ion torrent
by life technologies™

Discover variants in 46 cancer genes using the Ion AmpliSeq™ Cancer Panel v1

- Transformative technology that generates 190 amplicons surveying 739 mutations, using a simple, single-tube multiplex PCR assay
- Low–input DNA protocol (10 ng), compatible with FFPE research samples
- Workflow from DNA to annotated variants in just 10 hours
- Molecular barcodes enable cost-effective sample multiplexing
- Recent publication detects variant present at 5.5% frequency

Now available: Ion AmpliSeq™ Cancer Hotspot Panel v2

- Target list expanded to 50 genes, including *EZH2, GNA11, GNAQ,* and *IDH2*
- Higher coverage uniformity
- Improved variant detection with Torrent Suite Software v3.0

Introduction

Targeted resequencing can be utilized for efficient interrogation of specific genes or genomic regions. Current target selection methods are lengthy, and complex, and usually require large amounts of input DNA. Truly effective translational research depends on the development of rapid approaches that deliver reliable results from small amounts of sample material and from challenging research samples such as formalin-fixed, paraffin-embedded (FFPE) tissues.

Ion AmpliSeq™ target selection technology introduces a groundbreaking workflow that overcomes most known barriers to multiplex PCR, enabling the rapid sequencing of hundreds of known mutations for low-frequency allele detection or germline mutation detection. The single-tube workflow is as simple as setting up a PCR reaction and can be performed by any member of the lab. Superior coverage to detect low-frequency mutations, and automated analysis tools with Torrent Suite Software or Ion Reporter™ Software, enable you to go from extracted DNA to variant calls for relevant gene regions in just one day.

Ion AmpliSeq™ target selection technology offers researchers the convenience of fixed-content panels and the power to create custom-content panels for use with Ion PGM™ and Ion Proton™ Systems. Our ready-to-use panels include the Ion AmpliSeq™ Cancer Panel, the Ion AmpliSeq™ Comprehensive Cancer Panel, and the Ion AmpliSeq™ Inherited Disease Panel. For ultimate flexibility, Ion AmpliSeq™ Designer web-based software enables scientists to upload gene lists and create custom panels.

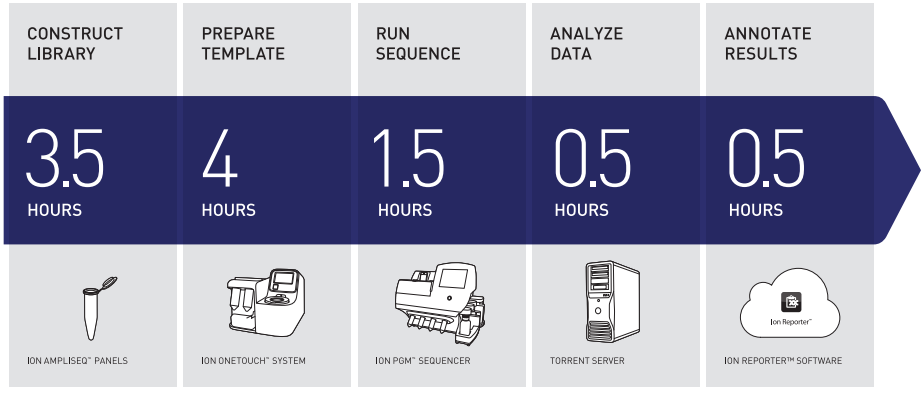

Figure 1. Ion AmpliSeq™ Cancer Panel single-day (10-hour) workflow. This workflow uses a single tube of pooled primers and a 100-base sequencing run with an Ion 314™ Chip and includes the option to annotate results with Ion Reporter™ Software. Complete automation options allow the three final steps—run sequence, analyze data, and annotate results—to be completed hands-free and initiated at the end of the work day.

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Low input DNA and single-day workflow

The Ion AmpliSeq™ Cancer Panel responds to the needs of clinical and translational researchers. The rapid, low-input DNA (10 ng) protocol makes it possible to screen FFPE samples that are out of reach for other target selection methods. A single-day workflow (Figure 1) allows rapid parallel assessment of hundreds of informative mutations. Targets are amplified using 10 ng of DNA and the Ion AmpliSeq™ Cancer Panel primer pool in a single-tube multiplex PCR reaction. After purification and phosphorylation of amplicons, standard library preparation follows. With this workflow, you can go from DNA to complete library generation in just 3.5 hours, with 30 minutes of hands-on time, for target selection of 190 amplicons. Template preparation follows with the automated Ion OneTouch™ System. Results are available for analysis after 90 minutes of sequencing run time on the Ion PGM™ System. The entire Ion AmpliSeq™ workflow—from DNA to variant calls—is complete in just 10 hours, with walk-away sequencing and data analysis. Variant calls from Torrent Suite Software are then viewed through a standard web browser.

Coverage of critical gene regions

The Ion AmpliSeq™ Cancer Panel combined with the Ion PGM™ System transforms oncology research with a single-tube assay that allows accurate and affordable detection of low-frequency mutations. Designed with input from premier oncologists and cancer researchers, the Ion AmpliSeq™ Cancer Panel v1 covers relevant regions across 46 well-established oncogenes and tumor suppressor genes. A single primer pool tube enables superior coverage of targeted mutations, with wide coverage of variants in the *KRAS, BRAF,* and *EGFR* genes, for the detection of somatic mutations in fresh-frozen or archived (FFPE) cancer samples.

To view the list of Ion AmpliSeq™ Cancer Panel v1 targeted genes and mutations, please visit http://lifetech-it.hosted.jivesoftware.com/docs/DOC-2182.

Table 1. Results from four samples processed using the Ion AmpliSeq™ Cancer Panel v1, Ion AmpliSeq™ Library Kit 2.0, and Torrent Suite Software v2.2.

Sample	1	2	3	4
Per-base accuracy	99.61%	99.60%	99.53%	99.52%
On-target reads	96.41%	96.34%	96.16%	96.4%
Coverage uniformity	98.06%	97.98%	98.47%	97.91%

High-sensitivity detection for low-frequency mutations

Incorporating the Ion AmpliSeq™ Library Kit 2.0, the Ion AmpliSeq™ Cancer Panel v1 is designed to deliver the data accuracy needed for detection of low-frequency mutations. Table 1 shows the high level of accuracy observed across three typical data sets obtained using the Ion AmpliSeq™ Cancer Panel v1, Ion AmpliSeq™ Library Kit 2.0, and Torrent Software Suite v2.2.

Three key metrics for experimental success are highlighted:

- **Per-base accuracy**, determined by aligning the reads to the reference. Highly accurate raw data enable detection of low-frequency variants with the appropriate coverage.
- **On-target reads** is the percentage of reads mapping to target regions. The more reads that map to the target, the more efficient and cost-effective the experiment will be.
- **Coverage uniformity** is the percentage of bases with >20% of mean coverage. The greater the percentage covered in this metric, the tighter the distribution and the fewer bases that have low coverage.

Discovery of critical variant at 5.5% frequency

In a recent publication,Yang et al. [1] described the histological and genetic analysis of two tumor samples (initial and reoccurrence) from the same individual. Following negative screening of candidate genes using Sanger sequencing, the Ion AmpliSeq™ Cancer Panel v1 was employed. Ten nanograms of FFPE-derived DNA from each sample was used for sequencing, and a variant in the *MET* gene that caused an amino acid change (a variant not previously observed in this particular tumor type) was present at 5.5% in the reoccurrence sample and at a very low but detectable level in the initial sample, demonstrating the ability of the Ion AmpliSeq™ Cancer Panel v1 to detect new variants at very low frequencies.

Automated variant analysis using Torrent Suite Software and Ion Reporter™ Software

For analysis of the results from the Ion AmpliSeq™ Cancer Panel, there are two methodologies available—Torrent Suite Software and Ion Reporter™ Software.

Torrent Suite Software

Sequence reads are generated and mapped to a reference sequence using Torrent Suite Software (TSS), v2.0 or higher. The Torrent Variant Caller Plugin utilizes the mapped reads to call SNPs and insertion and deletion variants.

Using the Torrent Variant Caller Plugin, the user can specify run type, target regions, hotspot regions, and somatic or germline variant detection. The user may also specify regions corresponding to known variant locations.

When using the Ion Xpress™ Barcode Adaptor Kit, the Torrent Variant Caller Plugin will separate the samples and report variants by sample based on the assigned barcodes (up to 96 barcodes are available). Data can be produced using the standard output files on the Ion PGM™ System, including FASTQ, BAM, and VCF, which facilitate analysis in a wide variety of third-party software applications.

Ion Reporter™ Software

Ion Reporter™ Software is a cloud-hosted software tool for automated variant analysis that enables laboratories of any size to create reproducible, quality-controlled analysis pipelines and produce annotated and classified variant calls. These annotations are derived from public databases (e.g., dbSNP, COSMIC, Ensembl, RefSeq). Users can also add their own data and utilize this information to classify and filter the variants.

The workflow is summarized in Figure 2 and consists of four basic activities:

- Import—initiate analysis pipeline on new data
  - Upload semiconductor sequencing data directly from Torrent Browser to the secure, centralized Ion Reporter™ Server
  - Select a preconfigured workflow
  - Launch analysis
- Analyze—review variants and annotations
  - Filter by evidence for confidence in variant calls
  - Filter by annotations for relevance data
- Report—finalize the annotated variant analysis
  - Classify variants by impact
  - Add interpretation for each variant
  - Write summary statement
- Archive—securely save the data
  - Gain access to individual files and audit logs
  - Store in the cloud or download to your local storage

Ion Reporter™ Software automates all the steps from the sequencing reads to the annotated variants (Figure 2). Researchers are able to concentrate on interpretation of the observed variants and decisions about their relevance.

Figure 2. Ion Reporter™ Software automates all the steps of your bioinformatics pipeline, starting with the chip loading/run planning and removing all hands-on time up to interpretation of annotated variants.

The Torrent Variant Caller Plugin identifies and provides standard variant information. The Ion Reporter™ Software takes variant analysis one step further by automatically searching numerous databases for genomic annotations for found variants. This automated feature provides the latest and most complete annotations, without researchers having to perform time-intensive searches among multiple databases (Table 2).

Orthogonal confirmation with TaqMan® Assays

To confirm the accuracy of the Ion AmpliSeq™ Cancer Panel v1, 62 of the variant positions targeted in the Ion AmpliSeq™ Cancer Panel v1 were also assayed using TaqMan® Mutation Detection Assays powered by castPCR™ technology. The castPCR™ technology uses competitive allele-specific TaqMan® PCR for mutation detection, with a limit of detection of 5 to 10 copies. These assays provide superior sensitivity (down to 0.1%) and specificity (>99.9%) to detect and quantitate very low amounts of mutant DNA in mixed sample populations.

Nine breast cancer sample pairs were tested, each sample pair consisting of an FFPE sample and a fresh-frozen sample taken at the same time. The results from the Ion AmpliSeq™ Cancer Panel v1 and TaqMan® Mutation Detection Assays at these 62 variant positions were compared. Of the 558 sites assayed, concordance was 99.5% between the two technologies for all mutations present at >1% frequency.

To enable easy selection of TaqMan® Genotyping Assays, the Torrent Variant Caller allows users to select called variants and automatically search for the matching TaqMan® SNP Genotyping Assays (recommended for germline mutation) or TaqMan® Mutation Detection Assays (specifically designed for somatic mutation detection). This feature greatly facilitates the design of variant confirmation or sample screening experiments.

Highly scalable and affordable

Targeted sequencing with Ion AmpliSeq™ technology is scalable in multiple ways—multiplexing of primer pairs in each tube, multiple Ion chips with different throughputs, and up to 96 barcodes for sample multiplexing. The number of primer pairs per pool is highly scalable, allowing researchers to target anything from a single gene to hundreds of genes while maintaining a low number of pools per panel. Ion AmpliSeq™ Custom Panels and Ion AmpliSeq™ Ready-to-Use Panels contain up to several thousand primer pairs per pool. For each experiment, you can use Ion Xpress™ Barcode Adaptors for multiplexing samples at equal or asymmetric concentrations.

Table 2. Analysis outputs from Ion Reporter™ Software, showing rich annotation derived from public databases for three variants.

RefSeq	Ensembl	Genomic	Transcript	Exon	Coding	Protein	Filtered coverage	Allele coverage	Allele frequency
MPL	MPL	chr1:43815008T	NM_005373	10	c. =	p.Trp515T	588	T = 587	T = 1.0
MPL	MPL	chr1:43815009G	NM_005373	10	c. =	p.Trp515T	431	T = 427	G = 0.99
NRAS	NRAS	chr1:115256528T	NM_002524	5	c. =	p.Gln61Gl	1,764	T = 1,749	T = 0.99

Table 3. Typical Ion AmpliSeq™ Cancer Panel v1 sequencing coverage on Ion 314™ Chip.

Depth of sequencing coverage	>100x	>500x	Average depth of coverage
All loci	96.4%	88.1%	2,107x
Loci in *KRAS*, *BRAF*, and *EGFR*	98.7%	94.1%	2,379x

The various Ion chip outputs provide high versatility for sample processing and experimental design. The output of the Ion 314™ Chip allows for affordable processing of one sample with the Ion AmpliSeq™ Cancer Panel. With at least 300,000 reads on an Ion 314™ Chip, this results in greater than 500x depth of coverage for more than 90% of the loci in the *KRAS, BRAF,* and *EGFR* genes, with average depth of coverage for all targeted mutations typically >2,000x (Table 3). This level of coverage allows for accurate detection of variants present at a 5% level, with 99% confidence.

The additional throughput of the Ion 316™ Chip and Ion 318™ Chip also allows for multiplexing using Ion Xpress™ Barcode Adapters Kits for both somatic and germline mutation detection, with increased depth of coverage (i.e., >3,000x) for detection of extremely low-frequency mutations. Four or more samples can be sequenced on the Ion 316™ Chip.

The Ion AmpliSeq™ target selection technology is the fastest and simplest approach to generating libraries of targeted regions of interest. A single-tube assay, as simple as setting up a PCR reaction, can generate up to 4,000 amplicons. The Ion AmpliSeq™ Cancer Panel v1 allows characterization of hundreds of mutations across 46 cancer genes in a single day—with high sensitivity and high accuracy using a single-tube multiplex PCR reaction and 10 ng of DNA.

Ordering information

Product	Cat. No.
Ion AmpliSeq™ Cancer Hotspot Panel v2 (primer pool targeting 50 genes)	4475346
Ion AmpliSeq™ Cancer Panel v1 (primer pool targeting 46 genes)	4471262
Ion AmpliSeq™ Library Kit 2.0 (8, 96, 384 reactions for both PCR amplification and library construction)	4475345, 4480441, 4480442
Ion Xpress™ Barcode Adapters Kits	4474517, 4471250, 4474009, 4474518, 4474519, 4474520, 4474521
Additional Ion AmpliSeq™ products	
Ion AmpliSeq™ Comprehensive Cancer Panel (primer pool)	4477685
Ion AmpliSeq™ Inherited Disease Panel (primer pool)	4477686
Ion AmpliSeq™ Custom Panels are orderable via Ion AmpliSeq™ Designer Learn more at **lifetechnologies.com/ampliseqcustom**	

References

1. Yang MMH, Singhal A, Rassekh SR et al. (2012). Possible differentiation of cerebral glioblastoma into pleomorphic xanthoastrocytoma: an unusual case in an infant. *J Neurosurg Pediatr 9:517–523.*

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