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DNA Extraction of Lung Cancer Samples for Advanced Diagnostic Testing

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Introduction

It is apparent that genetic testing leads to improved survival rates for lung cancer patients. Patients whose tumor DNA harbor oncogenic driver mutations respond favorably to drug treatments specifically tailored to those drivers. Currently, these lung targeted therapies are limited to mutations in 15-20 genes, but the number will grow as evidence emerges supporting novel drug-mutation interactions. A major challenge today is detecting therapeutically actionable driver mutations in lung cancer biopsies, most of which are sub-optimally preserved and contain limited amounts of evaluable tumor tissue.

Background

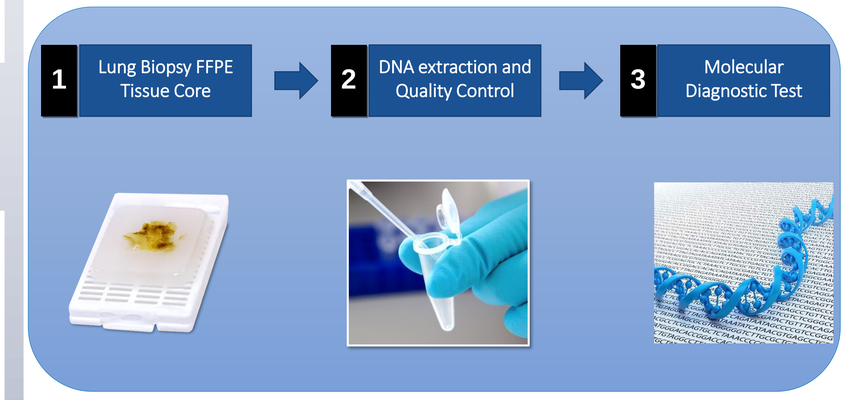
Lung cancer biopsies are routinely formalin fixed and paraffin embedded (FFPE) for histological evaluation by anatomical pathologists. This process preserves the tumor morphology and cellular features required for proper staining and microscopic review. However, this practice presents numerous challenges for the extraction of high quality DNA for genomic testing. An extraction process that consistently produces sufficient DNA yield and fragment size from these difficult but most precious tissue samples is a requirement for any Molecular Pathology laboratory. The data presented here compares the quantity and quality of DNA extracted using two methods, QIAGEN and Covaris, and the success of downstream molecular diagnostic testing platforms routinely utilized for detecting somatic alterations including single nucleotide variants (SNV), insertion/deletions (indel), copy number (CN) amplification, microsatellite instability (MSI) and tumor mutational burden (**Table 1**).

Alteration	Gene	Method	Platform	DNA requirement (ng)
Mutation	BRAF EGFR KRAS	Sanger sequencing Pyrosequencing single analyte	ABI 3500 PyroMark Q24	25-50
Mutation/amplification/ fusion	AKT1 ALK BRAF DDR2 EGFR ERBB2 FGFR1 KRAS MAP2K1 MET NRAS PIK3CA PTEN RET ROS1	Multi-analyte: NGS and digital detection	Illumina MiSeq Ion Torrent PGM, S5XL Nanostring nCounter	10-500
Tumor mutational burden	> 400	Multi-analyte: NGS	Illumina MiSeq Ion Torrent S5XL	10-200

Table 1: Commonly tested alterations in lung cancer with method and DNA requirements.

Methods

FFPE tumor samples from a variety of tumor types, including lung, were macro-dissected using 14-gauge needles, with one core extracted using the Covaris truXTRAC FFPE DNA isolation method and the other matched core using the QIAGEN DNeasy tissue kit (Figure 1). All samples were processed using manufacturer's recommended instructions. DNA metrics were measured using Qubit and NanoDrop for yield and purity, followed by fragment size estimation on a 2100 BioAnalyzer.



A subset of matched DNA sample pairs were used as template for mutation detection using pyrosequencing (PyroMark), Ion AmpliSeq (PGM) and Illumina TruSeq Custom Amplicon (MiSeq) NGS, and for copy number using the NanoString nCounter system. An additional set of Covaris prepared DNA samples were tested at 1:2 and 1:4 dilutions. OmniSeq Target[SM] for Lung, a NYS CLEP approved test, was used for all NGS and nCounter analyses.

Results

DNA yields and fragment lengths were substantially higher for Covaris extracted samples as compared to QIAGEN when measured by Qubit (Table 2) and Bioanalyzer electrophoresis (Figure 2). A higher degree of successful advanced molecular diagnostic test results was also observed for the truXTRAC DNA samples, especially for the MiSeq NGS system (improved clustering and coverage) and nCounter platform (improved counts) that prefer longer fragment lengths than PGM NGS.

Matched Core extraction		NanoDrop				Qubit	
Sample #	Disease Type	QIAGEN yield (ug)	Covaris yield (ug)	QIAGEN: DNA 260/280	Covaris: DNA 260/280	QIAGEN yield (ug)	Covaris yield (ug)
1	CRC	9.72	23.2	2.04	1.81	0.93	6.56
2	Melanoma	25.03	1.11	1.39	1.59	0.43	0.08
3	Breast CA	6.38	12.92	2.00	1.85	0.77	4.46
4	Breast CA	12.17	17.07	2.05	1.83	1.44	5.30
5	Breast CA	13.66	17.28	2.01	1.89	3.28	5.02
6	Melanoma	13.14	20.36	2.02	1.81	0.81	4.24
7	NSCLC	8.9	16.89	2.00	1.85	0.86	4.54
8	CRC	19.02	30.07	2.05	1.86	3.38	6.76
	Average	**13.50**	**17.36**	**1.95**	**1.81**	**1.49**	**4.62**

Table 2: DNA yields of matched FFPE tissue core extractions (1mm).

Results

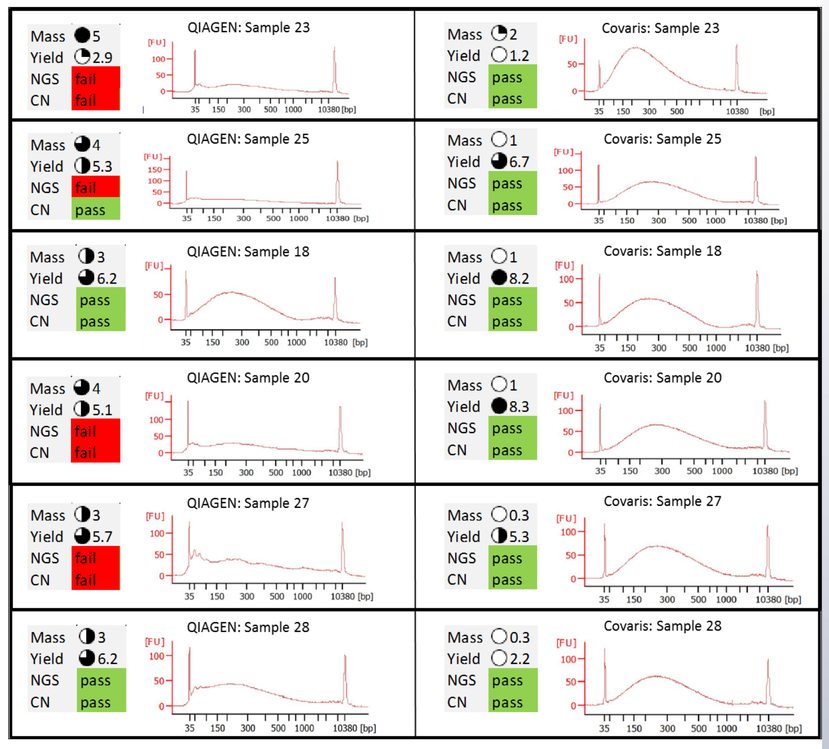


Figure 2: Tissue mass (# cores), DNA yield (ug), fragment traces, and NGS and nCounter CN test result success for six NSCLC samples extracted with QIAGEN (left) and Covaris (right) methodologies.

Covaris extracted DNA samples were also successfully validated for use with single analyte PyroMark assays, and when diluted 25-50% for NGS analysis (**Table 3**).

Sample #	PyroMark Test	QIAGEN result	Covaris result
22	BRAF	mutation ND	mutation ND
	EGFR	mutation ND	mutation ND
	KRAS	mutation ND	mutation ND
	JAK2	mutation ND	mutation ND
24	BRAF	p.Val600Glu	p.Val600Glu
	EGFR	mutation ND	mutation ND
	KRAS	mutation ND	mutation ND
	JAK2	mutation ND	mutation ND
26	BRAF	mutation ND	mutation ND
	EGFR	mutation ND	mutation ND
	KRAS	p.Gly12Asp	p.Gly12Asp
	JAK2	mutation ND	mutation ND

Sample #	DNA Dilution	NGS QC	NGS SNV calls (concordance)
27	1:1	pass	32
	1:2	pass	32 (100%)
	1:4	pass	32 (100%)
28	1:1	pass	27
	1:2	pass	27 (100%)
	1:4	pass	27 (100%)
29	1:1	pass	24
	1:2	pass	24 (100%)
	1:4	pass	24 (100%)
31	1:1	pass	24[#]
	1:2	pass	25 (96%)
	1:4	pass	25 (96%)

[#] RET c.2712C>G silent mut, VAF 0.40 not detected

Table 3: PyroMark results for QIAGEN and Covaris DNA (left) and OmniSeq Target NGS results for serially diluted Covaris DNA (right).

Conclusions

FFPE tumor samples prepared using the Covaris truXTRAC isolation kit provides an efficient system for generating high quality DNA samples even from lung cancer specimens that previously failed testing with QIAGEN extraction. The combination of improved yield and fragment size measured for nearly every sample tested suggests that even smaller biopsies can now be collected and extracted for advanced diagnostic testing. Additionally, DNA requirements for NGS based testing can be reduced 25-50% without loss of assay performance or analytic sensitivity as validated in our CLIA laboratory.