TARGETED NGS OF CLINICAL SPECIMENS FROM ASURAGEN'S TUMOR BANK

FFPE specimens form the bulk of samples from cancer biopsies and resections. DNA from fixed samples is typically lower in quality compared to DNA from fresh or frozen specimens and imparts unique challenges for mutational analysis. Next-generation sequencing (NGS) of FFPE DNA offers the opportunity for highly sensitive, comprehensive mutation detection and is particularly suited to address the cellular and molecular heterogeneity of FFPE samples.

STUDY DESIGN

The goal of this study was to identify mutations in 35 regions from 16 genes in FFPE cancer specimens using amplicon resequencing. Five specimens from each of five cancer types (breast, colon, lung, melanoma, and ovarian) were analyzed.

FOCUSED GENE PANEL

ABL1	FGFR1	HRAS	MET	BRAF	KIT	EGFR	RET
AKT1	FGFR3	JAK2	NRAS	FLT3	PDGFRA	KRAS	PIK3CA

Table 1: Focused gene panel (previously SuraSeq® 500 Cancer Panel) targets mutational hotspot regions in 16 cancer-associated genes. Now available as QuantideX® NGS Pan Cancer Kit with 21 genes.

RESULTS

A targeted panel of commonly mutated regions in 16 oncogenes was PCR-enriched and sequenced using the Illumina NGS platform to identify variants in 25 FFPE tumor bank specimens. Variants identified are listed in Table 2. Mutation frequencies within the samples ranged from 8 - 68%, and detection sensitivity was determined to be as low as 4%.

Sample ID	Cancer Type	Variant	Nucleotide Change	# of Variant Specific Reads	% of Total Reads	Confirmed
RS00855	COL	KRAS G13D	29C>T	30409	35	
RS00857	COL	KRAS G12V	32C>A	23714	27	V
RS00862	COL	KRAS G12D	32C>T	22420	31	V
RS00877	COL	KRAS G12S	33C>T	26464	43	V
RS00859	LUN	BRAF V600E	23T>A	26593	8	
RS00872	LUN	KRAS G12V	32C>A	13880	35	V
RS00858	MEL	BRAF V600E	23T>A	48747	68	V
RS00861	MEL	NRAS Q61R	42T>C	27848	43	V
RS00863	MEL	BRAF V600E	23T>A	43798	35	V
RS00865	MEL	NRAS G13R	46C>G	34689	27	
RS00866	MEL	NRAS Q61H PIK3CA H1047R	41T>G 50A>G	34784 27381	68 18	V NA
RS00869	OVA	PIK3CA H1047R	50A>G	22441	61	V

Table 2: Variants identified in Asuragen's tumor bank FFPE specimens on the Illumina NGS platform and confirmation on the Ion Torrent PGM.

CONCLUSION

PCR enrichment and NGS analysis were utilized to interrogate the mutational state of selected regions from 16 oncogenes in 25 FFPE samples from Asuragen's tumor bank, demonstrating suitability for DNA-based analysis in these clinically relevant specimens. Mutations identified were confirmed using an orthogonal NGS platform.

Accurate quantification and high analytical sensitivity of low abundance mutations are important considerations for molecular profiling of cancer. Asuragen's tumor bank offers a large collection of cancer subtypes with associated specimen data available including histology, demographic and molecular characteristics. This diverse sample collection can be utilized in model assays to identify both common and rare variants that may be clinically relevant to a given disease and successfully incorproated into both basic and clinical cancer research.

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