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AmpliSeq™ for Illumina *BRCA* Panel

Fast and accurate detection of somatic and germline mutations in *BRCA1* and *BRCA2*.

Highlights

- **Relevant gene content**
  Target all exonic regions and flanking intronic sequences of *BRCA1* and *BRCA2*
- **Fast, streamlined workflow**
  Prepare sequencing-ready libraries in a single day using as little as 1 ng high-quality DNA or 10 ng DNA from FFPE tissue
- **Accurate data**
  Detect germline and somatic mutations down to 5% frequency using local or cloud-based analysis

Introduction

The AmpliSeq for Illumina *BRCA* Panel is a targeted resequencing assay designed for detecting somatic and germline mutations across all exonic regions and the flanking intronic sequences of *BRCA1* and *BRCA2* (Table 1). *BRCA1* and *BRCA2* are human tumor suppressor genes that, when carrying specific mutations, have been implicated in an increased risk for breast and ovarian cancers.[1] Understanding BRCA status within the tumor may be a factor when researching potential therapies.[2]

To assist with quick and accurate assessment of genomic variation within *BRCA1* and *BRCA2*, Illumina offers the AmpliSeq for Illumina *BRCA* Panel. The *BRCA* panel is part of a streamlined workflow that includes PCR-based library preparation, Illumina sequencing by synthesis (SBS) chemistry and next-generation sequencing (NGS) technology, and automated analysis. Requiring as little as 1 ng high-quality DNA per pool, the two-pool panel can be used with low-quality and low-quantity samples, including formalin-fixed, paraffin-embedded (FFPE) tissues.

Simple, streamlined workflow

The AmpliSeq for Illumina *BRCA* Panel is part of a DNA-to-variant solution that offers streamlined content, easy-to-perform library preparation, push-button sequencing systems, and simplified data analysis.

Library preparation follows a straightforward, PCR-based protocol that can be completed in as little as 5 hours, with < 1.5 hours hands-on time. Resulting libraries can be normalized, pooled, and then loaded on to a flow cell for sequencing. Prepared libraries are sequenced using proven SBS chemistry on any compatible Illumina sequencing systemthe (Table 2).

Resulting data can be analyzed locally with Local Run Manager or easily streamed into BaseSpace™ Sequence Hub. Local Run Manager and BaseSpace Sequence Hub can access the DNA Amplicon analysis workflow to perform alignment and variant calling. BaseSpace Sequence Hub provides access to BaseSpace Variant Interpreter, which assists with turning variant call data into annotated results.

Table 1: AmpliSeq for Illumina *BRCA* Panel at a glance

Parameter	Specification
No. of genes	2
Targets	All exonic regions of the *BRCA1* and *BRCA2* tumor suppressor genes and flanking intronic sequences
Cumulative target size	22 kb
Variant types	SNVs, indels[a]
Amplicon size	98 bp on average
No. of amplicons	265
Input DNA requirement	1-100 ng (10 ng recommended per pool)
No. of pools per panel	2
Supported sample types	FFPE tissue, blood
Germline: percent targets covered at minimum 50× at recommended throughput	> 95%
Somatic: percent targets covered at minimum 500× at recommended throughput	> 95%
Coverage uniformity (percent of targets with >0.2× mean coverage)	> 95%
Percent on-target aligned reads	> 90%
Total assay time[b]	5 hours
Hands-on time	< 1.5 hours
DNA-to-data time	2.5 days

a. SNVs: single nucleotide variations; indels: insertions/deletions
b. Time represents library preparation only and does not include library quantification, normalization, or pooling.

Data on file at Illumina, Inc. 2017

Table 2: Illumina sequencing systems recommended for use with the AmpliSeq for Illumina *BRCA* Panel

Instrument	No. of Samples per Run	Run Time
For investigating somatic mutations		
iSeq™ 100 System	12	17 hours
MiniSeq™ System (mid output)	24	17 hours
MiniSeq System (high output)	80	24 hours
MiSeq System (v2 chemistry Nano)	3	17 hours
MiSeq System (v2 chemistry Micro)	12	19 hours
MiSeq System (v2 chemistry)	48	24 hours
MiSeq System (v3 chemistry)	80	32 hours
For investigating germline mutations		
iSeq 100 System	96	17 hours
MiSeq System (v2 chemistry Nano)	32	17 hours
MiSeq System (v2 chemistry Micro)	96	19 hours

A maximum of 96 samples can be multiplexed.

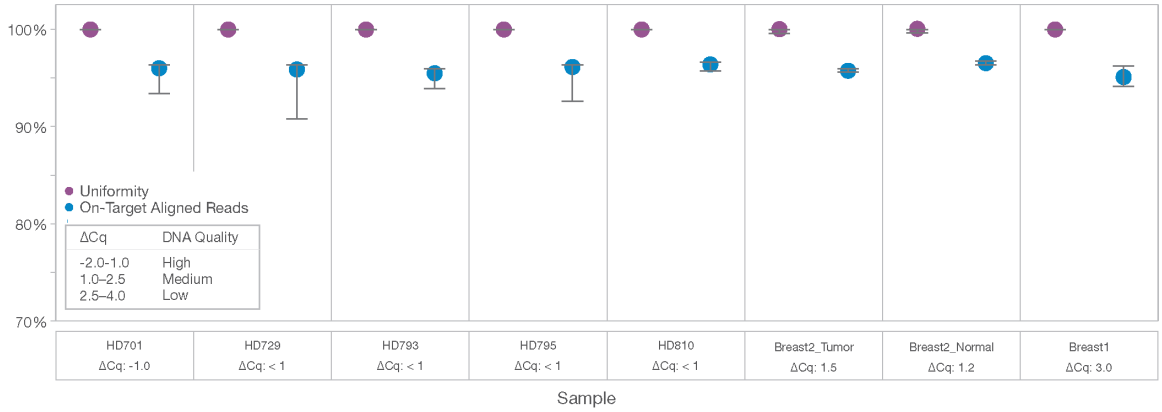

Figure 1: High Coverage Uniformity and On-Target Alignment—DNA extracted from HD and breast tissue samples of varying quality was evaluated using the AmpliSeq for Illumina *BRCA* Panel and sequenced on the MiSeq System. Error bars indicate variability of technical replicates. ΔCq is an indicator of the quality of the DNA.

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Accurate data

To demonstrate assay capabilities, Horizon Discovery (HD) and breast tissue (tumor/normal pair) samples were evaluated using the AmpliSeq for Illumina *BRCA* Panel on the MiSeq™ System. Results showed high coverage uniformity and on-target percentage of aligned reads, even with varying sample quality (Figure 1). In addition, two HD samples of varying quality were evaluated for variant calling accuracy. Data showed high concordance between expected and detected SNVs (Table 3).

Ordering information

Order AmpliSeq for Illumina products online at www.illumina.com

Product	Catalog No.
AmpliSeq for Illumina *BRCA* Panel (24 reactions)	20019168
AmpliSeq for Illumina Library PLUS (24 reactions)	20019101
AmpliSeq for Illumina Library PLUS (96 reactions)	20019102
AmpliSeq for Illumina Library PLUS (384 reactions)	20019103
AmpliSeq for Illumina CD Indexes Set A (96 indexes, 96 samples)	20019105
AmpliSeq for Illumina Direct FFPE DNA	20023378
AmpliSeq for Illumina Library Equalizer	20019171

Learn more

Learn more about the AmpliSeq for Illumina *BRCA* Panel

Learn more about the AmpliSeq for Illumina targeted sequencing solution

References

1. BRCA1 and BRCA2: Cancer Risk and Genetic Testing Fact Sheet - National Cancer Institute. https://www.cancer.gov/about-cancer/causes-prevention/genetics/brca-fact-sheet#q1. Accessed October 30, 2017.
2. Approved Drugs > FDA approves olaparib tablets for maintenance treatment in ovarian cancer. https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm572143.htm. Accessed November 21, 2017.

Table 3: High Concordance Between Expected and Detected Variant Frequency

Known Variant	Expected VF[a]	Detected VF[a]	No. of Samples	Call Rate
HD795 (Horizon Discovery high-quality sample)				
BRCA2 N289H	7.5%	7.4%	16	100%
BRCA2 N991D	7.5%	7.2%	16	100%
BRCA2 D1420Y	32.5%	32.5%	16	100%
BRCA2 V2466A	100%	99.9%	16	100%
BRCA1 S1613G	7.5%	7.4%	16	100%
BRCA1 R1443STOP	32.5%	32.8%	16	100%
*BRCA1*K1183R	7.5%	7.9%	16	100%
*BRCA1*P871L	15.0%	14.5%	16	100%
*BRCA1*K820E	7.5%	7.7%	16	100%
*BRCA1*D435Y	7.5%	6.9%	16	100%
HD810 (Horizon Discovery formalin-fixed sample)				
BRCA2 N289H	7.5%	7.0%	10	100%
BRCA2 N991D	7.5%	7.6%	10	100%
BRCA2 D1420Y	32.5%	33.9%	10	100%
BRCA2 V2466A	100%	99.9%	10	100%
BRCA1 S1613G	7.5%	7.3%	10	100%
BRCA1 R1443STOP	32.5%	33.9%	10	100%
*BRCA1*K1183R	7.5%	7.5%	10	100%
*BRCA1*P871L	15.0%	14.6%	10	100%
*BRCA1*K820E	7.5%	7.7%	10	100%
*BRCA1*D435Y	7.5%	7.1%	10	100%

a. VF: variant frequency

DNA from high-quality (HD795) and formalin-fixed (HD810) samples was evaluated using the AmpliSeq for Illumina *BRCA* Panel and sequenced on the MiSeq System. Results show that 100% of expected SNVs were detected.

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