# Evaluation of the Pillar NGS SLIMamp™ Lung and Colon Hot Spots Panel

Jason D. Peterson[1], Francine B. de Abreu[1], Zhaohui Wang[2], Wendy A. Wells[1], Gregory J. Tsongalis[1]

[1]Department of Pathology and Laboratory Medicine, DHMC and NCCC, Lebanon, NH and Geisel School of Medicine, Hanover, NH and
[2] Pillar Biosciences, Natick, MA

## ABSTRACT

**Introduction:** Next-generation sequencing (NGS) has become a critical technology in guiding patient treatment in clinical oncology. As laboratories are increasingly challenged to reduce testing time while managing increased sample volumes, there is a high demand for targeted panels that offer rapid library preparation and the ability to highly multiplex patient samples. Here we evaluate the Pillar SLIMamp™ Lung and Colon Hot Spots Panel and compare the results to the Ion Torrent Cancer Hotspot Panel v2 (CHPv2).

**Methods:** A total of 15 samples were included in this evaluation: six non-small cell lung carcinoma (NSCLC) and nine colon adenocarcinoma. All samples had DNA concentration higher than 50 ng/µL and high DNA quality (Q129bp/Q41bp: 0.8-0.92) according to the KAPA hgDNA Quantification and QC Kit. Library preparation was performed using 50 ng and 5 ng of gDNA of each sample. A total of 30 samples were normalized using Qubit, pooled and sequenced on the v3 cartridge on the Illumina's MiSeq® system. For data analysis, FASTq files were uploaded to the Pillar, where sequence alignment, annotation, and variant classification were performed. Variant calls within genomic regions covered by both panels were compared.

**Results:** For the 15 FFPE samples, there was a high degree of concordance between the SLIMamp™ Lung and Colon Hot Spots Panel and CHPv2 variant calls (90.0%, 27/30 variants). Three variants that were called by the Pillar panel were not called using the CHPv2 (two single base-pair deletions and one-point mutation). In addition, variant calls for the Pillar panel were highly reproducible using both 50 ng and 5 ng of input material (100.0% concordance, 30/30 variants). Allelic frequencies for the variants detected in the 50 ng and 5 ng replicates were also highly reproducible (average deviation of 1.5% between replicates).

**Conclusions:** As NGS tumor profiling becomes an increasingly integral component in determining patient treatment, clinical laboratories will need to accommodate high sample volumes and variable specimen quality. The Pillar SLIMamp™ Lung and Colon Hot Spots sequencing panel allows laboratories to perform accurate, highly-multiplexed, targeted NGS using benchtop instruments. In addition, this panel demonstrates a high degree of reproducibility in variant calls using both average and extremely low FFPE DNA inputs.

## INTRODUCTION

- Next-generation sequencing (NGS) has become a critical technology in guiding patient treatment in clinical oncology.
- As laboratories are increasingly challenged to reduce testing time while managing increased sample volumes, there is a high demand for targeted panels that offer rapid library preparation and the ability to highly multiplex patient samples.

**Aim.** To evaluate the Pillar SLIMamp™ Lung and Colon Hot Spots Panel and compare the results to the Ion Torrent Cancer Hotspot Panel v2 (CHPv2).

## METHODS

**Samples.**

- **Internal control:** the EGFR ΔE746-A750 50% FFPE Reference Standard used in our routine sequencing runs was included in this study.
- **Clinical:** 14 samples previously screened by our laboratory using CHPv2 were selected for this study: five NSCLC (non-small cell lung carcinoma) and nine colon adenocarcinoma.
  - All samples had DNA concentrations greater than 50 ng/µL according to Qubit and good DNA quality (Q129bp/Q41bp: 0.8-0.92) according to the KAPA hgDNA Quantification and QC Kit.

**SLIMamp™ Lung and Colon Hot Spots Panel.**

- **Sample dilution:** samples were diluted to 10 ng/µL and to 2.5 ng/µL.
- **Library Preparation** was performed using 50 ng and 5 ng of each sample.
- **Sequencing:** a total of 30 samples were normalized to 4 nM, pooled and sequenced on the MiSeq System.

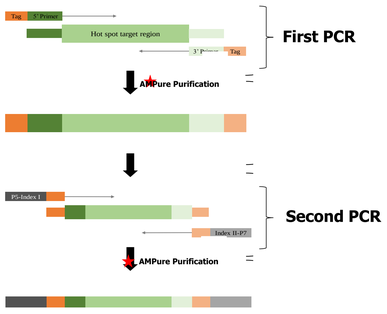


**Figure 1.** SLIMamp™ Lung and Colon Hot Spots Panel chemistry.

**Table 1.** Genes present in the Pillar Panel.

| Pillar SLIMamp™ Lung and Colon Hot Spots Panel | | | |
|---|---|---|---|
| AKT1 | ERBB2 | KRAS | PTEN |
| ALK | ERBB4 | MAP2K1 | SMAD4 |
| BRAF | FBXW7 | MET | STK11 |
| CTNNB1 | FGFR1 | NOTCH1 | TP53 |
| DDR2 | FGFR2 | NRAS | |
| EGFR | FGFR3 | PIK3CA | |

- **Panel:** 22 genes (1,800 hotspots)
- **Input:** > 2.5 ng FFPE gDNA (good quality)
- **Workflow:** 1 day library preparation, 1 day sequencing (MiSeq)

## METHODS cont.

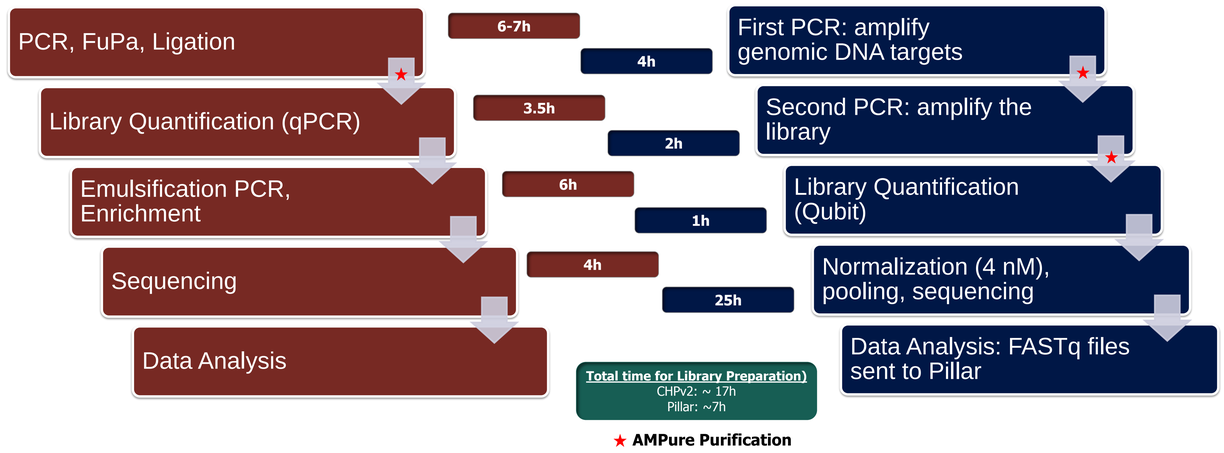


**Figure 3.** Cancer Hotspot Panel v2 (CHPv2) vs SLIMamp™ Lung and Colon Hot Spots Panel workflow.

## RESULTS

### CHPv2 vs Pillar SLIMamp™ Lung and Colon Hot Spots Panel.

- **Total of variant calls:** high degree of concordance between both panels' variant calls (90.0%, 27/30 variants).
- **Reproducibility:** high degree of reproducibility using both 5ng and 50ng of FFPE derived input DNA (100.0% concordance, 30/30 variants).

### Variant Allele Frequencies

- **Comparison** of Variant Allele Frequencies for 5ng and 50ng sample replicates (Only non-synonymous mutations).
- **Variant frequencies** were extremely reproducible using both 5ng and 50ng of FFPE derived input DNA (An average deviation of only 1.5% was observed between the 50ng and 5ng replicates)**.**

### Variant Quality Scores

- **Comparison** of Variant Quality Scores for 5ng and 50ng sample replicates (Only non-synonymous mutations.
- **High Degree** of reproducibility was also observed in the variant quality scores using both 50ng and 5ng of FFPE derived DNA.
- Variability between replicates was +/- 1.

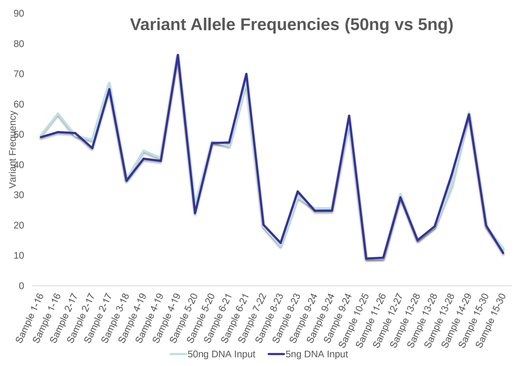


**Figure 4.** Variant Allele Frequency Comparison (50ng and 5ng DNA Input).

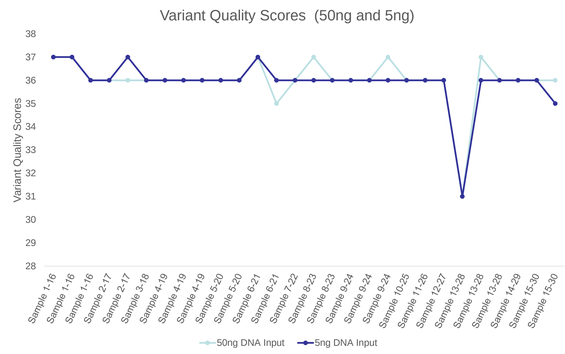


**Figure 5.** Variant Quality Scores Comparison (50ng and 5ng DNA Input).

## CONCLUSIONS

- The Pillar SLIMamp™ Lung and Colon Hot Spots sequencing panel demonstrates a high degree of reproducibility in variant calls using either average or extremely low DNA inputs.
- The Pillar SLIMamp™ Lung and Colon Hot Spots sequencing panel allows laboratories to perform accurate, highly-multiplexed, targeted NGS using benchtop instruments.