Ion AmpliSeq Colon and Lung Cancer Research Panel v2 and Ion AmpliSeq RNA Fusion Lung Cancer Research Panel

NGS workflows and analysis for both DNA and RNA

Designed with leading researchers from the OncoNetwork Consortium, these two lung cancer research panels are designed to deliver fast answers with minimal sample input. The OncoNetwork Consortium spans twelve institutions across the globe with years of experience in adopting next-generation sequencing (NGS) technologies to pioneer colon and lung cancer research.

Introducing the improved Ion AmpliSeq™ Colon and Lung Cancer Research Panel v2 for DNA analysis, and the Ion AmpliSeq™ RNA Fusion Lung Cancer Research Panel for RNA fusion transcript analysis. Compared to other testing methods, such as qPCR, FISH, or Sanger sequencing, research methods like NGS can deliver more variant or fusion detection results per sample. This is achieved with Ion AmpliSeq™ technology, which enables high multiplex capability with limited tumor samples, such as small formalin-fixed, paraffin-embedded (FFPE) samples or archived samples from fine needle aspiration biopsies.

With as little as 10 ng of DNA or RNA sample input, the Ion Torrent™ NGS workflows enable more answers with fewer samples:

- Small sample input volume requirements
- Minimizes samples rejected due to insufficient sample quantity
- A lower failure rate after workflow is complete—e.g., quantity not sufficient
- Helps reduce retesting
- Higher allowance for tissue heterogeneity

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Sequencing on the Ion PGM™ Sequencer and analysis on Torrent Suite™ and Ion Reporter™ Software completed in 24–48 hours (Figure 1) have these advantages:

- Faster next steps
  - Less waiting
- Differentiated service level
  - Provides information faster compared to alternative methods

"With these two panels, we finally have a tool to determine the main mutations involved in lung cancer research starting from a single archived tumor sample with a limited amount of material."

Nicola Normanno, MD
Chief, Laboratory of Pharmacogenomics
Centro Ricerche Oncologiche Mercogliano
Avellino, Italy

Our team of experienced and dedicated field application specialists enables:

- Faster time to implementation
  - Personalized service specific to your laboratory's unique needs
  - Verified workflows and protocols for sequencing and analysis available
- Increased sequencing expertise
  - Broad sequencing knowledge base, from NGS to Sanger sequencing
  - Previous NGS end users

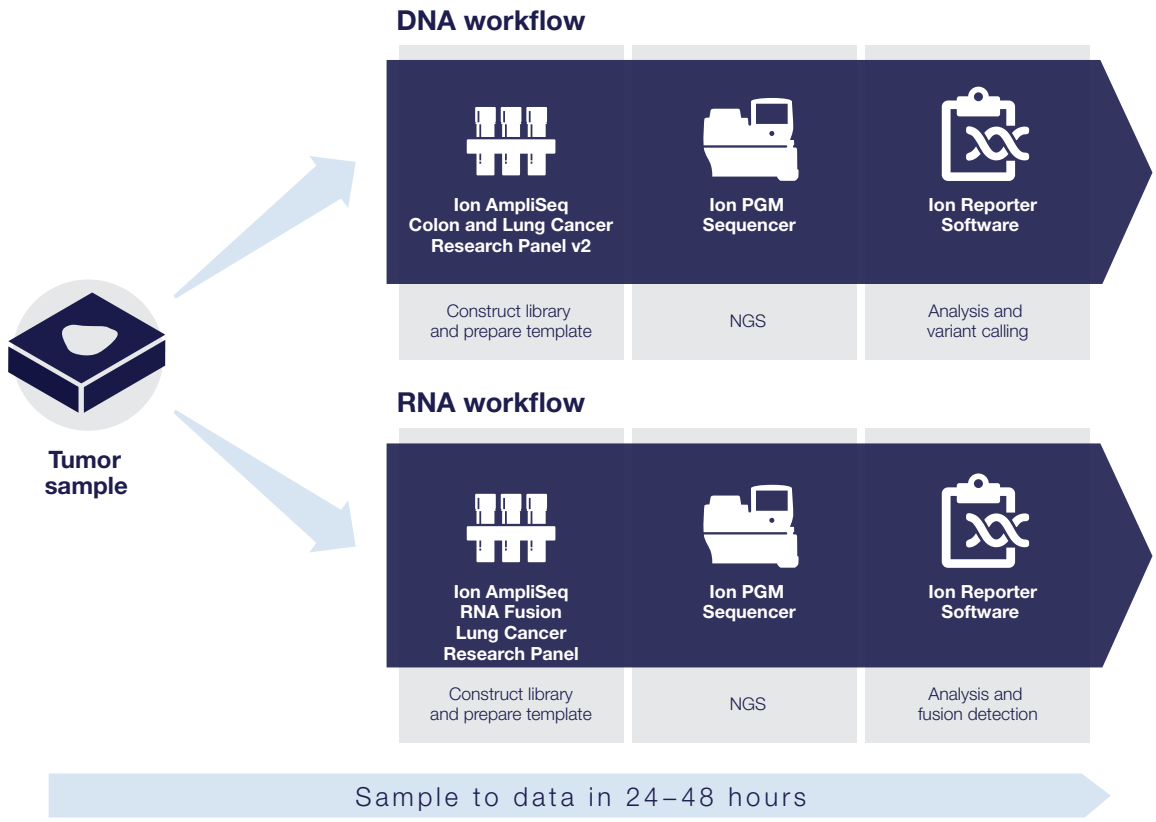

Figure 1. Parallel NGS Ion Torrent workflows for DNA and RNA analysis—resulting in single nucleotide variant and indel data from the DNA workflow, and fusion detection and expression data from the RNA workflow.

Ion AmpliSeq Colon and Lung Cancer Research Panel v2

The Ion AmpliSeq Colon and Lung Cancer Research Panel v2 (Table 1, Figure 2) has primer pairs in a single pool for hotspots and targeted regions for 22 known genes associated with colon and lung tumor tissue. In addition, the panel now also includes primers for 3 additional amplicons covering target regions of the *NRAS* and *ALK* genes:

- *NRAS* exon 4 variants (p.117, p.146)
- *ALK* variants (G1269A, p.S1206Y)

These known rare variants are important in colon and lung cancer research. The first version of the panel, the Ion AmpliSeq Colon and Lung Cancer Research Panel, was tested and verified by the OncoNetwork Consortium, with 155 unique FFPE samples. The Ion AmpliSeq Colon and Lung Cancer Research Panel v2 is ready-to-use and optimized for data analysis with Torrent Suite and Ion Reporter Software.

Table 1. Ion AmpliSeq Colon and Lung Cancer Research Panel v2.

Sample type	FFPE samples
Application	Somatic mutation detection
Genes	*KRAS*, *EGFR*, *BRAF, PIK3CA*, *AKT1, ERBB2*, *PTEN*, *NRAS*, *STK11*, *MAP2K1*, *ALK*, *DDR2*, *CTNNB1*, *MET*, *TP53*, *SMAD4, FBX7*, *FGFR3*, *NOTCH1*, *ERBB4*, *FGFR1*, and *FGFR2*
Primer pairs, amplicon length	92 pairs of primers in a single pool 92 amplicons with an average length of 162 bp
Input DNA required	10 ng
Observed performance	Percent of amplicons with the target base coverage at 500x: >95% Average panel uniformity: 95% Average percent reads on target: 98%
Multiplexing	2 samples per Ion 314™ Chip with at least 500x sequencing coverage 8 samples per Ion 316™ Chip with at least 500x sequencing coverage 16 samples per Ion 318™ Chip with at least 500x sequencing coverage

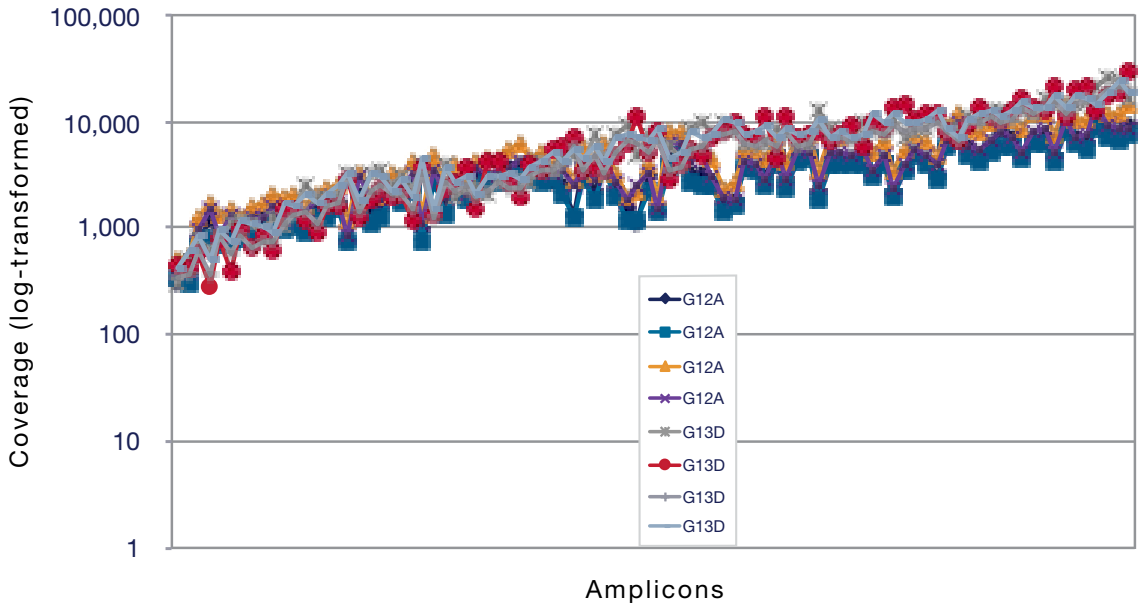

Figure 2. High coverage uniformity demonstrated with the Ion AmpliSeq Colon and Lung Cancer Research Panel v2 using Applied Biosystems™ AcroMetrix™ *KRAS* FFPE Process Controls (Cat. No. 950450) in quadruplicate runs with 8 samples on an Ion 316 Chip v2.

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Ion AmpliSeq RNA Fusion Lung Cancer Research Panel

The Ion AmpliSeq RNA Fusion Lung Cancer Research Panel targets over 70 fusion transcripts relevant to lung cancer research, with additional targets for major fusion gene families (Table 2). The panel also includes 5 positive control genes.

With Ion Reporter Software, you can quickly and simply detect known and novel gene fusions. With tunable analysis parameters and multisample heat map visualizations, Ion Reporter Software makes it easier to customize gene fusion workflows and perform multisample comparisons.

Additional features of the Ion AmpliSeq RNA Fusion Lung Cancer Research Panel include:

- RNA quality controls with housekeeping gene expression targets
- 5′ and 3′ *ALK* gene expression assays as indicators of a translocation
- Primer designs that target *ALK*, *ROS1*, *RET*, and *NTRK1* fusions that were previously detected from FFPE archived samples using immunohistochemistry, FISH, or qPCR by the OncoNetwork Consortium

Table 2. Ion AmpliSeq RNA Fusion Lung Cancer Research Panel.

Sample type	FFPE samples
Application	Somatic mutation detection
Fusion transcripts	Over 70 transcripts, including *ALK*, *RET*, *ROS1*, and *NTRK1*
Primer pairs, amplicon length	83 pairs of unique primers in a single pool 85 amplicons with an average length of 136 bp
Input RNA required	10 ng of total RNA
Observed performance	Fusion transcript detection down to 1% of the total RNA using cell line dilution
Recommended multiplexing	16 samples per Ion 318 Chip with at least 20,000 on-target reads per library

The OncoNetwork Consortium members include these researchers:

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