AmpliSeq™ for Illumina Oncology Panels on the iSeq™ 100 System

Proven amplicon chemistry meets industry-leading sequencing technology for cancer-related targeted resequencing applications.

Highlights

- **Highly accessible sequencing solution**
  Low cost, easy to implement, NGS system
- **Relevant gene content for cancer research**
  Expertly selected, cancer-focused content
- **High analytical sensitivity with low DNA input**
  Accurate variant detection as low as 5% allele frequency, using 1–100 ng input DNA or RNA
- **Validated workflow for easy implementation**
  Streamlined, comprehensive workflow with minimal hands-on time and automatic variant calling

Introduction

Next-generation sequencing (NGS) has transformed cancer research, enabling variant detection in numerous genes in a single assay. By focusing on genes related to a particular disease or pathway, targeted sequencing offers the ability to conduct NGS on smaller platforms, while also providing the advantage of higher depth of coverage on targeted genes. The increased analytical sensitivity that comes with higher coverage may be particularly important for detection of low-frequency variants in heterogenous tumor tissues.

The iSeq 100 System was designed to execute small-scale NGS applications. With immediate access to run various types of experiments on the benchtop, the iSeq 100 System provides autonomy and flexibility to small laboratories. The system is well suited for targeted sequencing applications that focus on disease-related genes.

This application note demonstrates the performance of the iSeq 100 System with four distinct targeted resequencing panels, each designed with proven amplicon chemistry to focus on different aspects of cancer research:

AmpliSeq for Illumina Cancer Hotspot Panel v2 is designed to detect somatic mutations across the hotspot regions of 50 genes with known associations to cancer. Starting with as little as 1 ng DNA from high-quality DNA, or DNA from formalin-fixed, paraffin-embedded (FFPE) tissues, the panel enables analytically sensitive mutation detection within multiple cancer types, including lung, colon, breast, ovarian, melanoma, and prostate. The low-input requirement enables use with precious samples.

AmpliSeq for Illumina Focus Panel was developed for biomarker analysis in both DNA and RNA concurrently. The panel enables high analytical sensitivity for mutation detection in 52 genes with known relevance to solid tumors, including lung, colon, breast, ovarian, melanoma, and prostate. The Focus Panel allows quick and accurate assessment of genomic variation for translational and clinical research studies.

AmpliSeq for Illumina *BRCA* Panel is a DNA sequencing panel that detects somatic and germline mutations across all exonic regions and the flanking intronic sequences of the *BRCA1* and *BRCA2* genes. *BRCA1* and *BRCA2* are tumor suppressor genes that have been implicated in an increased risk for breast and ovarian cancers when carrying specific mutations.[1] Understanding *BRCA* status within the tumor may be a factor when researching potential therapies.[2]

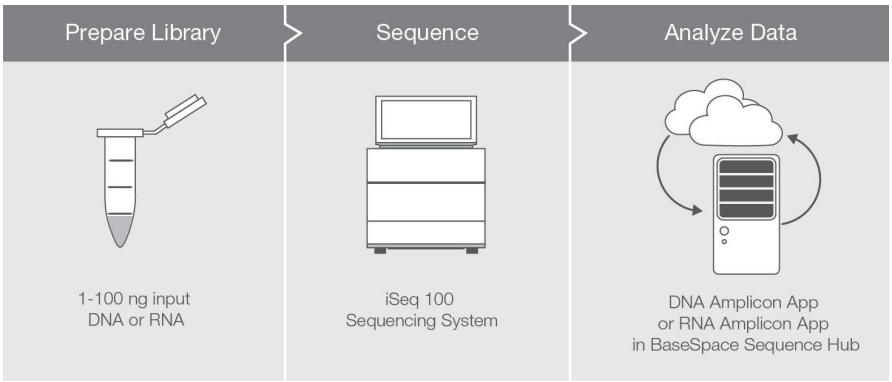

Figure 1: Simple, integrated workflow—Illumina offers integrated library preparation, sequencing, and automated data analysis, creating a streamlined workflow that can go from DNA/RNA input to data in 25 hours.

AmpliSeq for Illumina Immune Response Panel provides detection of biomarkers associated with different leukocyte subsets, antigen presentation, checkpoint pathways, and tumor progression. Starting with 1-100 ng input RNA, the Immune Response Panel enables quick and accurate analysis of 395 genes involved in tumor–immune system interactions, and evaluation of biomarkers that can be used for developing new immunotherapy treatments.

High operational efficiency

The integrated workflow (Figure 1) includes AmpliSeq for Illumina PCR-based library preparation, Illumina sequencing by synthesis (SBS) NGS technology, and automated analysis with BaseSpace™ Sequence Hub or Local Run Manager.

The Ampliseq for Illumina panels were optimized to provide high-quality, accurate data with low requirements for sample input. The library prep workflow requires less than 1.5 hours of hands-on-time, providing an efficient, comprehensive assay. The assay is also designed to obtain high coverage of targeted genes using as little as 1 ng of nucleic acid input. The low input requirement, combined with the ability to analyze hundreds of gene simultaneously, can be a significant benefit for labs with limited samples.

Simple, comprehensive workflow

The AmpliSeq for Illumina workflow is comprehensive and can be easily integrated into laboratory operations, going from DNA or RNA to data in approximately 25 hours (Figure 1). AmpliSeq for Illumina PCR-based library preparation can be completed in as little as 5 hours. Following library prep, sequencing with the iSeq 100 System is highly automated, requiring ~ 5 minutes hands-on time for loading of samples into the flow cell and starting the run. Data can be analyzed on the instrument with Local Run Manager software, or streamed automatically to BaseSpace Sequence Hub for more advanced analysis using the DNA Amplicon App or the RNA Amplicon App.

Reproducibility

The iSeq 100 System was designed to provide affordable access to NGS, while also providing the recommended depth of coverage for high-confidence variant calling (≥ 500× mean coverage). To demonstrate high performance of AmpliSeq chemistry on the iSeq 100 System, the same DNA sample was analyzed on the iSeq 100, MiniSeq™, and MiSeq™ Systems for each of the AmpliSeq for Illumina oncology panels (Table 1). For gene expression analysis using the AmpliSeq for Illumina Immune Response assay, reproducibility between samples was demonstrated by read count comparison performed by the RNA Amplicon App (Figure 2).

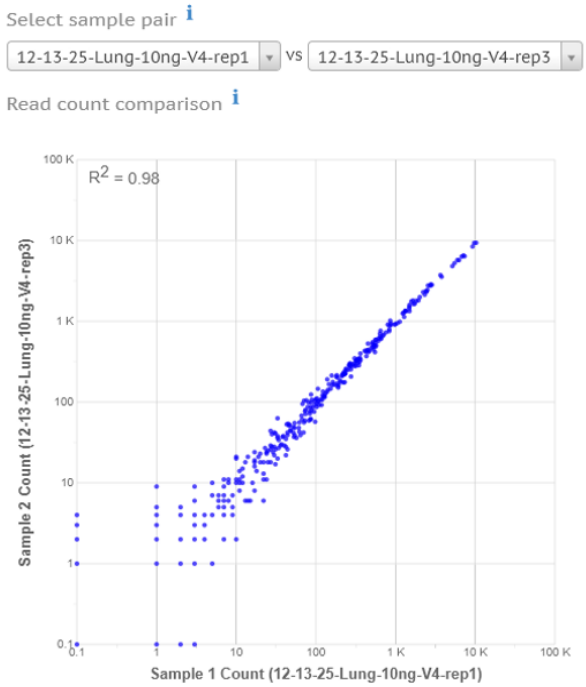

Figure 2: Reproducibility with the AmpliSeq for Illumina Immune Response Panel—Reproducibility of gene expression is confirmed using the RNA Amplicon App in BaseSpace Sequence Hub. A read count comparison for two replicates of the same lung tumor sample is shown.

Table 1: DNA analysis reproducibility between the iSeq 100, MiSeq, and MiniSeq Systems

AmpliSeq panel	System	SNV precision	SNV recall	Uniformity	Specificity	Percent on-target/aligned[a]	Mean coverage
Cancer Hotspot v2	iSeq 100	1.0000 +/-0	1.0000 +/-0	100 +/-0	93.5 +/-0.5	84.7 +/-0.7	3230
	MiniSeq	1.0000	1.0000	99.0	94.4	85.4	13950
	MiSeq	0.9861	1.0000	100	94.7	85.9	7190
Focus	iSeq 100	1.0000 +/-0	0.9458 +/-0.017	99.3 +/-0.2	89.5 +/-0.7	81.5 +/-0.9	1640
	MiniSeq	0.9286	1.0000	98.9	91.5	83.1	7120
	MiSeq	0.9400	1.0000	99.0	91.8	83.6	2890
BRCA	iSeq 100	1.0000 +/-0	0.9002 +/-0.013	100 +/-0	92.2 +/-0.2	81.3 +/-0.3	5500
	MiniSeq	0.8910	1.0000	100	92.8	81.9	22900
	MiSeq	0.8910	1.0000	100	93.1	82.3	9860

One replicate was analyzed on the MiSeq and MiniSeq Systems. The same sample was then analyzed with multiple replicates on the iSeq 100 System (n = 6 for Cancer Hotspot v2, n = 3 for Focus, and n = 3 for BRCA) with +/- values indicating standard deviation between replicates.

a. Percent on-target is the ratio of number of bases within a target region to total number of bases output by the sequencer.

Reliable, accurate performance

Combining the high coverage of targeted sequencing with the performance of the iSeq 100 System provides cancer researchers with high confidence for accurate variant detection. Using AmpliSeq chemistry to enrich for relevant targets, followed by Illumina sequencing chemistry, variant calling as low as 5% is possible with true variants distinguishable from background noise (Figure 3).

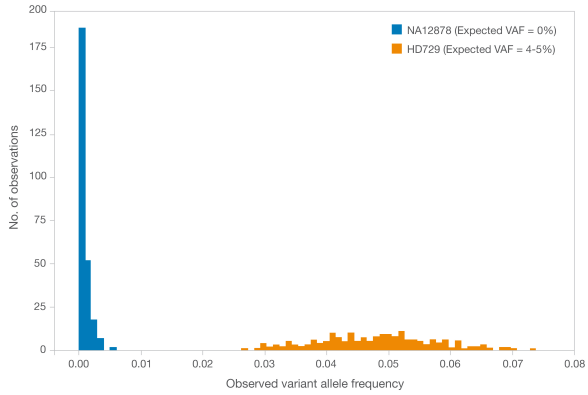

Figure 3: Distribution of observed variant frequencies for DNA with low-frequency variants versus blank DNA—A DNA standard (HD729) with nine single-nucleotide variants of expected variant allele frequencies (VAF) of 4-5% was analyzed with the AmpliSeq for Illumina Cancer Hotspot Panel v2. Shown are the number of times these variants were detected at different frequencies, using both the HD729 DNA standard and a blank DNA standard (NA12877) known to lack these variants. There are 48 observations for each variant, half from NA12877 and half from HD729. Each DNA standard was sequenced using two runs of 12 replicates each.

User-friendly analysis and reporting

Illumina sequencing systems offer the option to connect to BaseSpace Sequence Hub, the Illumina genomics computing environment for sequencing data analysis and management. Researchers can securely store, analyze, archive, and share sequencing data. The DNA Amplicon App and the RNA Amplicon App are designed to perform numerous analyses at the click of a button, such as variant calling, differential expression analysis, or sample comparison (Figure 2). Raw data outputs are provided, as well as user-friendly, focused reports.

Summary

With Illumina SBS chemistry and the AmpliSeq for Illumina oncology panels, the iSeq 100 System provides flexible, affordable access to expert-defined content for comprehensive investigation of cancer-related targets in the genome and transcriptome. The optimized amplicon sets provides comprehensive coverage of regions known to be frequently mutated in several areas of cancer research. With AmpliSeq for Illumina panels, hundreds of genes can be analyzed in a single assay. Combined with the iSeq 100 System, these NGS-based solutions are highly accessible to small independent laboratories.

Learn more

To learn more about the iSeq 100 System, visit www.illumina.com/systems/sequencing-platforms/iseq.html

To learn more about AmpliSeq for Illumina sequencing panels, visit www.illumina.com/products/by-brand/ampliseq.html

Ordering information

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

System and reagent kits	Catalog no.
iSeq 100 Sequencing System	20021532
iSeq 100 E1 Reagent	20021533
iSeq 100 E1 Reagent 4 Pack	20021534
Library prep	**Catalog no.**
AmpliSeq for Illumina Cancer Hotspot Panel	20019161
AmpliSeq for Illumina Focus Panel	20019164
AmpliSeq for Illumina *BRCA* Panel	20019168
AmpliSeq for Illumina Immune Response Panel	20019169
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

References

1. *BRCA1* and *BRCA2*: Cancer Risk and Genetic Testing Fact Sheet - National Cancer Institute. www.cancer.gov/about-cancer/causes-prevention/genetics/brca-fact-sheet#q1. Accessed November 29, 2017.
2. Approved Drugs > FDA approves olaparib tablets for maintenance treatment in ovarian cancer. www.fda.gov/drugs/informationondrugs/approveddrugs/ucm572143.htm. Accessed January 12, 2018.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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