Evaluation of ONCOReveal Lung & Colon Cancer Panel in a CRC Cohort and Effect of DNA Damage in Variant Detection

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Poster Number: 1428

Introduction

The ONCOReveal Lung&Colon Cancer Panel (LC103, Pillar Biosciences Inc.) interrogates regions in 22 genes that are frequently mutated in NSCLC and CRC. To evaluate the performance of the panel, we assessed a cohort of 207 colorectal cancer FFPE samples collected by a top-tier hospital in Shanghai between 2015 and 2016. Among them, 27 FFPE samples showed abnormally high numbers of low frequency variants (<2%) and were further investigated to assess the effect of DNA damage in somatic variant detection.

Materials and Methods

DNA library preparation and sequencing: 10-20 ng of FFPE DNA (Quantitated by Qubit, Life Tech.) was used to prepare libraries for 207 samples using the LC103 panel. All libraries were subsequently sequenced on Illumina MiSeq sequencer.

FFPE DNA repair: DNA extracted from 27 FFPE samples with a high number of low VAF (<2%) variants was treated with NEBNext FFPE DNA Repair Mix prior to library preparation. FFPE and DNA repaired FFPE were compared to analyze the effect of DNA damage in variant detection. Matched fresh frozen tumor tissue samples were also tested.

Data analysis: PiVAT™ (Pillar Biosciences Inc.) was used for data analysis. PCR errors and sequencing errors are reduced to be well below 1% VAF through the PiVAT error correction algorithm.

Results

100% success rate of library preparation and sequencing: All of 207 FFPE samples yielded high quality sequencing data that detected mutant alleles at frequencies as low as 1% **(Figure 1).**

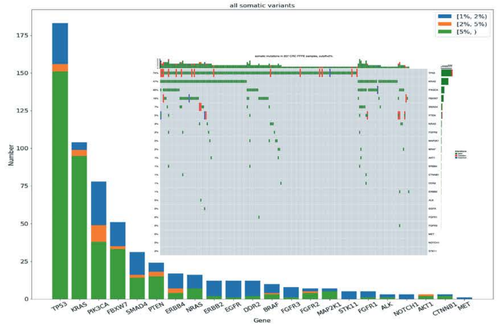

Figure 1. Somatic Variants in 207 CRC Patients

Variant detection: A total of 414 somatic variants, including SNV and small indels, were identified above 2% VAF in 193 out of 207 samples **(Figure 1).** TP53 (38)%, KRAS (24%), PIK3CA (12%), FBXW7 (9%) and PTEN (4%) were the most frequently mutated genes **(Figure 2).** CNVs were identified in EGFR, MET, ERBB2, KRAS and FGFR1 genes (Data are not shown).

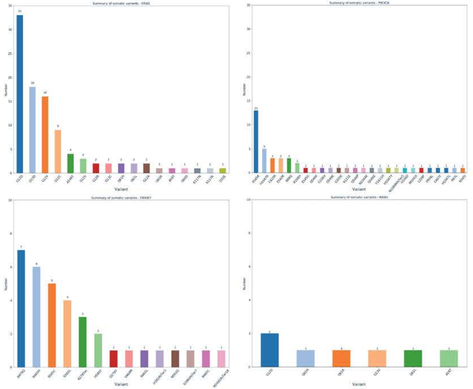

Figure 2. Somatic Variants Detected in KRAS, PIK3CA, FBXW7 and NRAS Genes

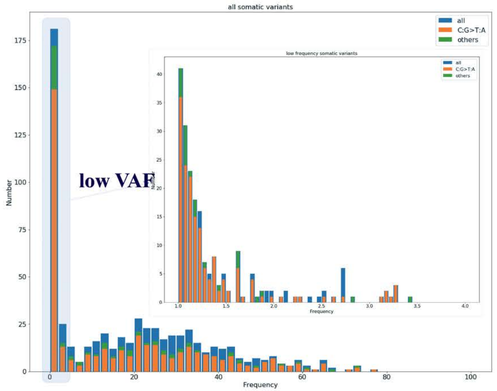

Figure 3. VAF Distribution of All Somatic Variants
all - all variants; C:G>T:A – variants where C:G was mutated to T:A; others - T:A>C:G

The effect of DNA damage: The majority of detected variants between 1% and 2% VAF, are not known hotspot mutations. C:G>T:A mutations account for 73.8% of variants between 1% and 2% VAF **(Figure 3).** DNA repair by NEBNext FFPE DNA Repair Mix or other UDG enzymes reduced 1-2% VAF calls significantly, indicating that these variants are false positive calls **(Figure 4).** However, DNA repair enzymes used in our study could not eliminate false positive calls completely**(Figure 5).** Other mechanisms are suspected to contribute to the remaining low frequency calls (Data not shown).

Variant between 2% and 5% VAF: In total, 33 somatic variants were detected at 2-5% VAF. There are many clinically actionable mutations or common driver mutations, including KRAS G12C (2.10%), KRAS G12D (2.21%), KRAS Q61L (2.74%), BRAF G469E (2.51%), PIK3CA E545K (2.08%, 2.39%, 4.78%), PIK3CA E545G (4.61%), PIK3CA Q546K (4.51%), PIK3CA H1047R (2.80%) (Data not shown).

DNA repaired FFPE vs Fresh frozen tissue: The concordance rate of somatic mutation calls between frozen and matched FFPE samples with or without the treatment of DNA repair enzymes is 49.3% and 11.4% for cut-off=1%, 73.1% and 73.9% at cutoff=2%, 72.1% and 72.9% at cutoff=5%, reflecting DNA damage of FFPE DNA samples and tumor heterogeneity **(Figure 4 and Table 1).**

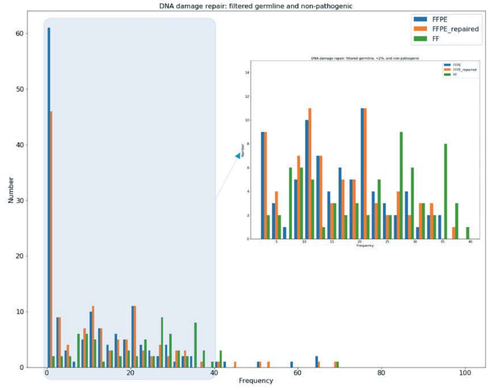

Figure 4. VAF Distribution of Matched Fresh frozen (FF), FFPE,and FFPE_repaired Samples (variants below 2%VAF were removed from the inlet figure)

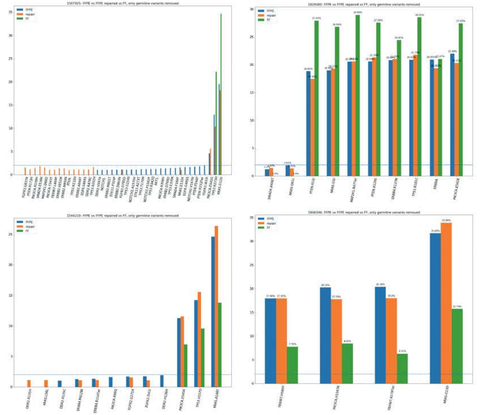

Figure 5. Examples of FFPE DNA damage.
1) severe damage - still a lot of false positive variants after DNA repair; 2) and 3) medium damage – no false positive variants after DNA repair; 4) high VAF – no false positive variants.

Table 1. Concordance at Different VAF Cutoff Level

VAF cutoff	number of somatic variants			concordance- (a&b) / (a | b)		
	FF	FFPE	repair	FF vs FFPE	FF vs repair	FFPE vs repair
1%	77	595	132	11.40%	49.30%	15.00%
2%	75	85	86	73.90%	73.10%	92.10%
3%	73	74	75	11.40%	72.10%	98.70%

Conclusions

The ONCOReveal Lung&Colon Cancer Panel is a robust and sensitive NGS assay for the detection of somatic variants. DNA damage confounds variant identification in FFPE samples between 1% and 5% frequency.