

CleanPlex® UMI Lung Cancer Panel

Ultrafast and precise analysis of hotspots mutations in lung cancer

Highlights

- Relevant Gene Content**
 Interrogate 23 genes containing variants that are frequently mutated in lung cancer using a hotspot panel designed to work with cell-free DNA (cfDNA)
- Fast, Single-Tube Workflow**
 Generate high-quality molecular-barcoded and target-enriched NGS libraries in just 3.5 hours using a three-step, single-tube protocol
- Enhanced Error Correction**
 Remove PCR and sequencing errors with powerful error correction enabled by novel unique molecular identifiers (UMIs) that can distinguish the two strands of DNA.
- High-Confidence Variant Detection**
 Detect low-frequency variants down to 0.1% allele frequency with high specificity using just 50 ng of DNA

The CleanPlex® UMI Lung Cancer Panel is a targeted resequencing assay designed for rapid and high confident detection of low-frequency variants across the hotspot regions of 23 genes associated with lung cancer. This panel is specifically designed to work with cell-free DNA (cfDNA) and FFPE DNA for precise analysis of tumor DNA in liquid biopsy and tumor profiling applications. The panel is powered by Paragon Genomics' CleanPlex UMI Technology which incorporates novel unique molecular identifiers (UMIs) to enable correction of PCR and sequencing errors. Starting with just 20 ng of DNA, sequencing-ready libraries can be prepared using a single-tube workflow in just 3.5 hours. Low-frequency variants at 0.1% allele frequency can be confidently detected with high specificity using just 30 ng of DNA.

CleanPlex UMI Lung Cancer Panel Gene List

AKT1	CDKN2A	FGFR3	KRAS	RET
ALK	CTNNB1	GNAS	MET	STK11
APC	EGFR	IDH1	MLH1	TP53
ATM	ERBB2	IDH2	NRAS	
BRAF	FBXW7	JAK3	PIK3CA	

CleanPlex UMI Lung Cancer Panel Specifications

Parameter	Specification
Enrichment Method	Multiplex PCR
Platform	Illumina®
Molecular Barcoding	Inline 16 bp degenerate UMI on each end of reads
Number of Genes	23
Targets	20 genes containing variants that are frequently mutated in lung cancer
Cumulative Target Size	1,889 bp
Variant Types	SNVs, indels ^A
Number of Amplicons	53
Amplicon Size	70 – 100 bp, one at 166 bp (82 bp on average)
Number of Primer Pools	1
Input DNA Requirement	20 – 80 ng per pool (≥30 ng cfDNA or FFPE DNA per pool recommended for 0.1% LOD)
Sample Types	Cell-free DNA, FFPE DNA, gDNA
Total Assay Time	3.5 hours
Hands-On Time	85 minutes
Design Coverage	100%
Coverage Uniformity (targets with >0.2X mean coverage)	≥ 95%
On-Target Aligned Reads	≥ 95%

A. SNVs: single nucleotide variations; indels: insertions-deletions

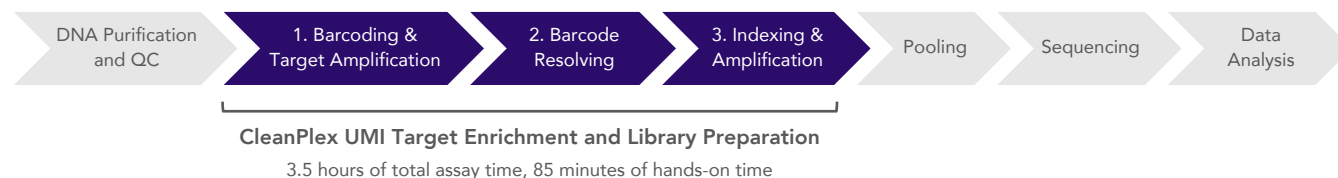
Recommended Sample Multiplexing for CleanPlex UMI Lung Cancer Panel

Instrument	Samples per Run ^A
MiniSeq™ System (mid-output)	3
MiniSeq System (high-output)	9
MiSeq System (v2 chemistry Micro)	1
MiSeq System (v2 chemistry)	5
MiSeq System (v3 chemistry)	9
NextSeq™ System (mid-output)	49

A. Samples per run at an intended average read depth of 100,000X to detect variants at 0.1% allele frequency using 30 ng of input DNA.

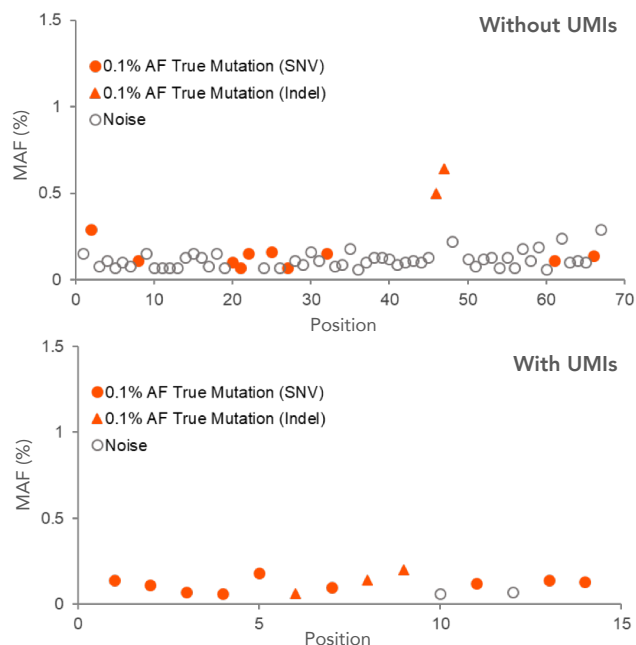
CleanPlex UMI Single-Tube Workflow

The CleanPlex UMI Lung Cancer Panel offers a ultrafast and streamlined workflow. Starting from purified and quantitated DNA, the multiplex PCR-based protocol can be completed in just 3.5 hours, with 85 minutes of hands-on time, using a three-step, single-tube workflow. Each step consists of a thermal-cycling or incubation condition, followed by “with bead” purification using magnetic beads.



UMI Error Correction Facilitates Confident Detection

The CleanPlex UMI Lung Cancer is powered by Paragon Genomics’ CleanPlex UMI Technology, which uses a proprietary multiplex PCR-based molecular barcoding chemistry to uniquely label and differentiate the two strands of each DNA molecule. Consensus sequences can be constructed from the sequencing data to remove PCR and sequencing errors to enable confident detection of low-frequency variants.



50 ng of SeraCare Seraseq™ ctDNA Complete™ Mutation Mix at minor allele frequency (MAF) of 0.1% was used to prepare targeted NGS libraries using the CleanPlex UMI Lung Cancer Panel, which covers 13 mutations present in the reference material. The resulting data was analyzed with and without the use of UMIs. The bottom panel demonstrates that UMI-enabled error correction results in a significant reduction in false positives, allowing true mutations (orange circles and triangles) to be distinguished from background noise (white circles).

High Detection Sensitivity

The CleanPlex UMI Lung Cancer Panel can detect the majority of the 13 mutations present in the SeraCare reference material.

DNA Input	Mutations Expected	Mutations Detected		
		0.5% MAF	0.25% MAF	0.1% MAF
20 ng	13	13	11	9
30 ng	13	13	11	10
50 ng	13	13	13	12
75 ng	13	13	13	11

Ordering Information

The CleanPlex UMI Lung Cancer Panel contains CleanPlex UMI Multiplex PCR Primers and CleanPlex Targeted Library Kit. CleanPlex Unique Dual-Indexed PCR Primers and CleanMag® Magnetic Beads are purchased separately to complete the workflow from input DNA to sequencing-ready NGS libraries. For more indexing options and additional product configurations visit www.paragongenomics.com/store/

Product	SKU
CleanPlex UMI Lung Cancer Panel (8 reactions)	916064
CleanPlex UMI Lung Cancer Panel (32 reactions)	916065
CleanPlex UMI Lung Cancer Panel (96 reactions)	916066
CleanPlex Unique Dual-Indexed PCR Primers for Illumina® Set A (16 indexes, 32 / 96 reactions)	716011 / 716012
CleanPlex Unique Dual-Indexed PCR Primers for Illumina® Set B (16 indexes, 32 / 96 reactions)	716013 / 716014
CleanMag Magnetic Beads (5 mL)	718002
CleanMag Magnetic Beads (60 mL)	718003

Learn More

To learn more about CleanPlex UMI Ready-to-Use NGS Panels, visit www.paragongenomics.com/cleanplex_umi_panels/
To learn more about CleanPlex Technology UMI, visit www.paragongenomics.com/cleanplex_umi_technology/

Paragon Genomics, Inc. | 3521 Investment Blvd Suite 1, Hayward CA 94545, USA | +1.650.822.7545
www.paragongenomics.com | techsupport@paragongenomics.com

© 2018 Paragon Genomics, Inc. All rights reserved. All trademarks are the property of Paragon Genomics, Inc. or their respective owners.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

