Ion AmpliSeq™ RNA Panels—quantitative targeted gene expression analysis

Introduction

The Ion AmpliSeq™ technology is well established as a leading methodology to target desired genomic regions for sequence analysis using the Ion Torrent™ Personal Genome Machine [PGM™] Sequencing System. We have leveraged the highly reproducible Ion AmpliSeq™ workflow and included adaptations to selectively amplify specific RNA targets. With the launch of the Ion AmpliSeq™ RNA applications, customers can choose from over 20,000 targeted genes with which to interrogate their samples. Additional ready-to-use panels are available, targeting both cancer and apoptosis pathways. After sequencing the RNA-derived amplicons on the Ion Torrent™ sequencing platform, the number of reads mapped to each gene corresponds to the abundance of the assayed gene in the test sample. Relative quantitation of the target genes is maintained between test samples.

Ion AmpliSeq™ RNA amplicons are designed to provide the greatest coverage of multiple transcripts that are often assigned to a single gene in standard databases such as RefSeq. This approach of highest coverage allows gene-level detection with greater confidence across a broad range of samples types. Amplicons are also designed to bridge an exon–exon boundary whenever possible to minimize the impact of residual genomic DNA in isolated total RNA. The unique Ion AmpliSeq™ technology coupled with RNA-specific primer design parameters enables up to 300 customer-defined genes in one amplification reaction from very low amounts of input total RNA (down to 500 pg).

The new capabilities created with Ion AmpliSeq™ RNA primer design and optimized library preparation reagents allow researchers to specifically sequence genes from their own custom-designed pools or our fixed-panel content. This flexibility allows maximum utilization of sequencing depth to deliver key gene abundance information to the translational researcher. Additionally, the nucleotide sequence of the target amplicon is provided, allowing for gene-specific information at the nucleotide level using Ion AmpliSeq™ RNA tools. For added flexibility, the current Ion Xpress™ Barcode Adaptors can be utilized to multiplex multiple libraries on one of the larger-capacity sequencing chips, such as the Ion 318™ Chip. An Ion AmpliSeq™ RNA-specific reference and BED file for the targets in a panel are deployed using the RNA Coverage Analysis Plug-in, which enables fast and efficient data analysis. Taken together, this methodology offers a complete sequencing solution for targeted RNA analysis using Ion Torrent™ post-light technology.

Currently available:

- Ion AmpliSeq™ RNA Library Kit (8, 96, and 384 rxns)
- Ion AmpliSeq™ RNA Custom Panel
- Ion AmpliSeq™ RNA Cancer Panel
- Ion AmpliSeq™ RNA Apoptosis Panel
- Ion Xpress™ Barcode Adaptors
Targeted RNA analysis with AmpliSeq™ technology

The Ion AmpliSeq™ RNA Library Kit employs the current Ion AmpliSeq™ reagents and adds reverse transcription (RT) enzyme and Dynabeads® Cleanup Modules required for targeted RNA analysis. The new library kit’s user manual includes specific recommendations for extremely low input of RNA (down to 500 pg, unfixed) and FFPE (down to 5 ng) samples. This enables translational researchers to perform RNA sequencing analysis from precious sample types such as formalin-fixed and fresh-frozen tumor samples, serum samples, and circulating tumor cells—materials that typically don’t yield sufficient quantities of RNA for classic RNA-Seq methods. Additionally, the Ion AmpliSeq™ RNA sequencing workflow is ideal for researchers who want to query a subset of genes involved in a specific disease pathway. Ion AmpliSeq™ RNA sequencing of targeted genes addresses these and other challenges facing basic and translational researchers.

In addition to custom panels, two ready-to-use panels are currently available. The Ion AmpliSeq™ RNA Cancer Panel was developed to target the 50 genes covered by the Ion AmpliSeq™ Cancer Hotspot Panel v2 (targets DNA “hotspot” regions frequently mutated in human cancer genes). This RNA panel detects 50 tumor suppressor genes and oncogenes, including KRAS, BRAF, and EGFR. The second ready-to-use panel—the Ion AmpliSeq™ RNA Apoptosis Panel—is a single pool of primers representing 267 genes involved in the cellular apoptosis pathway, including genes associated with death receptor-, c-Myc, and p53-mediated apoptosis.

Incorporating improvements in amplicon primer design, library preparation, and coverage analysis, Ion AmpliSeq™ RNA technology can provide relative abundance data for the assayed genes in only 1.5 days. The cost per sample decreases with the ability to multiplex as many as 10 Ion AmpliSeq™ RNA Cancer Panel libraries on a single Ion 318™ Chip. The multiplexing capacity is dependent on the number of genes in a library and the dynamic range of the targeted gene expression levels in each library.

Gene expression levels from Ion AmpliSeq™ RNA libraries correlate with those from TaqMan® Assays

An experiment was designed to compare relative gene expression levels measured using the new Ion AmpliSeq™ RNA Cancer Panel reagents with those obtained using TaqMan® Gene Expression Assays. We used Universal Human Reference RNA (UHRR) and the Human Brain Reference RNA (HBRR) as test samples. Because UHRR and HBRR have been extensively evaluated as part of the Microarray Quality Control (MAQC) studies, relative gene expression data are publically available (based on microarray analysis and TaqMan® assay qRT-PCR data), making them suitable control RNA samples.

Libraries using the Ion AmpliSeq™ RNA Library Kit and Ion AmpliSeq™ RNA Cancer Panel oligos were generated from 10 ng of total RNA, clonally amplified using the automated Ion OneTouch™ DL template preparation reagents, and sequenced on Ion 318™ Chips. Five independent libraries were evaluated in the Ion AmpliSeq™ RNA workflow in total, two UHRR libraries and three HBRR libraries. A representative example showing mapping information from both UHR and HBR control RNAs using the fixed-content cancer panel is shown in Table 1.

The HBRR/UHRR fold change for each of the 50 genes included in the ready-to-use cancer panel was generated and compared to fold-change calculations of the same genes with TaqMan® Gene Expression Assays. TaqMan® assays were chosen that amplify the same transcripts interrogated by the Ion AmpliSeq™ RNA primers. The impressive correlation of 0.9892 is shown in Figure 1 comparing the Ion AmpliSeq™ RNA Cancer Panel fold-change with the TaqMan® fold-change measurements. All 50 genes targeted in the ready-to-use panel are included in the correlation analysis. These results show the accuracy of the gene expression data obtained with the Ion AmpliSeq™ RNA workflow.

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<th>RNA type</th>
<th>Reads mapped to targets</th>
<th>Off-target reads</th>
<th>Percent reads on target</th>
<th>Targets detected</th>
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<td>UHRR</td>
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<td>98%</td>
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<tr>
<td>HBRR</td>
<td>4.43 million</td>
<td>21,171</td>
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Table 1. Representative mapping information for Ion AmpliSeq™ RNA Cancer Panel libraries.
Simple data processing using the Torrent Suite™ Coverage Analysis Tool

The Ion AmpliSeq™ RNA design tool at ampliseq.com utilizes a unique RNA reference containing the highest-coverage consensus transcript sequence identified for each gene in RefSeq. For custom gene pools, simply input a list of gene symbols and you receive recommendations for amplicons that will target the specified genes, based on proprietary Ion AmpliSeq™ technology that minimizes off-target amplification; primers specific for your custom targets are then ordered through the online tool. You receive a single primer pool for use with the Ion AmpliSeq™ RNA Library Kit, which you can then use for targeted RNA sequencing on the Ion PGM™ Sequencer. The necessary canonical transcript reference and BED files for the fixed panels or your custom gene panel are provided for use with the Coverage Analysis Plug-in—updated for RNA applications to analyze your data quickly on the Torrent Server (Figure 2). After appropriate selections are made for your specific Ion AmpliSeq™ RNA experiment, the plug-in generates an analysis report for each library sequenced. Select the finished plug-in report to view Ion AmpliSeq™ RNA results (Figure 3).

Up to 10 libraries created using the Ion AmpliSeq™ RNA Cancer Panel have been successfully multiplexed with Ion Xpress™ molecular barcodes and evaluated on an Ion 318™ Chip. The extent of multiplexing will depend on the number of genes in your targeted panel, the expression levels of those genes in the test RNAs, and the sequencing capacity of the chip used. General guidelines are provided in the Ion AmpliSeq™ RNA Library Kit user guide.

Figure 1. Correlation of Ion AmpliSeq™ RNA Cancer Panel with TaqMan® Assays. Fold change between HBRR and UHRR samples was determined for 50 target genes, by the two methods of targeted gene expression analysis.

Figure 2. Example of Torrent Coverage Analysis setup for targeted RNA sequencing.

Figure 3. Example of a completed Torrent Coverage Analysis Report Selection.
Conclusions

The Ion AmpliSeq™ RNA targeted gene sequencing technology now enables targeted RNA-Seq from limited samples. The ability to sequence only the genes you’re interested in optimizes the information you are able to obtain from precious samples. The simple workflow consisting of RT reactions, PCR amplification, and bead-based clean-up steps enables high-throughput sample processing when required. The choice of user-identified custom gene panels as well as ready-to-use content for key diseases offers researchers the ability to sequence specific RNAs. In addition, Ion AmpliSeq™ RNA technology allows users to leverage its high reproducibility to generate sensitive and accurate quantitative gene expression analysis from test samples of as little as 500 pg of unfixed or 5 ng of fixed total RNA. This new technology has been developed to generate dependable gene expression profiles from small amounts of RNA from precious samples such as formalin-fixed tumor tissues, enabling gene expression analysis with archived samples—key to translational research.

Ordering information

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