# Evaluation of the Pillar NGS SLIMamp™ Cancer Hotspot Panel

Francine B. de Abreu[1], Jason D. Peterson[1], Brian Dugan[2], Zhaohui Wang[2], 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) methods for somatic mutation detection have been rapidly integrated in clinical oncology. The main considerations for the clinical laboratories to adopt the NGS test include the ability of using low-quality and low amount of FFPE DNA as input, sequencing quality, assay turn-around-time, ease-of-use, cost, and sample multiplexing capacity. To address these needs, Pillar Biosciences has developed a streamlined single-tube multiplex-PCR-based library-prep technology (SLIMamp) and an accompanying NGS data analysis pipeline. In this study, we evaluated the performance of the Pillar SLIMamp™ Cancer Hotspot Panel for 56 genes and assessed the concordance of variant detection against the previously reported clinical results tested by the Ion Torrent Cancer Hotspot Panel v2 (CHPv2).

**Methods:** A total of 15 FFPE samples were included in this evaluation from disparate indications: glioma and colon adenocarcinoma. All samples had DNA quality Q129bp/Q41bp above 0.4 according to the KAPA hgDNA Quantification and QC Kit. A range of 17 – 72 ng of gDNA was used as input for SLIMamp library preparation. A total of 24 samples were normalized, pooled, and sequenced on Illumina's MiSeq® system using v3 chemistry. For data analysis of the Pillar panel, FASTQ files were uploaded to the Pillar pipeline, where sequence alignment, variant calling and annotation were performed. Variant calls within genomic regions covered by both panels were compared. The data was then compared to clinical samples which had previously been tested in a CLIA lab.

**Results:** For the 15 FFPE samples, the SLIMamp™ Cancer Hotspot Panel displayed both high median on-target percentages (99.57%) and mapping rates (99.11%) across targeted regions with the mean base coverage ranging from 2816x-4857x. The SLIMamp Cancer Hotspot Panel demonstrated 100% concordance between the panel and previous clinical results. In addition, the panel demonstrated excellent sensitivity detecting the presence of alleles above 5% frequency.

**Conclusions:** The Pillar SLIMamp Cancer Hotspot Panel allows for the interrogation of a diverse set of solid tumor samples and a high degree of sample pooling on a MiSeq instrument, providing quick turn-around time from extracted DNA to data analysis.

## INTRODUCTION

- Next-generation sequencing (NGS) methods for somatic mutation detection have been rapidly integrated in clinical oncology.
- The main considerations for clinical laboratories to adopt NGS test include the ability of using low-quality and low amount of FFPE DNA as input, sequencing quality, assay turn-around-time, ease-of-use, cost, and sample multiplexing capacity. To address these needs, **Pillar Biosciences** has developed a streamlined single-tube multiplex-PCR-based library-prep technology (SLIMamp) and an accompanying NGS data analysis pipeline.

**Aim.** To evaluate the performance of the Pillar SLIMamp™ Cancer Hotspot Panel for 56 genes and assessed the concordance of variant detection against the previously reported clinical results tested by the Ion Torrent Cancer Hotspot Panel v2 (CHPv2).

## METHODS

**Evaluation Project: Study Design (Figure 1).**

- **Goal:** to assess the concordance of variant detection between the Ion Torrent Cancer Hotspot Panel v2 (CHPv2) and Pillar SLIMamp™ Cancer Hotspot Panel.
- **Samples:** a total of 178 samples were blinded at DHMC and sent to Pillar Biosciences.
- **Library Preparation:** DNA input and library quantification
  - DNA input: 16 ng- 101 ng (samples were not normalized).
  - Library quantification: 6.5 nM – 126.0 nM.
- **Sequencing:** all samples were normalized to 4 nM, pooled and sequenced on an Illumina MiSeq, MiSeqDx or NextSeq500.
- **Data Analysis:** Pillar PiVAT software pipeline was used for analysis (allelic frequency (AF)>2.5%).
- **Data comparison:** files were sent to DHMC for data comparison.

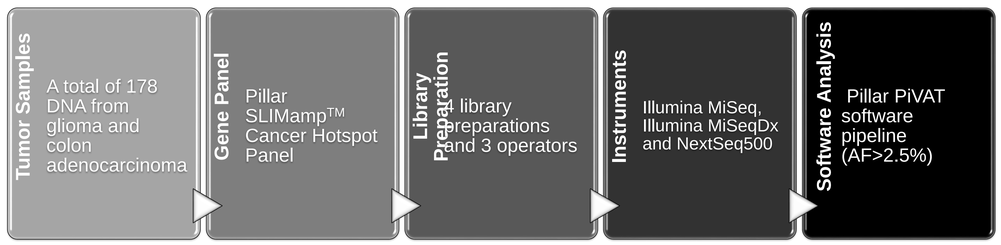


**Figure 1.** Evaluation Project: study design.

## METHODS cont.

**Samples.**

- **Clinical:** 146 colon adenocarcinoma and 32 glioma.
- **Sample information (Figure 2):** DNA extraction procedure, DNA quantification and DNA quality.
- **NGS.** All samples were previously sequenced at DHMC using the CHPv2 panel.

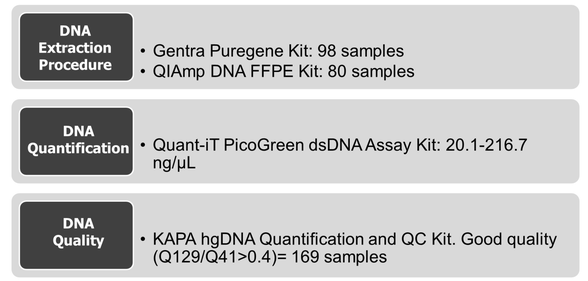


**Figure 2.** Detailed information of each sample prior to library preparation.

**Ion Torrent Hotspot Cancer Panel v2 (CHPv2).**

- **Panel:** 50 genes with 207 amplicons (Table 1), which includes single nucleotide variants (SNVs) and insertions/deletions (INDELs)
- **DNA input:** 10 ng FFPE gDNA
- **Workflow:** 3 days library preparation + sequencing
- **Data analysis:** Torrent Server 4.0.2 and Golden Helix Software 8.3.4

**Pillar SLIMamp™ Cancer Hotspot Panel.**

- **Panel:** 56 genes with 251 amplicons (Table 2), which includes single nucleotide variants (SNVs), insertions/deletions (INDELs), splice variants and CNVs
- **DNA input:** 5 ng FFPE gDNA
- **Workflow:** 2 days library preparation + sequencing
- **Data analysis:** Pillar PiVAT software pipeline

**Table 1.** Gene list of the Ion Torrent Hotspot Cancer Panel v2 (CHPv2).

| Ion Torrent Hotspot Cancer Panel v2 (CHPv2) | | | | |
|---|---|---|---|---|
| ABL1 | EGFR | GNAQ | KRAS | PTPN11 |
| AKT1 | ERBB2 | GNAS | MET | RB1 |
| ALK | ERBB4 | HNF1A | MLH1 | RET |
| APC | EZH2 | HRAS | MPL | SMAD4 |
| ATM | FBXW7 | IDH1 | NOTCH1 | SMARCB1 |
| BRAF | FGFR1 | IDH2 | NPM1 | SMO |
| CDH1 | FGFR2 | JAK2 | NRAS | SRC |
| CDKN2A | FGFR3 | JAK3 | PDGFRA | STK11 |
| CSF1R | FLT3 | KDR | PIK3CA | TP53 |
| CTNNB1 | GNA11 | KIT | PTEN | VHL |

**Table 2.** Gene list of the Pillar SLIMamp™ Cancer Hotspot Panel.

| Pillar SLIMamp™ Cancer Hotspot Panel. | | | |
|---|---|---|---|
| ABL1 | EZH2 | JAK2 | PIK3CA |
| AKT1 | FBXW7 | JAK3 | PTEN |
| ALK | FGFR1 | KDR | PTPN11 |
| APC | FGFR2 | KIT | RAC1 |
| ATM | FGFR3 | KRAS | RB1 |
| BRAF | FLT3 | MAP2K1 | RET |
| CDH1 | FOXL2 | MET | ROS1 |
| CDKN2A | GNA11 | MLH1 | SMAD4 |
| CSF1R | GNAQ | MPL | SMARCB1 |
| CTNNB1 | GNAS | NOTCH1 | SMO |
| DDR2 | HNF1A | NPM1 | SRC |
| EGFR | HRAS | NRAS | STK11 |
| ERBB2 | IDH1 | NTRK1 | TP53 |
| ERBB4 | IDH2 | PDGFRA | VHL |

## RESULTS

**CHPv2 vs Pillar SLIMamp™ Cancer HotSpot Panel** (only overlapping regions)

- **Targeted region:** a total of 15,049 bp overlap between both panels (Figure 3).
- **Library yield (nM):** samples with higher quality Q129/Q41≥0.6 had library quantification higher than 50 nM.

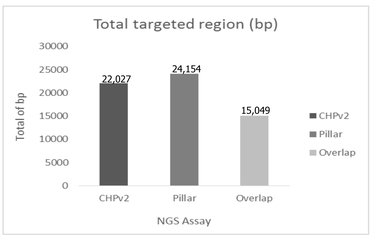


**Figure 3.** Comparison targeted region coverage.

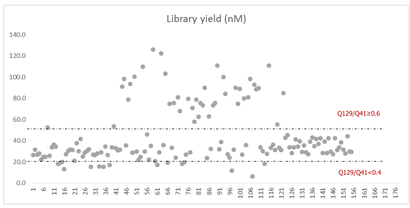


**Figure 4.** Library yield vs DNA quality.

- **SNVs and INDELs calls with AF> 5% only:** 100% of concordance between both panels (15/178 samples).
- **Variant Allelic Frequency:** high degree of similarity between both panels.

**Table 3.** AF and Variant types detected by each panel.

| | CHPv2 Panel | Pillar SLIMamp™ Cancer HotSpot |
|---|---|---|
| **Allelic Frequency** | | |
| LOD | > 5% | > 2.5% |
| **Variant Types** | | |
| SNVs | ■ | ■ |
| Insertions | ■ | ■ |
| Deletions | ■ | ■ |
| Splice Variants | ■ | ■ |
| CNVs | ■ | ■ [1] |

[1] Five samples previously screened for CNVs using array. NGS panel had concordant results with 3 samples (discordant results were due to panel coverage).

## CONCLUSIONS

- The **Pillar SLIMamp™ Cancer Hotspot Panel** allows for the interrogation of a diverse set of solid tumor samples and a high degree of sample pooling on a MiSeq instrument, providing quick turn-around time from extracted DNA to data analysis.