleterious *BRCA1/2* variants [21]. However, detection of large *BRCA1/2* rearrangements typically requires specialized analytical tests such as multiplex ligation-dependent probe amplification (MLPA™) assays. To detect large rearrangements from NGS data, a novel bioinformatics algorithm was developed. In total, 193 samples (FFPE and blood) were analyzed in parallel using the Oncomine *BRCA* Research Assay and MLPA assay. The MLPA assay identified 17 samples that were positive for 14 different exon-level deletions or duplications (Table 14). By comparison, the Oncomine *BRCA* Research Assay identified the identical exon-level deletion or duplication in 16 of these samples (94% sensitivity).

Table 14. Detection of *BRCA1/2* large rearrangements.

Gene	Mutation	Unique samples	Total replicates	MLPA positive	NGS positive
BRCA1	Exon 2 deletion	4	4	4	4
BRCA1	Exon 2 duplication	1	1	1	1
BRCA1	Exon 2–6 deletion	1	1	1	1
BRCA1	Exon 2–13 deletion	1	1	1	1
BRCA1	Exon 3 duplication	1	1	1	1
BRCA1	Exon 4–6 duplication	1	1	1	1
BRCA1	Exon 4–7 deletion	1	1	1	1
BRCA1	Exon 4–9 deletion	1	1	1	1
BRCA1	Exon 12–15 deletion	1	1	1	1
BRCA1	Exon 18–20 deletion	1	1	1	1
BRCA1	Exon 23–24 deletion	1	6	6	6
BRCA1	Exon 8–24 deletion	1	1	1	1
BRCA2	Exon 4–26 duplication	1	1	1	0
BRCA2	Exon 21–27 deletion	1	1	1	1

Figure 3 shows an example of the results obtained from a sample with wild-type *BRCA1/2* and a sample with *BRCA1* containing a deletion in exons 18–20. The visualization of normalized read counts per exon is part of the analysis workflow in Ion Reporter Software.

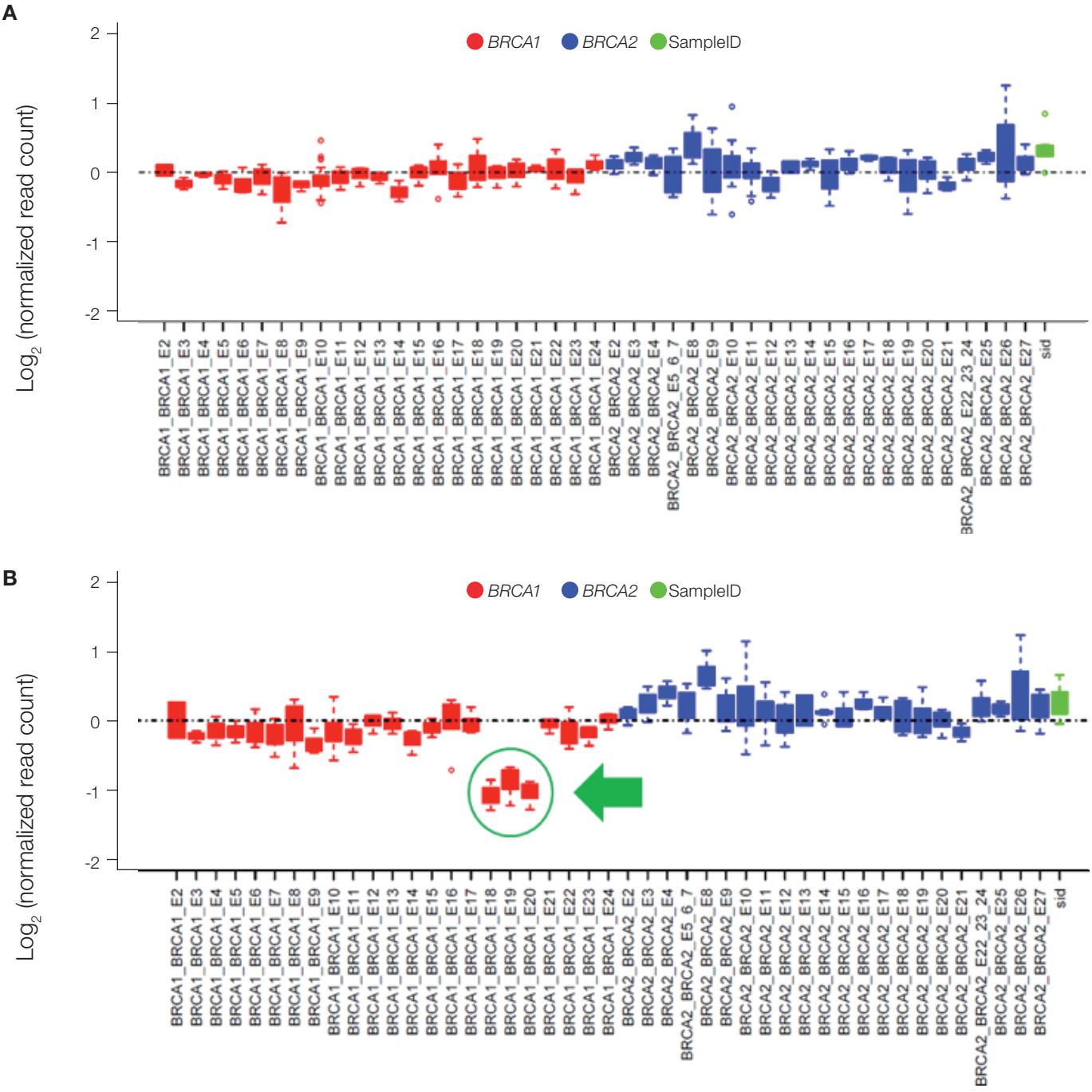

Figure 3. Examples of FFPE samples analyzed with the large rearrangement algorithm in the Oncomine *BRCA* Research Assay workflow. **(A)** An example of a sample with wild-type *BRCA1/2*. **(B)** An example of an FFPE sample with a deletion of *BRCA1* exons 18–20 (indicated by the green circle and arrow). SampleID: sample identification primer pairs included with the Oncomine BRCA Research Assay.

Reporting

The Oncomine informatics workflow includes a fully integrated solution that goes from primary signal processing to variant calling and basic variant annotation to tertiary-level variant interpretation (Figure 4).

Oncomine Reporter is customizable so that laboratories can design reporting solutions that are optimized for their laboratory and applications. Each section can be selected or omitted. Once an optimal format is determined, it can be saved to use as a default report template. An example report for *BRCA1/2* variants is shown in Figure 5.

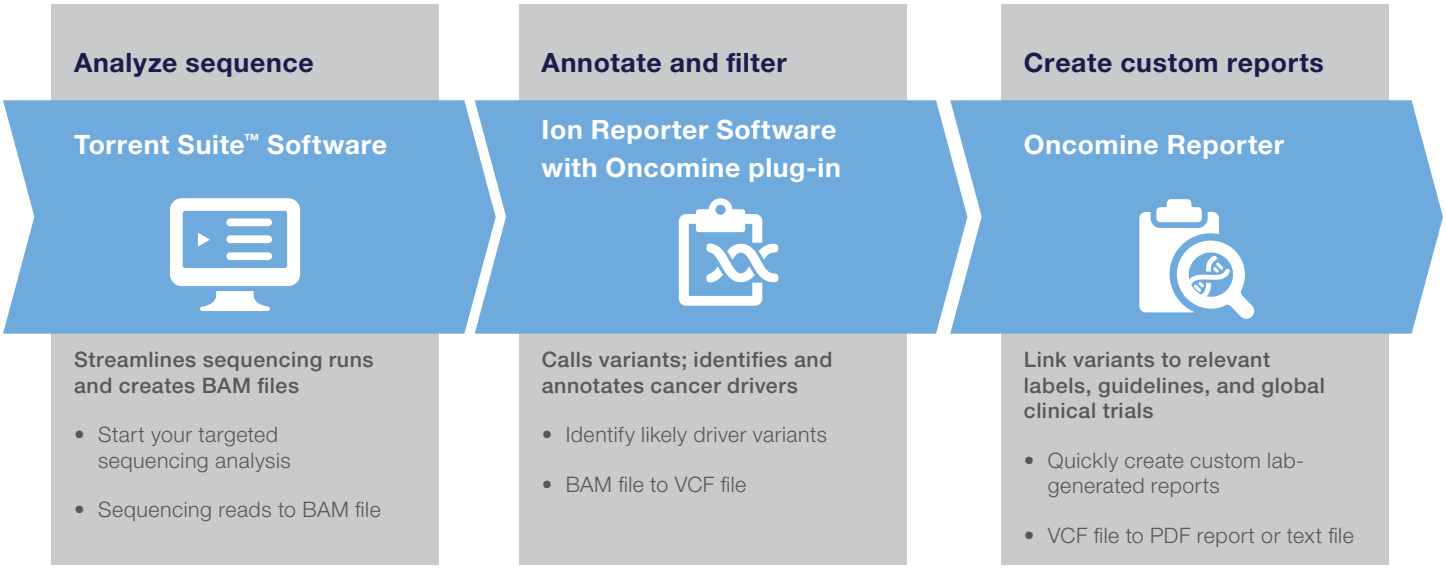

Figure 4. Standard Oncomine informatics workflow. This workflow helps you to identify and prioritize variants, as well as link driver variants with associated evidence in a final report.

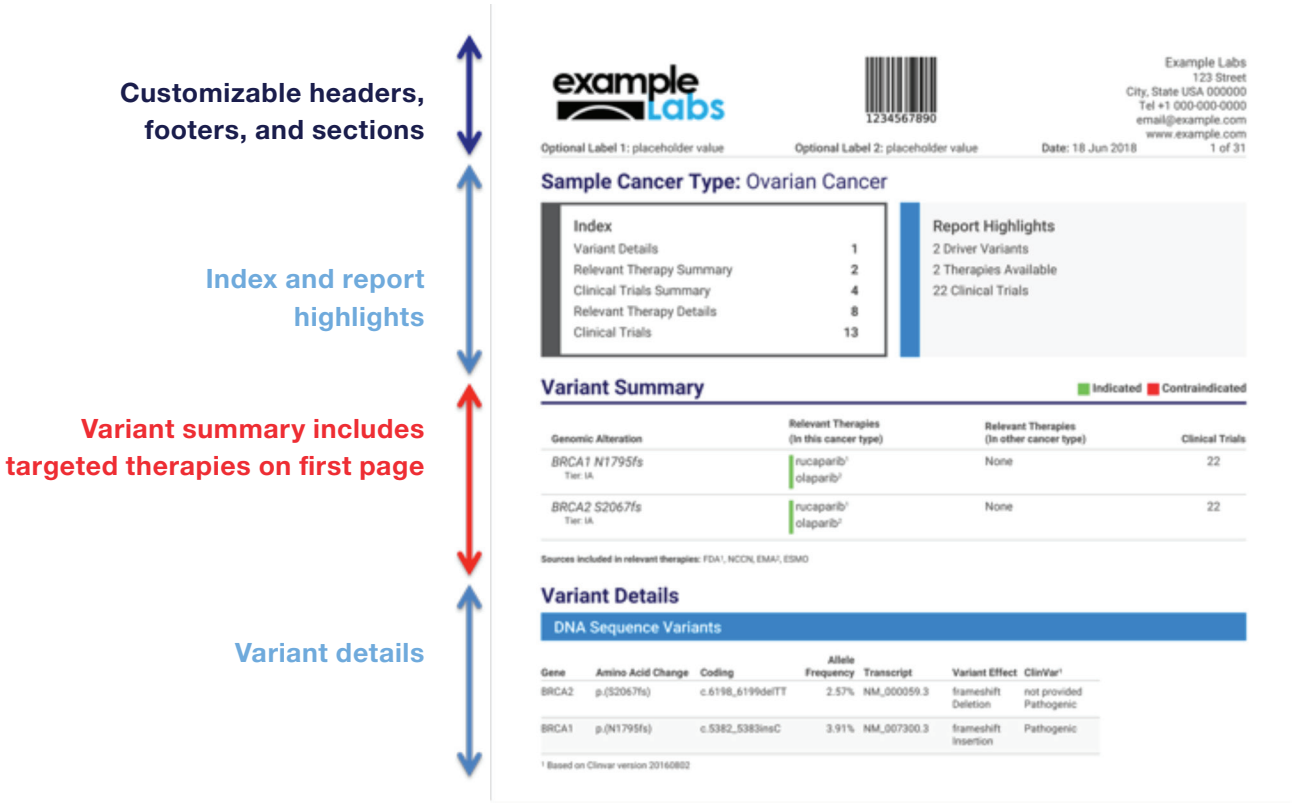

Figure 5. Example report from Oncomine Reporter for a sample run processed with the Oncomine informatics workflow.

ion torrent

The evidence annotations derived in Oncomine Reporter are continually curated by multiple independent reviewers. Variants identified in Ion Reporter Software are linked to relevant evidence from labels, guidelines, and clinical trials, and a custom report is created to help users interpret evidence from multiple global sources. Report templates are available in 11 different languages.

Oncomine Reporter enables integrated reporting for a complete workflow, and supports somatic variants detected by the Oncomine *BRCA* Research Assay.

Conclusion

The Oncomine *BRCA* Research Assay sensitively detects germline and somatic SNVs, indels, and large rearrangements across the complete coding sequence of *BRCA1/2* from as little as 20 ng of DNA derived from blood or FFPE samples. The assay achieved coverage uniformity of >97% for all workflows and sample types, and showed robust performance and sufficient depth to detect variants down to an allele frequency of 5%. The assay detected somatic and germline SNVs and indels with high sensitivity (98–100%) and high PPV (92–100%) across a broad set of clinical research samples, including challenging long indels and indels in homopolymer contexts. High sensitivity (94%) was demonstrated for large rearrangements in blood and FFPE samples. Combined with an effective reporting solution in Ion Reporter Software and Oncomine Reporter, the Oncomine *BRCA* Research Assay provides a rapid and robust workflow for analysis of all types of variations in *BRCA1/2* genes.

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