Comparative Laboratory Performance Evaluation of Pillar Biosciences, ArcherDx, and ThermoFisher Sequencing Chemistries for the Targeted Characterization of CRC & NSCLC Samples

Cameron Sammons[1], Heather Amidon[1], Devon Hemnauth[3], Virginia C. Thurston, PhD[2], Holli M. Drendel, PhD[2]
1. Atrium Health, Charlotte, NC; 2. Carolinas Pathology Group, Charlotte, NC; 3. Pillar Biosciences, Natick, MA

Background

Numerous variants have been categorized as diagnostic, prognostic and/or therapeutic in colorectal cancer (CRC) and non-small cell lung (NSCLC) cancer. To be efficient, minimize laboratory costs, and maximize patient samples, somatic sequencing panels are utilized to evaluate multiple clinically significant genes at one time. Rapidly developing technology leads to discontinuation of instruments and the need for new workflows. Here we report the results of the evaluation and comparative workflow assessment of three different methodologies utilizing CRC and NSCLC samples.

Materials & Methods

Twelve formalin-fixed paraffin-embedded (FFPE) tissue samples (macrodissected from slides) that were previously determined to be positive or negative on the 50-gene Ion Ampliseq Hotspot Panel v2 (ThermoFisher) were tested on the oncoReveal 47-gene Solid Tumor Panel (Pillar Biosciences) and the VariantPlex 20-gene HGC v2 (ArcherDx/IDT) panel. DNA was isolated using the cobas DNA sample preparation kit (Roche Diagnostics) and prepared prior to loading on the Personal Genome Machine (PGM) or Illumina NextSeq 550Dx. The Ampliseq panel utilizes clonal amplification and Ion Sphere Particles, while the oncoReveal utilizes a proprietary SLIMamp technology and the VariantPlex utilizes anchored multiplex PCR (Figure 1). Data analysis occurred using the ThermoFisher Torrent Browser, Pillar PiVAT pipeline and the Archer Analysis Unlimited pipeline.

Results

Tumor percentages and input DNA for the FFPE samples ranged from 40-90% and 10ng-100ng, respectively. Accuracy, sensitivity, on-target reads, mean depth, LOD, cost, and tech time were determined/compared (Table 1). The accuracy and sensitivity for all three methods was 100%. However, when comparing the average on-target read percentage, mean depth, and LOD, slight differences were noted between the panels (Table 2).

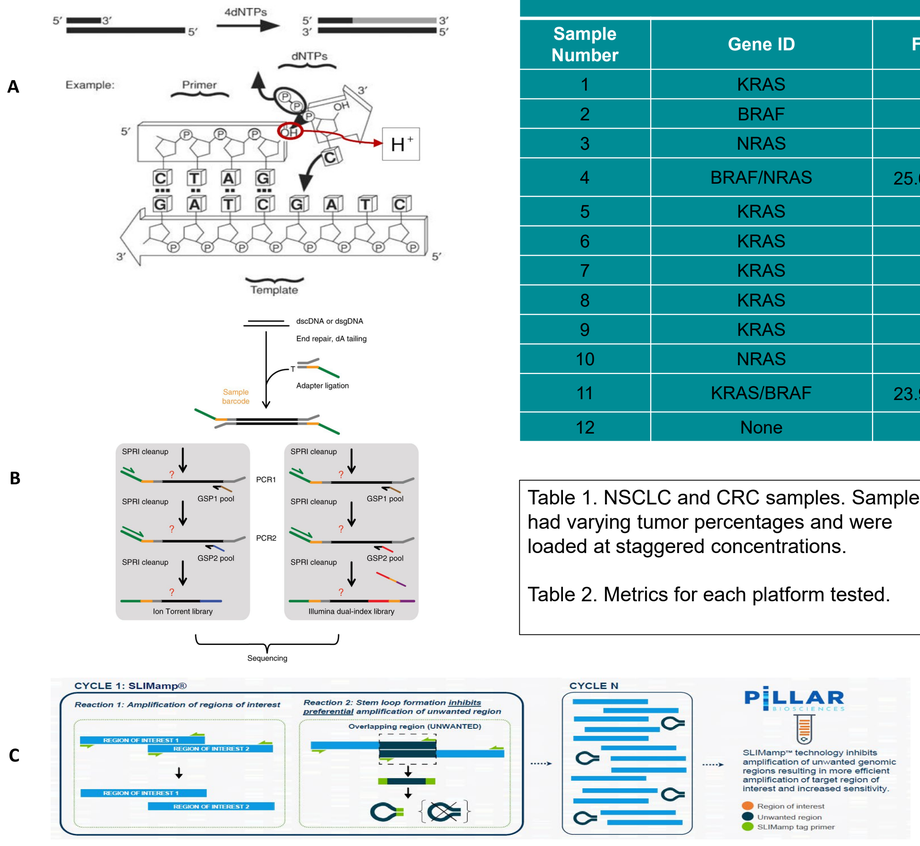

Figure 1. A. IonTorrent chemistry with Ampliseq panel. Nucleotides flow over the chip and DNA extension is detected by individual pH meters when an H^+ ion is released. B. Anchored Multiplex PCR (AMP) chemistry. Molecular barcodes are added to DNA that allows for identifying samples. Two target-specific PCR cycles amplify areas of interest for sequencing. C. SLIMamp chemistry with oncoReveal panel. Targeted regions are amplified and unwanted regions are selected against by creating stem loops.

Table 1

Sample Number	Gene ID	Frequency	Tumor Type	Tumor %	[Sample] Loaded (ng)
1	KRAS	69.40%	Lung	70%	20
2	BRAF	40.60%	Colon	90%	30
3	NRAS	37.80%	Colon	90%	40
4	BRAF/NRAS	25.60%/24.10%	CAP	N/A	88
5	KRAS	26.60%	Colon	60%	48
6	KRAS	21.70%	Lung	70%	60
7	KRAS	24.30%	Lung	60%	60
8	KRAS	11.20%	Lung	70%	70
9	KRAS	34.60%	Colon	90%	90
10	NRAS	7.50%	Colon	80%	80
11	KRAS/BRAF	23.90%/23.60%	Lung	75%	10
12	None	Neg	Colon	40%	10

Table 1. NSCLC and CRC samples. Samples had varying tumor percentages and were loaded at staggered concentrations.

Table 2. Metrics for each platform tested.

Table 2

Platform	On-Target Reads (Avg)	Mean Depth (Avg)	LOD	Mapped Reads (Avg)
Pillar	98.6%	197,512.6	1%	91,422,534.83
Archer	92.1%	7,195.5	2%	1,870,865
Thermo Fisher	98.1%	2,494.1	5%	568,805.08

Table 3

Platform	Cost per test (16 samples/ run)	Number of samples (YTD)	Potential cost (YTD)
Pillar	$597.82	173	$103,422.86
Archer	$1,084.71	173	$187,654.83
Thermo Fisher	$2,194.94	173	$379,724.62

Table 3. Cost per test analysis for each platform tested.

Discussion & Conclusion

All panels performed very well in our hands. Because FFPE samples are typically of low-quality with minimal DNA, it was beneficial that the oncoReveal requires smaller amounts of input DNA (10ng) than the VariantPlex (100ng), as compared to the Ampliseq (10ng). Due to the difference in chemistries, the oncoReveal hands-on workflow was faster versus the Ampliseq and VariantPlex workflows. This allowed for library prep and sequencer loading to be completed in one 8-hour shift versus two 8-hour shifts; thus, providing at least one day faster turn-around time and reduced tech time. Additionally, the cost of the oncoReveal reagents were less as compared to the VariantPlex and Ampliseq. Overall, all panels/platforms performed well; however, based on the efficiency, cost, minimal DNA input and coverage, the oncoReveal panel outperformed the others.

References

1. PMID: 25384085
2. Images courtesy of ThermoFisher and Pillar Biosciences