Using the versatile Ion S5 System for cancer genomics and transcriptomics research

Ion S5 System and cancer research

- Easily detect mutations in tumor- associated genes from as little as 1 ng of tumor DNA research samples with Ion AmpliSeq™ cancer research gene panels, or create custom assays with the Ion AmpliSeq™ Designer
- Identify single nucleotide variants (SNVs), indels, copy number variants (CNVs), and gene fusions as demonstrated with the Oncomine™ Comprehensive Assay
- Perform analysis on small archived formalin-fixed, paraffin-embedded (FFPE) solid tumor and fine needle aspirate research samples
- The easy-to-use Ion S5™ System, with cartridge-based reagents, a straightforward user interface, and adjustable output, is highly optimized for sequencing multiplexed Ion AmpliSeq cancer research gene panels

Figure 1. The Ion S5 System for cancer research. The massively parallel Ion S5 System can rapidly sequence up to 80 million DNA clones per run directly on semiconductor chips. Specific regions of the cancer genome can be amplified by the power of the proprietary Ion AmpliSeq technology that can enable highly multiplexed PCR (~20,000 amplicons/reaction), offering an effective method for targeted sequencing of cancer-related regions in the genome.

Introduction

In cancer research, scientists approach the complexity of cancer using multiple strategies including, but not limited to: (1) focusing on targeted genes and important variants within the genome, and analyzing tumor research samples for somatic mutations, (2) studying inheritance of cancer genes within the germline to better understand the nature of cancer predisposition, and (3) studying cancer gene regulation, the potential effects of oncology drug candidates, and future post-treatment recurrence surveillance by analyzing transcriptomes. Next-generation sequencing (NGS), which enables the analysis of somatic and inherited DNA variants, as well as RNA analysis, has transformed cancer genomics research.

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The Ion S5 System (Figure 1) enables massively parallel sequencing of hundreds to thousands of targeted genomic regions and multiple tumor research samples in one run. The high-throughput nature of the Ion S5 System offers significant advantages over traditional PCR or Sanger sequencing approaches by enabling substantially greater information per sequencing run, thereby maximizing the information obtained from each precious sample.

When combined with the Oncomine Comprehensive Assay and Ion AmpliSeq cancer research panels (see Table 2) that enable the survey of genes and genomic regions studied in cancer research, a high level of detection sensitivity can be achieved from a variety of samples such as fresh, frozen, or FFPE tissues. Ion AmpliSeq technology, which is also leveraged with the Oncomine Comprehensive Assay, enables multiplex PCR of up to ~20,000 amplicons in a single reaction from as little as 1 ng of DNA (Figure 2). The tunable sequencing output of the Ion S5 System (Table 1), from ~5 million to ~80 million reads, enables a broad set of cancer research applications and sample throughputs to be addressed effectively.

In this application note, we describe effective approaches for cancer research using the Ion S5 System, Oncomine Comprehensive Assay and Ion AmpliSeq™ Transcriptome Panel for mutational analysis and gene expression profiling.

Sequencing workflow

The sequencing workflow (Figure 3) consists of four main steps: preparation of a targeted sequencing library from genomic DNA or RNA, clonal amplification of the library on beads by emulsion PCR, sequencing of the clonally amplified (templated) beads, and data generation and analysis.

Table 1. Number of cancer research samples that can be run on the chips (barcodes are available for up to 96 samples per run).

	 Ion 520™ Chip	 Ion 530™ Chip	 Ion 540™ Chip
Reads (million/chip)	3–5	15–20	60–80
Read length (bp)	200–400	200–400	200
Output (Gb/chip)	1.2–2	6–8	10-15
Maximum number of samples/run			
Oncomine Comprehensive Assay	1	4	8
Ion AmpliSeq Transcriptome Panel	–	~2	~8

Table 2. Benefits of Ion AmpliSeq panels and Oncomine assays.

Differentiators	Ion AmpliSeq panels	Oncomine assays
Market leadership in targeted sequencing	• >600 tests implemented in clinical research labs • Hundreds of thousands of test results generated in clinical research labs • >100 peer-reviewed clinical oncology research publications • >1,000 total publications • Results from more samples, including fine-needle aspirate research samples	
Fast turnaround time	• Affordable sequencing of smaller sets (batching) • Ion Torrent™ technology enables faster run times	
Informed assay design using the Oncomine Knowledgebase	–	**Content informed by one of the world's largest collections of curated oncology genetic information used by pharma companies globally**
Oncomine™ Knowledgebase Reporter	–	**Oncomine Knowledge Reporter aids in variant interpretation** • FDA labels • NCCN guidelines • Clinical trial evidence
Lot matched reagents	–	**Lot-matched for high-quality consistency in performance and user convenience**

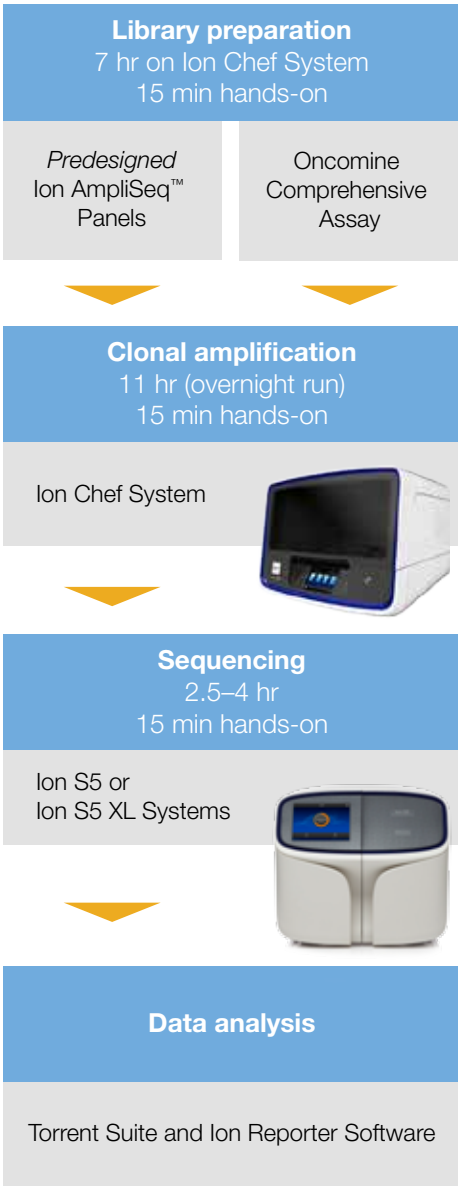

Figure 2. Targeted sequencing workflow. Ion AmpliSeq or Oncomine Comprehensive Assay libraries are prepared manually or with the Ion Chef™ Instrument. Libraries are then placed in the Ion Chef System for emulsion PCR, enrichment, and loading on Ion S5 Chips. Chips are placed in the Ion S5 System with reagents for sequencing. Primary data analysis is performed using Torrent Suite™ Software, with further analysis and annotation using Ion Reporter™ Software.

1. Library preparation for targeted sequencing

Genomic DNA (as little as 1 ng) isolated from the sample is converted to a sequencing library by selective amplification of regions of interest. Ion AmpliSeq technology enables highly multiplexed PCR of up to ~20,000 amplicons in a single tube. A fraction of the target-specific Ion AmpliSeq primers is digested away from the amplified product (eliminating primer-dimers), followed by a ligation step to add adapter sequences to the amplicons.

Predesigned panels are available, such as the Oncomine Comprehensive Assay and the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit, or custom panels can be easily developed using the Ion AmpliSeq Designer online tool.

Automation on the Ion Chef™ System offers the option to automatically prepare 8 Ion AmpliSeq libraries directly from DNA, producing normalized, pooled libraries ready for downstream templating and sequencing.

2. Clonal amplification (templating)

Libraries prepared manually or by automation are clonally amplified on the Ion Chef System by emulsion PCR on beads.

The Ion Chef System performs all the steps, from creating the emulsion mixture, performing PCR, carrying out post-PCR purification, and loading purified templated beads onto the Ion semiconductor chips. The prepared chips are then loaded into the Ion S5 System for sequencing.

3. Sequencing

A sequencing run on the Ion S5 and Ion S5 XL Systems is initiated by loading a reagent cartridge, buffer, cleaning solution, and waste container. The templated chip is then loaded and the run is started. Up to 400 base pairs of sequence information can be produced from the three available semiconductor chips, with ~5, ~20, or ~80 million reads per chip.

4. Data analysis

The Ion S5 System generates base calls and variants from the raw data. Torrent Suite Software coordinates it all—run setup to data processing—to complete the primary analysis of the sequencing data. The data can also be uploaded to Ion Reporter™ Software for panel-specific workflows, annotation, and reporting of common and rare variants.

Results

The flexibility, simple workflow, and cost-effectiveness of the Ion S5 System enables highly efficient sequencing-based analysis.

Oncomine Comprehensive Assay

The Oncomine Comprehensive Assay is based on Ion AmpliSeq technology and is informed by the Oncomine™ Knowledgebase. It enables detection of variants across 143 genes (~244 kb) in DNA or RNA isolated from fresh-frozen and FFPE samples, fine needle biopsies, and core needle aspirate research samples. In addition to hotspots, SNVs, indels, and CNVs, gene fusions are also detected from extracted RNA. Integrated Ion Reporter Software analysis produces annotated variant reports. The resulting annotated variant data, including hotspots, CNVs, indels, and gene fusions, can be further analyzed using the Oncomine™ Knowledgebase Reporter, which helps users prepare a report meeting their specific laboratory needs.

Targeted regions were amplified using the Oncomine Comprehensive Assay on samples made from 3 DNA and 3 RNA sources, as shown below, and sequenced on the Ion 530 Chip using the Ion S5 XL System.

DNA samples:

- Cell line CRL-1619 with known SNVs
- Cell line CRL-2321 with known CNVs
- AcroMetrix™ Oncology Hotspot Control with >500 SNVs, 19 insertions, 29 deletions, and 3 complex tumor-associated mutations across 53 genes

RNA sample—cocktail of RNA from the following human lung adenocarcinoma cell lines with known fusions:

- H2228
- HCC78
- LC-2/ad

Three sample mixtures were prepared: mixtures of Sample 1: 80% AcroMetrix Oncology Hotspot Control with 20% RNA cocktail; Sample 2: 80% CRL-1619 DNA with 20% RNA cocktail; and Sample 3: 80% CRL-2321 DNA with 20% normal colon cell RNA. The 3 samples were run in duplicate on an Ion 530 Chip. Primary data analysis was performed with Torrent Suite Software followed by variant calling in Ion Reporter Software.

Targeting efficiency was 97.93% of reads on target, with a mean coverage depth of 2,565x, with 99.95% targeted bases covered at 10x base coverage uniformity of 97.29%. Combined SNV and indel concordance for hotspot positions that intersect with the variants within the AcroMetrix Oncology Hotspot Control was 99.15%. The overall precision for SNVs and indels was 99.43%. All 3 of the fusion transcripts were detected.

Ion AmpliSeq Transcriptome Human Gene Expression Kit

The Ion AmpliSeq transcriptome approach offers a robust method to determine the global gene expression profile of >20,800 genes from as few as 40 cells. A range of sample types of small quantities, including total RNA isolated from FFPE samples, and tissue and cells isolated by laser capture microdissection (LCM), can be analyzed at low cost using the Ion AmpliSeq Transcriptome Panel with the Ion S5 System.

Two replicates each of 4 different resected invasive ductal breast carcinoma (IDC) research samples, which varied in stage, prognosis, and estrogen- and progesterone-receptor positivity, were sequenced on the Ion 540 Chip. Replicates clustered tightly with gene expression profiling of the top 50 genes (exhibiting the largest sample-to-sample expression differences) revealed subtle differences that might elucidate IDC sample differences (Figure 4).

Conclusion

Multiple sequencing applications can be perfomed effectively on the Ion S5 System. The three chips available for use with the Ion S5 platform offer multiple throughputs: 3–5 million reads for the Ion 520 Chip, 15–20 million reads for the Ion 530 Chip, and 60–80 million reads for the Ion 540 Chip, with up to 400 bp read lengths (on the Ion 520 and Ion 530 Chips). Sample throughput can be optimized by using the chip that most closely fits the sequencing depth requirements of the desired application. The PCR multiplexing capability of Ion AmpliSeq gene panels and the ease of use of the Ion S5 System provide an optimal solution for cancer researchers.

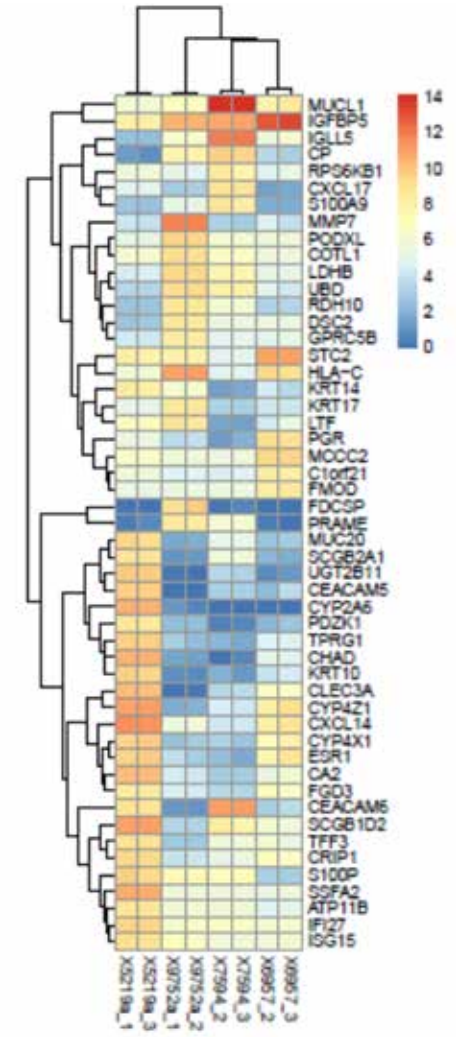

Figure 4. Global gene expression of invasive ductal breast carcinoma research samples. Eight Ion AmpliSeq transcriptome libraries sequenced on an Ion 540™ Chip were analyzed by multisample, hierarchical clustering of the top 50 most variable genes, which show distinct clustering by subsample type. **Clustering parameters:** complete linkage, Euclidean distance metric; **filtering:** row sum across samples ≥500 read counts; **per-library normalization:** RPM values taken directly from the Torrent Suite Software AmpliSeq RNA plugin.

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Ordering information

Product	Description	Size	Cat. No.
Targeted sequencing panels for cancer research			
Oncomine Comprehensive Assay	143 genes, 3 primer pools	Inquire	Inquire
Ion AmpliSeq Transcriptome Human Gene Expression Kit	20,800 genes, 20,800 amplicons, 1 primer pool	24 reactions	A26325
Ion AmpliSeq library preparation			
Ion AmpliSeq Library Kit 2.0	Manual Ion AmpliSeq library preparation	8 reactions	4475345
		96 reactions	4480441
		384 reactions	4480442
Ion Xpress Barcode Adapters 1-96 Kit	96 unique barcode adapters	1 kit	4474517
Ion Library Equalizer Kit	Bead-based solution replacing the need for library quantification and library dilutions for library normalization	96 reactions	4482298
Automated Ion AmpliSeq library preparation			
Ion AmpliSeq Kit for Ion Chef DL8	Automated Ion AmpliSeq library preparation supplied with IonCode barcodes	4 x 8 reactions	A29024
Template preparation			
Ion Chef System	Automates template preparation and chip loading	1 system	4484177
Ion 520/530 Kit-Chef	Template preparation for Ion 520 and Ion 530 Chips on the Ion Chef System	8 reactions	A27757
Ion 540 Kit-Chef	Template preparation for Ion 540 Chips on the Ion Chef System	8 reactions	A27759
Sequencing			
Ion S5 System	Ion S5 next-generation sequencing system	1 system	A27212
Ion S5 XL System	Ion S5 XL next-generation sequencing system	1 system	A27214
Ion 520 Chip Kit	Sequencing reagents including an Ion 520 Chip	8 reactions	A27762
Ion 530 Chip Kit	Sequencing reagents including an Ion 530 Chip	8 reactions	A27764
Ion 540 Chip Kit	Sequencing reagents including an Ion 540 Chip	8 reactions	A27766
Data analysis			
Ion Reporter Software		thermofisher.com/ionreporter	

Find out more at **thermofisher.com/ions5**

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