Streamlined and Accurate Detection of Actionable Fusions with the oncoReveal® Multi-Cancer RNA Fusion v2 Panel

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Background

The laboratory analysis of RNA fusions by next-generation sequencing (NGS) is becoming standard of care (SOC) in assisting with therapeutic management of lung cancer and other solid tumors. It is critical that actionable alterations are identified quickly and tracked to help ensure patients receive targeted treatments prior to the initiation of other SOC cytotoxic chemotherapies. However, many existing fusion assays can be expensive, time-consuming, and require high input material, limiting their accessibility in many clinical settings. We compare the performance of two commercially available fusion panels and assess each panel's performance, useability, and accessibility in resource-constrained environments.

Materials & Methods

We compared the performance of the Pillar oncoReveal® Multi-Cancer RNA Fusion v2 (PFP) panel against the Archer™ FUSIONPlex™ Pan Solid Tumor v2 (AFP) panel (Figure 1 and Figure 2). RNA was extracted and library preparation was performed per manufacturer protocols. Libraries were quantified using the QIAxcel Connect system (QIAGEN) and normalized to an arbitrary concentration. Once pooled into one library, true quantification was measured using the Qubit 4 fluorometer (ThermoFisher Scientific). Sequencing was carried out on the Illumina NextSeq 550 Dx and data were analyzed using Pillar's analysis software, PiVAT®.

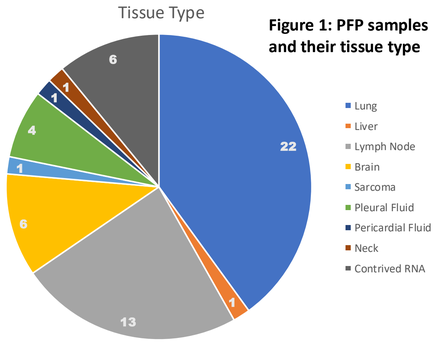

Figure 1: PFP samples and their tissue type

Results

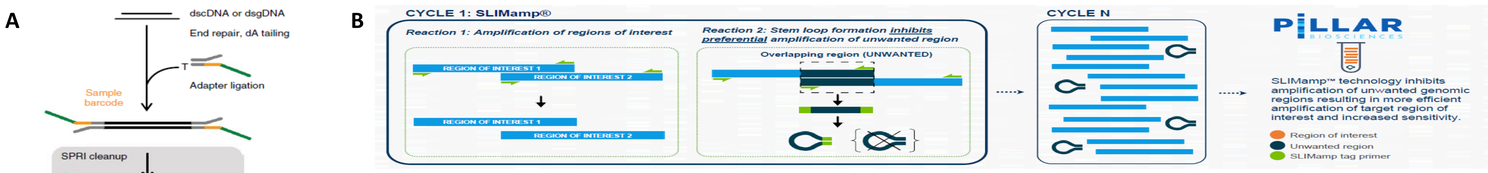

Figure 2. Schematic diagrams of Archer panels and Pillar panels. A. Anchored Multiplex PCR (AMP) for targeted sequencing for the Archer FP ST panel[4]. Following cDNA synthesis/gDNA end repair, dA tailing and ligation, the fragments of interest are amplified by utilizing gene-specific primers (GSP1/2) and are subject to sequencing. **B.** Stem-loop inhibition mediated amplification (SLIMamp) for the Pillar ST and MPN panels[5]. The regions of interest are amplified by primers that can form stem loops in the overlapping regions which inhibits amplification of unwanted fragments.

Figure 3: Most frequently observed fusions of AFP & PFP

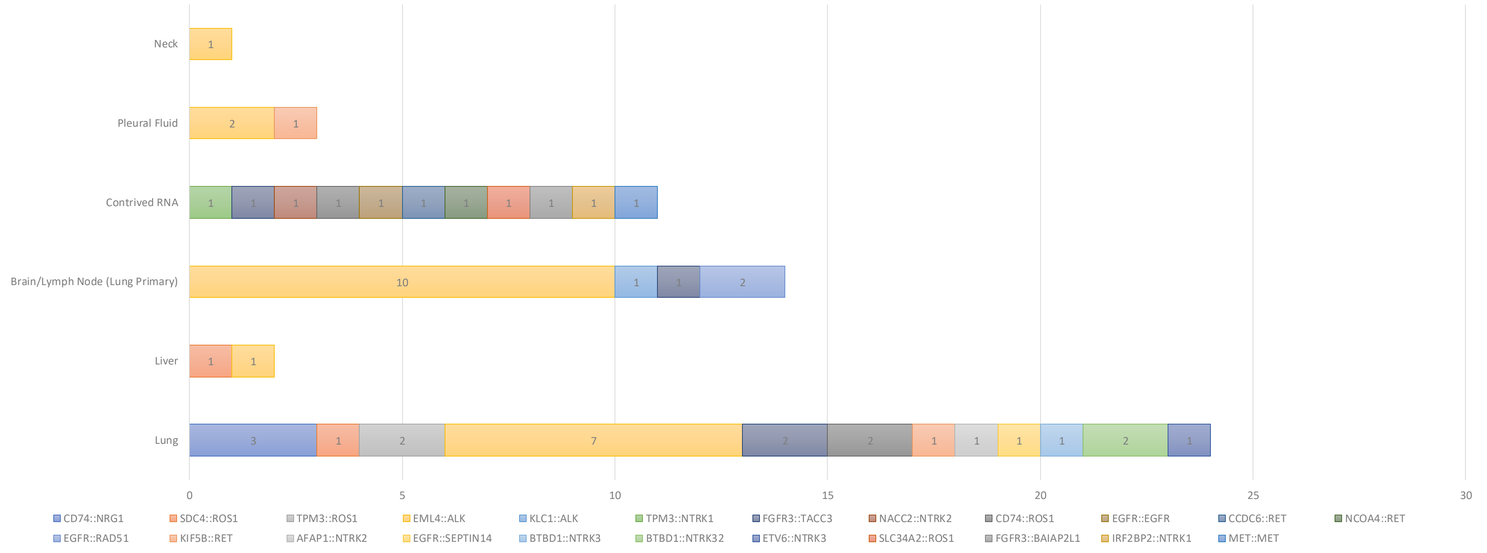

Table 1: Metrics for each panel used

Platform	Targeted Genes	Input Range	Fusion Chemistry	Fusions Detected	Cost/Test	Library Prep Duration	Hands on Time
Pillar	18	10-50ng	Targeted	82/82 fusions	$600-$320 (PFP) (8-24 samples)	~4.5 hr	~3 hr
Archer	17	150-200ng	Novel	81/82 fusions	$1190-$732 (AFP) (8-16 samples) $535-$475 (LungV2)	~36 hr	~3.5 hr

Discussion & Conclusions

The RNA input amount ranged from 25-100ng. The recommended read depths per sample for PFP and AFP are 50k and 3.5M reads, respectively. Of the 96 total fusions, 82 were covered on both panels, 13 were only covered on the AFP panel and 1 was not covered on either panel. The AFP panel detected 81/82 fusions, whereas PFP detected 82/82 fusions. The 1 false negative sample was repeated from library prep on the AFP panel and the missing fusion was detected. This sample was initially undetected due to not meeting minimum thresholds for AFP panel (≥3 start sites (SS); ≥5 reads; ≥10% reads). Overall, the *oncoReveal*® panel was 100% concordant with the FUSIONPlex™ panel for these actionable fusions.

The *oncoReveal® Multi-Cancer RNA Fusion v2* panel offers a fast, cost-effective and accurate alternative to traditional fusion assays, with performance comparable to the Archer FUSIONPlex™ panel. Its ability to work with low input materials, fewer reads, and integration with other panels makes it suitable for routine clinical use. Further, the hands-on time was significantly different between the two panels with PFP saving approximately 1 day. While this panel can be used to rapidly screen for actionable fusions prior to comprehensive genomic profiling, it will not pick up novel fusions. However, PFP may potentially offer a fast and effective means to monitor fusion alterations during cancer treatment.

References

1. Archer™ FusionPlex™ Protocol for Illumina, RA-DOC-047 / REV01
2. Pillar oncoReveal™Multi-Cancer RNA Fusion v2 User Manual, UM-0026 version