Patient Name:		**Booking ID:**	
Age:		**Sample Type:**	
Gender:		**Sample collection date:**	
Referring Clinician:		**Sample receiving date:**	
Test Requested:		**Reporting date:**	

LUNG CANCER NGS PANEL REPORT

CLINICAL INFORMATION

(Received one FFPE block labeled as 1964/UL, Tumor content-40%)
D/o CA Lung with SVCO

RESULT SUMMARY

(No clinically actionable variant were detected in provided FFPE block tissue sample)

KEY VARIANT (DNA LEVEL) FINDING

Genes & Transcript	Exon number	Locus	Genetic variants	Allele Frequency/ Depth	Function of gene in this cancer	Variant class & effect	AMP Classification*
NA							

*Genetic test results are reported based on the recommendations of AMP-ASCO-CAP guidelines.

*Tier Reference: Li et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017 Jan;19(1):4-23.

Note/Remark: As per existing raw data processed in repeats too, no variant detected because of on target data were not sufficient to get good quality variant. This is because of very high level degraded DNA quality yield in provided FFPE block sample. For further evaluation of mutation in this case, A fresh or recent FFPE block is suggested to get a good quality sample.

QC metrics of NGS data for reference:

Number of mapped reads	1,240,233
Percent reads on target	**5.40%**
Average base coverage depth	210.2
Uniformity of base coverage	89.39%
Target base coverage at 1x	100.00%
Target base coverage at 20x	99.13%
Target base coverage at 100x	61.40%

RECOMMENDATION

- ✓ Please correlate clinically.
- ✓ Genetic counseling for accurate interpretation of test results is recommended.
- ✓ For about this report, or for assistance in locating nearby genetic counseling services, please contact the Laboratory: geneticcounselors@redcliffelabs.com, or ccsupport@redcliffelabs.com.

TEST DESCRIPTION

This somatic lung cancer gene panel (35 genes) include hot-spots through next generation sequencing (NGS) allows the identification of different type of variants i.e. targeted hot-spot mutations, Indels, mutation to understand their prognostic and therapeutic implications in various carcinomas of an affected individuals. Targeted regions of genes analysis by NGS method allows detection of specific mutations (SNVs, INDELs,) that can provide treatment opportunities to the affected patients and their predictive response of therapy against the FDA/NCCN/ESMO approved drugs as per key findings. This gene panel with improved primer design and low input requirement of as 10-20 ng of DNA enable to sequence challenging samples such as Formalin fixed, paraffin embedded (FFPE) tissue which exhibit variable quality.

TEST METHODOLOGY

Next Generation Sequencing: These clinically relevant genes have been selected on the basis of their known impact as actionable targets of existing and emerging anti-cancer therapies, and prognostic features in various tumor types. The sensitivity of the assays depends on the quality of the FFPE tissue block/slide, and its tumor percentage (>10-15%). In the multiple validation studies, the limit of detection (LOD) were observed at 5% with minimum depth at >500x. In process, quality controls were determined for prepared library. The libraries were sequenced at range mean depth: >500-1500x on Ion Torrent next generation sequencing platform. Reference sequence to the GRCh37 (hg19) assembly of the human genome were used. Genomic DNA were isolated from FFPE tissue block sample using commercial kit according to manufacturer's instructions and the target regions of interest were amplified using the targeted gene panel. Library preparation was performed and sequenced on the Ion Torrent Gene Studio S5 plus sequencer. The FASTQ reads were aligned against the hg19 in the Torrent suite software (v5.18.1). Variant calling and annotations were done using Variant Annotator v3.3. AMP-ASCO-CAP guidelines were followed for variant classification. Clinically relevant mutations were identified and annotated using published variants in literature and a set of databases. The effect of non-synonymous variant is calculated using multiple prediction algorithms such as PolyPhen, SIFT, Mutation Taster2

TEST LIMITATIONS

- ✓ It should be noted that this test is limited to a limited number of genes and does not include all intronic and non-coding regions.
- ✓ This report only includes variants that meets a level of evidence threshold for cause or contribute to disease. Test results are interpreted in the context of clinical & pathological findings and laboratory data.
- ✓ The accuracy and completeness may vary due to variable information available in different databases. Synonymous mutations were not considered while preparing this report.
- ✓ The variants have not been confirmed using Sanger sequencing and/or alternate technologies. To rule out germ line mutations i.e. variant with variant allele frequency at nearly 50% or 100%, whole blood sample is recommended to process along with tissue sample.
- ✓ The scope of this assay limits to SNV and small deletions/duplication. Due to poor quality of FFPE DNA, indeterminate result due to low gene coverage or low variant depth cannot be ruled out. The sensitivity of the assays depends on the quality of the block, and tumor content.
- ✓ The sensitivity of this assay to detect small deletions/duplication is upto certain number of bases only. The CNVs if detected with this assay have to be confirmed by alternate method such as MLPA & Microarray.
- ✓ Variations with high minor allele frequencies which are benign/likely benign will be given upon request if required.
- ✓ Due to inherent technology limitations, coverage is not uniform across all regions. Hence, pathogenic variants present in areas of insufficient coverage as well as those variants which currently do not correlate with the provided phenotype may not be analyzed/ reported. Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity.
- ✓ This assay is not meant to interrogate most promoter regions, deep intronic regions, or other regulatory elements. Incidental or secondary findings (if any) that meet the AMP-ASCO-CAP guidelines can be given upon request.
- ✓ Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS).
- ✓ The transcript used for clinical reporting generally represents the canonical transcript, which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.
- ✓ Detailed clinical trial summary for clinically relevant variant is available with specific case and can be given upon request to authorized person.

DISCLAIMER

- Test has been performed assuming that the sample received belongs to the above-named individual(s) and that any stated relationships between individuals are accepted as true. It is also assumed that consent for the same was provided after pre-test counseling at the point of collection/referral.
- The results should be interpreted in the context of the patient's medical evaluation, family history and racial/ethnic background. Please note that variant classification and/or interpretation may change over time if more information available. Re-interpretation of multi gene next generation sequencing data is recommended on an annual basis and may be requested by a medical provider.
- More evidence for disease association of genes and causal pathogenic variants are discovered every year, and it is recommended that genetic variants are re-interpreted with updated software and annotations periodically.
- Rare polymorphisms may lead to false negative or positive results. False negative results may be due to sampling error/errors in sample handling as well as clonal density below the limit of detection. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Identification of a mutation in one or more of these genes does not guarantee activity of the drug in a given indication due to the presence of contraindicated mutation in the gene not covered by the panel.
- The information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician.
- Patient care and treatment decisions should only be made by the physician after taking into account all relevant information available including but not limited to the patient's condition, family history, findings upon examination, results of other diagnostic tests, and the current standards of care.
- This report should only be used as an aid and the physician should employ sound clinical judgment in arriving at any decision for patient care or treatment. Since only a portion of the tumor was tested, it is possible that this result may not represent the entire tumor population.

VARIANT REPORTING CLASSIFICATION BASED ON AMP ASCOCAP RECOMMENDATIONS

Variants	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).
Tier I	**Variants with Strong Clinical Significance (Level A and B Evidence)** ***Level A,*** biomarkers that predict response or resistance to US FDA-approved therapies for a specific type of tumor or have been included in professional guidelines as therapeutic, diagnostic, and/or prognostic biomarkers for specific types of tumors; ***Level B,*** biomarkers that predict response or resistance to a therapy based on well- powered studies with consensus from experts in the field or have diagnostic and/or prognostic significance of certain diseases based on well-powered studies with expert consensus.
Tier II	**Variants with Potential Clinical Significance (Level C and D Evidence)** ***Level C,*** biomarkers that predict response or resistance to therapies approved by FDA or professional societies for a different tumor type (i.e., off-label use of a drug), serve as inclusion criteria for clinical trials, or have diagnostic and/or prognostic significance based on the results of multiple small studies. ***Level D,*** biomarkers that show plausible therapeutic significance based on preclinical studies or may assist disease diagnosis and/or prognosis themselves or along with other biomarkers based on small studies or multiple case reports with no consensus.
Tier III	**Variants of Unknown Significance** Not observed at a significant allele frequency in the general or specific sub population or pan cancer or tumor specific variant databases. No convincing published evidence of Cancer Association
Tier IV	**Benign or Likely Benign**

928-909-0609 ccsupport@redcliffelabs.com www.redcliffelabs.com

Terms and Conditions of Reporting

1. The presented findings in the Reports are intended solely for informational and interpretational purposes by the referring physician or other qualified medical professionals possessing a comprehensive understanding of reporting units, reference ranges, and technological limitations. The laboratory shall not be held liable for any interpretation or misinterpretation of the results, nor for any consequential or incidental damages arising from such interpretation.

2. It is to be presumed that the tests performed pertain to the specimen/sample attributed to the Customer's name or identification. It is presumed that the verification particulars have been cleared out by the customer or his/her representation at the point of generation of said specimen / sample. It is hereby clarified that the reports furnished are restricted solely to the given specimen only.

3. It is to be noted that variations in results may occur between different laboratories and over time, even for the same parameter for the same Customer. The assays are performed and conducted in accordance with standard procedures, and the reported outcomes are contingent on the specific individual assay methods and equipment(s) used, as well as the quality of the received specimen.

4. This report shall not be deemed valid or admissible for any medico-legal purposes.

5. The Customers assume full responsibility for apprising the Company of any factors that may impact the test finding. These factors, among others, includes dietary intake, alcohol, or medication / drug(s) consumption, or fasting. This list of factors is only representative and not exhaustive.