

Ion AmpliSeq™ Cancer Panel

- Low-DNA input protocol (10 ng) compatible with research FFPE samples
- Transformative technology that generates 190 amplicons using a simple, single-tube, multiplex PCR assay
- Rapid, affordable sequencing in just 90 minutes
- Go from extracted DNA to variant report in just 9.5 hours
- Molecular barcodes enable cost-effective sample multiplexing
- Scalable technology for deep sequencing of low-frequency mutations in heterogeneous samples or for germline mutation detection

Introduction

Targeted resequencing can be utilized for efficient sequencing of specific genes or genomic regions. Current target selection methods are lengthy, complex, and usually require large amounts of input DNA. Translational and clinical research require rapid approaches that are compatible with small input amounts of nucleic acids derived from challenging research samples such as formalin-fixed, paraffin-embedded (FFPE) tissues.

By overcoming known barriers of multiplex PCR, the Ion AmpliSeq™ Target Selection Technology introduces a groundbreaking workflow enabling the rapid sequencing of hundreds of known mutations with low-frequency allele detection. Utilizing low DNA input, this single-tube workflow is as simple as setting up a PCR reaction and can be performed by any laboratory technician. Superior coverage to detect low-frequency mutations and push-button analysis tools with Torrent Suite Software enable you to go from extracted DNA to variant calls in just one day.

For research use only, the Ion AmpliSeq™ Target Selection Technology offers researchers the convenience of fixed-content panels and the power to create custom-content panels. The Ion AmpliSeq™ Cancer Panel is the first available fixed-content panel, and coming soon are the Ion AmpliSeq™ Comprehensive Cancer Panel and the Ion AmpliSeq™ Inherited Disease Panel. For ultimate flexibility, the soon-to-be-released Ion AmpliSeq™ Designer web-based software will enable scientists to upload custom human content for assay designs.

Low-DNA input protocol and a single-day Ion AmpliSeq™ workflow

The Ion AmpliSeq™ Cancer Panel matches the needs of clinical and translational research. The rapid, low-DNA (10 ng) input protocol enables the screening of FFPE samples that cannot be addressed by other target selection methods. A single-day workflow (shown in Figure 1), allows for rapid parallel assessment of hundreds of informative mutations.



Figure 1. Single-day (9.5 hours) workflow using a single primer tube and an Ion 314™ Chip.

Targets are amplified using 10 ng of DNA and the Ion AmpliSeq™ Cancer Primer Pool in a single-tube multiplex PCR reaction. After purification and phosphorylation of amplicons, standard library preparation follows. This allows you to go from DNA to complete library generation in just 3.5 hours, with 15 minutes of hands-on time for target selection of 190 amplicons. Template preparation follows with the fully 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 goes from DNA to variant calls in just 9.5 hours, with walk-away sequencing and data analysis. Variant calls from Torrent Suite Software are then viewed through a standard web browser. For those situations where multiple samples need to be analyzed, the scalability of the Ion PGM™ system can be utilized by running a higher-capacity chip (Ion 316™ Chip or Ion 318™ Chip), which decreases cost per sample and maintains the high coverage needed to call low-frequency variants. All that is needed to enable the use of up to 32 barcodes is the addition of the Ion Xpress™ Barcode Adaptor Kit to the workflow.

Complete coverage of critical gene regions with the Ion AmpliSeq™ Cancer Panel

With the Ion AmpliSeq™ Cancer Panel, the Ion PGM™ system transforms oncology research with a single-tube assay that allows for the detection of low-frequency mutations accurately and affordably. The Ion AmpliSeq™ Cancer Panel covers relevant regions across 46 oncogenes and tumor suppressor genes, with emphasis on variants in the *KRAS*, *BRAF*, and *EGFR* genes, for the detection of somatic mutations in archived cancer samples. Designed with input from premier oncologists and cancer researchers, a single primer pool tube enables superior coverage of all targeted mutations in key cancer genes, including well-established tumor suppressor genes and oncogenes.

To view the Ion AmpliSeq™ Cancer Panel list of targeted genes and mutations, please visit <http://products.invitrogen.com/ivgn/product/4472395>.

Table 1. Typical Ion AmpliSeq™ Cancer Panel sequencing coverage on Ion 314™ Chip.

Depth of sequencing coverage	>100x	>500x	Average depth of coverage
All mutations	96.4%	88.1%	2,107x
Mutations in <i>KRAS</i> , <i>BRAF</i> , and <i>EGFR</i>	98.7%	94.1%	2,379x

High-sensitivity detection for low-frequency mutations

Genomic DNA was extracted from FFPE samples. The extracted DNA was quantitated with the Qubit® Fluorometer and the Qubit® dsDNA HS Assay Kit. Samples were then sequenced using the Ion AmpliSeq™ Cancer Panel and the Ion PGM™ sequencer. Using 10 ng of FFPE DNA, samples were processed according to the Ion AmpliSeq™ Library Preparation User Guide. The amplicon library from each sample was sequenced on the Ion PGM™ sequencer using an Ion 314™ Chip, following template preparation.

Given the accuracy of the Ion PGM™ system and the sensitivity of the Ion AmpliSeq™ technology, baseline noise levels are very low across amplicons targeted by the Ion AmpliSeq™ Cancer Panel for various samples (Table 2). This gives a high level of confidence in the detection of low-frequency alleles in heterogeneous populations with the Ion AmpliSeq™ Cancer Panel.

Figure 2 shows one amplicon in the *MET* gene with a 5.3% allele frequency mutation at position 61 of the amplicon. This highlights the sensitivity and accuracy of the Ion PGM™ system when using Ion AmpliSeq™ Cancer Panel. This mutation was detected at 3,426x depth of coverage with a median baseline noise level of 0.32% (signal-to-noise ratio >16). This high level of accuracy and the high depth of coverage achieved with the Ion PGM™ system allow for reliable detection of low-frequency mutations, with high confidence, in heterogeneous samples.

Table 2. Baseline noise levels using the Ion AmpliSeq™ Cancer Panel.

Sample	Baseline noise level (all amplicons)	Baseline noise level (<i>KRAS</i> , <i>BRAF</i> , and <i>EGFR</i> amplicons)
Cell line sample	0.43%	0.40%
FFPE tonsil control sample	0.41%	0.40%

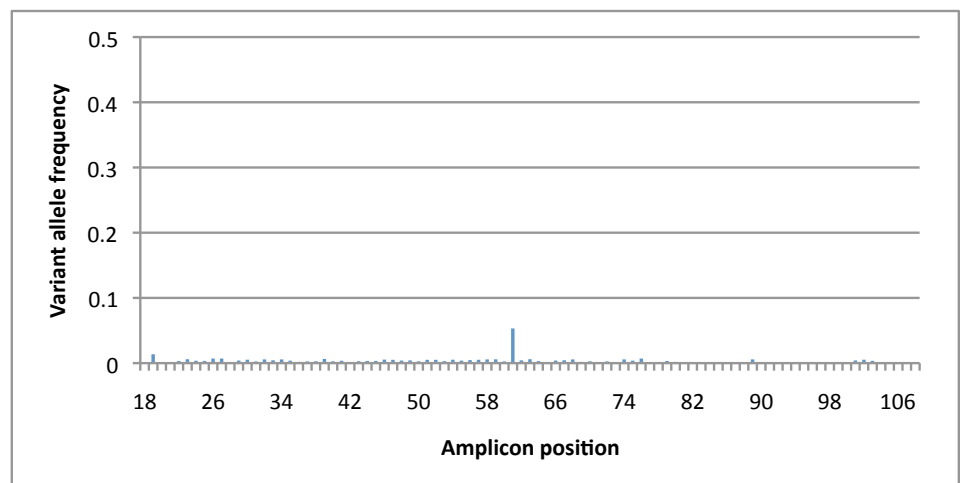


Figure 2. Low-frequency allele mutation detection using the Ion AmpliSeq™ Cancer Panel.

ION Variant Caller Plugin

Reference Genome: hg19

Library Type:

Variant Frequency:

Targeted regions:

Hotspot regions:

Figure 3. The data input panel for the Torrent Variant Caller plugin.

Variant Caller Report

Barcode Coverage and Variants Report								
Variant Caller Reports	Mapped Reads	Reads On-Target	Bases On-Target	Read Depth	1x Coverage	20x Coverage	100x Coverage	Variants Detected
lonXpress_001	179,515	97.18%	86.17%	893.21	99.267%	98.033%	90.067%	10
lonXpress_002	246,842	97.05%	86.09%	1,218.46	99.267%	98.542%	93.882%	10

Figure 4. Barcode Summary Report. Each line is a summary of a single barcoded sample.

Allele Coverage for all bases in HotSpot Regions												
Show <input type="text" value="50"/> entries		Search: <input type="text" value="HSM347"/>										
Chromosome	Position	Region ID	HotSpot ID	Ref	Coverage	A	C	G	T	INDEL	Cov (+)	Cov (-)
chr10	43609951	AMP43609942	HSM347	C	208	0	208	0	0	0	80	128
chr10	43609952	AMP43609942	HSM347	G	208	0	0	208	0	0	80	128
chr10	43609953	AMP43609942	HSM347	C	209	0	209	0	0	0	80	129
chr10	43609954	AMP43609942	HSM347	A	209	209	0	0	0	0	80	129
chr10	43609955	AMP43609942	HSM347	C	209	0	208	0	1	0	80	129
chr10	43609956	AMP43609942	HSM347	G	209	0	0	209	0	0	80	129
chr10	43609957	AMP43609942	HSM347	G	209	0	0	209	0	0	80	129
chr10	43609958	AMP43609942	HSM347	T	209	0	0	0	209	0	80	129
chr10	43609959	AMP43609942	HSM347	G	209	0	0	209	0	0	80	129
chr10	43609960	AMP43609942	HSM347	A	209	208	0	1	0	0	80	129
chr10	43609961	AMP43609942	HSM347	T	209	0	0	0	209	0	80	129
chr10	43609962	AMP43609942	HSM347	C	209	0	209	0	0	0	80	129
chr10	43609963	AMP43609942	HSM347	G	209	0	0	209	0	0	80	129
chr10	43609964	AMP43609942	HSM347	C	209	0	209	0	0	0	80	129
chr10	43609965	AMP43609942	HSM347	A	209	209	0	0	0	0	80	129
chr10	43609966	AMP43609942	HSM347	G	209	0	0	209	0	0	80	129

Showing 1 to 16 of 16 entries (of 837) First Previous 1 Next Last

Figure 5. Torrent Variant Caller Report: panel showing allele coverage for all HotSpot Regions.

Push-button variant analysis using Torrent Suite Software

Sequence reads are generated and mapped to a reference sequence using the Torrent Suite Software (TSS), version 2.0 or higher. The Torrent Variant Caller utilizes the mapped reads to call SNPs and insertion and deletion variants.

In the Torrent Variant Caller plugin (Figure 3), the user can specify run type, target regions, hotspot regions, and high- or low-frequency variant detection. The user may also specify regions corresponding to known variant locations, using the hotspot regions BED file.

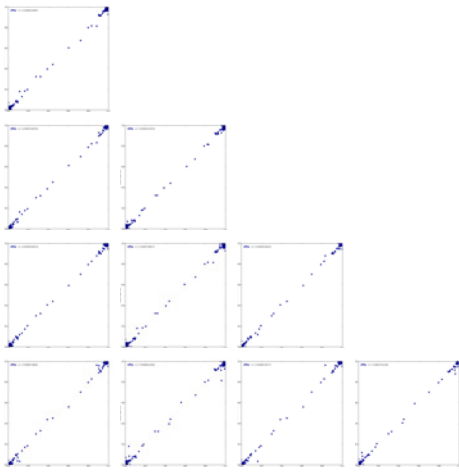
When using the Ion Xpress™ Barcode Kit, the Torrent Variant Caller will generate a Barcode Summary Report (Figure 4), with each row corresponding to the reads assigned to a single sequence barcode. In this example, two barcodes were used.

Each row in the table shown in Figure 5 shows detailed information for each “HotSpot” base, including the chromosomal location, HotSpotID, the expected base, and the number of reads that cover this base. The right-most columns of the table report numbers of reads showing variant bases, the presence of indels, and reads separated by strandedness.

Data can be output using the standard output files of the Ion PGM™ system, including FASTQ, BAM, and VCF, which facilitate analysis in a wide variety of third-party applications.

Orthogonal confirmation with castPCR™ technology

For confirmation of *KRAS*, *BRAF*, and *EGFR* mutations covered by the Ion AmpliSeq™ Cancer Panel, Life Technologies offers 44 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 1 to 5 copies. These assays provide superior sensitivity (0.1%) and specificity (>99.9%) to detect and quantitate very low amounts of mutant DNA in mixed sample populations.



	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5
Replicate 1	1.00000				
Replicate 2	0.99988	1.00000			
Replicate 3	0.99992	0.99991	1.00000		
Replicate 4	0.99992	0.99988	0.99994	1.00000	
Replicate 5	0.99989	0.99981	0.99989	0.99992	1.00000

Figure 6. Reproducibility of the Ion AmpliSeq™ workflow.

High workflow reproducibility

Reproducibility of the Ion AmpliSeq™ workflow is extremely high (Figure 6). Five replicate AcroMetrix® KRAS FFPE control samples were processed using the Ion AmpliSeq™ Cancer Panel and sequenced on the Ion PGM™ sequencer in single replicates using 10 ng DNA. The average correlation coefficient across all sample runs was 0.99989.

Ion AmpliSeq™ scalability

While the Ion AmpliSeq™ Cancer Panel utilizes a single tube with 190 primer pairs, this approach is entirely scalable to increasing the number of primer pairs per tube. The Ion AmpliSeq Custom Solutions, soon to be released, will generate up to 1,536 amplicons in a single tube. For more information on Ion AmpliSeq™ Custom Panels,* please visit the Ion Community.

The simple Ion AmpliSeq™ workflow can also be easily adapted to accommodate multiple primer pool tubes, each using only 10 ng of DNA. For example, 8 tubes with 480 primer pairs each would require 80 ng of DNA in total, for 3,840 primer pairs. Additionally, protocols can be optimized for manual or automated processing of 96-well or 384-well plates.

The various chip outputs from Ion Torrent 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; four or more samples can be sequenced on the Ion 316™ Chip. 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 *KRAS*, *BRAF*, and *EGFR*, with average depth of coverage for all targeted mutations typically >2,000x (Table 1). This level of coverage allows for accurate detection of 5% frequency alleles with 99% confidence.

The additional throughput of the Ion 316™ Chip and Ion 318™ Chip* also allows for multiplexing with Ion DNA barcoding kits for both somatic and germline mutation detection. With increased system accuracy expected in coming months, samples can also be run on the Ion 316™ Chip or Ion 318™ Chip* with increased depth of coverage (i.e., >3,000x) for detection of extremely low-frequency alleles.

Conclusions

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 1,536 amplicons. The Ion AmpliSeq™ Cancer Panel allows the characterization of hundreds of mutations across 46 key 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 Panel (includes primer pools and reagents required for library preparations)	4472395
Ion Xpress™ Barcode Adaptors 1-16 Kit (10 sets of 16 libraries)	4471250
Ion Xpress™ Barcode Adaptors 17-32 Kit (10 sets of 16 libraries)	4474009

* The content provided herein may relate to products that have not been officially released and is subject to change without notice.

Additional reagents for template preparation, sequencing, FFPE controls, nucleic acid extraction and quantitation, and TaqMan® assays are available for purchase through Life Technologies.

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Ion Torrent by Life Technologies | 7000 Shoreline Court | Suite 201 | South San Francisco, CA 94080 USA
Phone +1-203-458-8552 | Toll Free in North America 1-87-SEQUENCE (1-877-378-3623)

www.lifetechnologies.com | www.iontorrent.com | http://ioncommunity.iontorrent.com C001585 0112

