

AccuFusion™ RNA Lung Cancer Panel

Fast and reliable assay for targeted gene fusion detection

Highlights

- Targeted identification of RNA fusions associated with lung cancer**
 Interrogate ~280 known RNA fusions using a target specific dual-primer multiplex PCR method.
- Fast, streamlined workflow**
 Generate sequencing-ready libraries in just 6 hours using a rapid, four-step protocol from extracted RNA to sequence ready libraries.
- Excellent performance with high on-target rate**
 Prepare high-quality targeted NGS libraries using AccuFusion™ RNA Fusion Technology to achieve high on-target rate for efficient sequencing

AccuFusion™ RNA Lung Cancer Panel for next generation sequencing enables the detection of 280 gene fusions associated with non-small cell lung cancer. In contrast to the OmniFusion™ panels, the AccuFusion™ workflow is for targeted and focused detection of known fusion partners. The AccuFusion™ dual-primer based amplification technology uses expertly designed target specific primers to generate libraries without fragmentation of the input material. Compared to other methods such as qPCR, FISH, or Sanger sequencing, Paragon Genomics' targeted fusion sequencing method allows robust multiplexed detection of variants using minimum sample input and a simple workflow.

The chemistry allows sensitive detection of rare variants while using as little as 10ng of RNA input. The panel was also designed to be compatible with degraded FFPE samples for improved performance, even under less than ideal sample conditions.

AccuFusion Streamlined Workflow

The AccuFusion™ RNA Panels offer a simple and streamlined workflow. Starting from purified and quantitated RNA, the protocol includes cDNA synthesis by reverse transcription (RT), followed by the mPCR-based CleanPlex® workflow, which in total can be completed in 6 hours with minimal hands-on time. The CleanPlex® 3-step workflow requires minimal tube-to-tube transfers that can be easily automated on liquid handling platforms.



AccuFusion™ Target Enrichment and Library Preparation

6 hours of total assay time, 70 minutes of hands-on time

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1 | PS6002-01

AccuFusion™ RNA Lung Cancer Panel Specifications

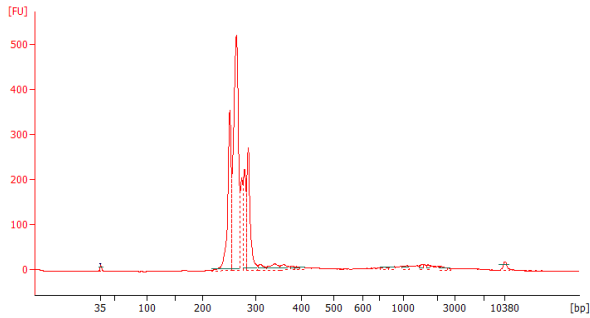
Parameter	Specification
Enrichment Method	Multiplex PCR with dual-Primer target specific primers
Sequencing Platforms	Illumina®
Number of Fusion Genes	Fusions associated with ALK, CIT, EML4, FGFR1, MBIP, MET, NRG1, NTRK1, NTRK3, PDGFRA, RET, ROS1, TACC3 1 control gene (TBP)
Targets	~280 known gene fusions associated with non-small cell lung cancer
Variant Types	RNA fusion, translocation
Number of Amplicons	98
Amplicon Size	150 - 200 bp
Number of Primer Pools	2
Input RNA Requirement	10-100 ng per pool
Sample Types	FFPE, FNA, Fragmented RNA
Total Assay Time	6 hours
Hands-On Time	70 minutes
Detection	Somatic detection down to 1% allele frequency
On-Target Aligned Reads	~93%

The AccuFusion™ technology was built upon the foundations of Paragon Genomics' CleanPlex® chemistry that produces high quality and clean libraries for sequencing. Therefore, the AccuFusion™ technology is able to achieve high on-target and mapping rates with minimal primer-dimer rates. AccuFusion™ libraries allow robust, efficient, and high-throughput fusion detection by sequencing.

AccuFusion™ RNA Lung Cancer Panel | Product Sheet

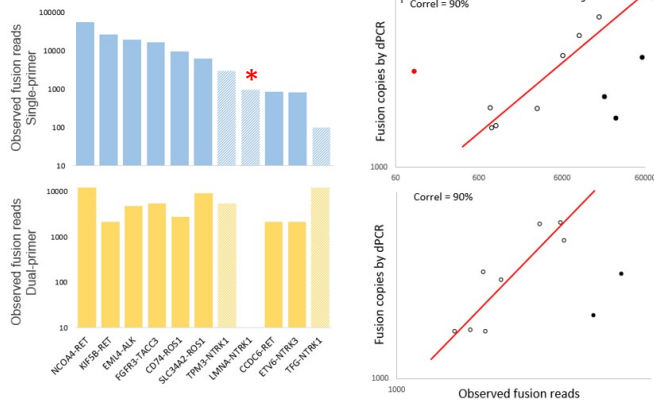
CleanPlex® Background Cleaning Chemistry

The AccuFusion™ RNA Lung Cancer Panel is powered by Paragon Genomics' CleanPlex® technology, which uses a proprietary multiplex PCR background cleaning chemistry to effectively remove non-specific PCR products, resulting in best-in-class target enrichment performance and efficient use of sequencing reads.

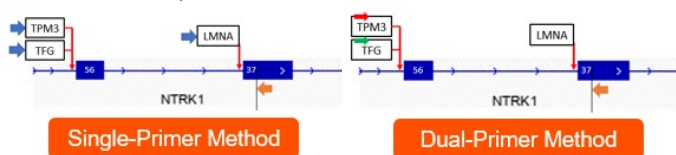


Identification of novel and known gene fusions

Seraseq® Fusion RNA mix v4 was used to generate libraries with the OmniFusion™ and AccuFusion™ RNA Lung Cancer Panels. The AccuFusion™ panel was able to detect all 10 expected fusions in the control material (yellow bar graph), whereas OmniFusion™, which is also able to detect unknown fusions (blue bar graph), identified one additional novel fusion (LMNA-NTRK1, as indicated by * below). In addition, the uniform amplification of the CleanPlex® chemistry is highlighted by the high correlation seen between observed reads and fusion copies (determined by dPCR).



Paragon Genomics offers two fusion detection methods: OmniFusion™ for the detection of known and novel fusions, and AccuFusion™ for the targeted detection of known fusions. The single-primer method of OmniFusion™ is able to identify novel fusions with unknown information (i.e., LMNA-NTRK1 fusion), whereas the dual-primer method of AccuFusion™ is used for focused interrogation of known fusion targets (i.e., TPM3/TFG-NTRK1 fusions).



AccuFusion™ RNA Lung Cancer Panel Performance

	Mapping Rate %	On-Target Rate %	Primer-Dimer Rate %
Average	99.6	93.7	0.45
STDV	0.4	1.3	0.06

The table above displays the performance of AccuFusion™ RNA Lung Cancer Panel using 25ng of Seracare® Fusion Reference RNA as input. The generated libraries were sequenced at 0.1 million reads per sample.

Recommended Sample Multiplexing AccuFusion™ RNA Lung Cancer Panel

Instrument	Samples per Run ^A
iSeq™ i1 System	16
MiSeq™ System (v2)	62
MiniSeq™ System (High-output)	103
NextSeq™ System (Mid-output)	536

A. Samples per run at an intended average read depth of 2,500X for 1% MAF detection with 2x 150bp sequencing.

Ordering Information

The AccuFusion™ RNA Lung Cancer Panel contains the primers and AccuFusion™ RNA Library Kit. CleanPlex® Indexed PCR Primers and CleanMag® Magnetic Beads are ordered separately to complete the workflow from input RNA to sequencing-ready NGS libraries. For more indexing options and additional product configurations visit www.paragongenomics.com/store/

Product	SKU
AccuFusion™ RNA Lung Cancer Panel (8 Rxns)	917103
AccuFusion™ RNA Lung Cancer Panel (96 Rxns)	917104
AccuFusion™ RNA Lung Cancer Panel (384 Rxns)	917105
CleanPlex® Dual-Indexed PCR Primers for Illumina® Set A (96 indexes, 96 reactions)	716006
CleanPlex® Dual-Indexed PCR Primers for Illumina® Set B (96 indexes, 96 reactions)	716018
CleanMag® Magnetic Beads (5 mL)	718002
CleanMag® Magnetic Beads (60 mL)	718003

Learn More

To learn more about OmniFusion™ and AccuFusion™ technologies, visit <https://www.paragongenomics.com/targeted-sequencing/amplicon-sequencing/rna-fusion-detection/>

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2 | PS6002-01

