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CASE STUDY

Validation and Clinical Utilization of the SLIMamp-based Columbia Solid Tumor Panel, a Sensitive and Robust Single-Vial Amplification NGS Assay

Next-generation sequencing (NGS) is a multifaceted technique that can generate sequencing data at an unprecedented rate. In the realm of cancer, this can be used to look for somatic alterations in numerous tumor types for the purposes of therapeutic application, diagnostic confirmation, prognosis, and classification.

Target enrichment methods in NGS are typically divided into two categories: hybrid capture and amplicon-based enrichment. While hybrid capture-based enrichment is highly scalable and good for large gene panels, it typically requires high DNA input requirements, a complicated and lengthy library preparation process, and higher costs, making it less viable for small-scale panels or for smaller labs.

Amplicon-based enrichment broadly uses PCR to amplify target sites for study and generally requires less time, resources, and starting material. However, the overlapping regions between the adjacent overlapping amplicons will be preferentially amplified and dominate the reaction. Traditionally, to get rid of these overlapping regions, PCR reactions are separated to amplify each target region individually before they are pooled together, which adds time and resources.

To combat these problems, Pillar Biosciences developed a single-tube multiplexed approach to amplicon-based enrichment. Stem-Loop Inhibition Mediated amplification (SLIMamp) adds tags to the primer pairs so that overlapping regions have an affinity for each other, causing them to anneal to form stem-loop structures that cannot be further amplified (Figure 1). This approach reduces overlapping amplicons while preferentially driving target region amplification. Furthermore, this technique amplifies target regions in a single tube, creating a multiplexed approach that saves time and resources for optimal output. Lastly, the workflow is simple and integrates well into existing NGS workflows to reduce time and shorten turnaround times.

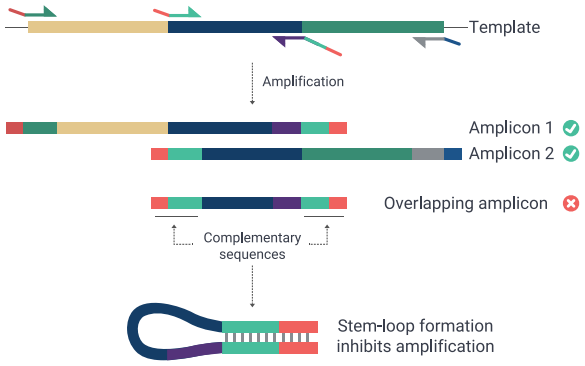

Figure 1 Overview of SLIMamp. Overlapping regions have tags that cause them to anneal into loop structures that prevent amplification.

This case study details the efforts of Columbia University Irving Medical Center (CUIMC) and how they validated and secured New York State Department of Health approval for the SLIMamp technology for their clinical solid tumor panel. The group, headed by Dr. Helen Fernandes, designed a SLIMamp-based custom solid tumor panel, known as the Columbia Solid Tumor

Panel (CSTP), as a hot spot panel to analyze 47 genes covering SNV's and indels and spanning lung, colon, thyroid, brain, melanoma, GIST and other solid tumor cancers.

Dr. Subit Barua presented data on CUIMC's validation and use of the CSTP at the CSCO 2020 conference. To validate the panel, CUIMC analyzed 146 samples, comprising various variant types including SNVs and insertions and deletions (indels), including variants that were part of homopolymers, and splice sites and promoter regions. Additionally, coding regions of two genes, STK11 and PTEN, were entirely sequenced. The CUIMC group found that coverage across the 47 genes of interest from the 146 samples was consistently high among all samples **(Figure 2)**. They also found broad coverage of all STK11 and PTEN coding regions, including pseudogenes and GC-rich domains, areas that are traditionally difficult to sequence using NGS.

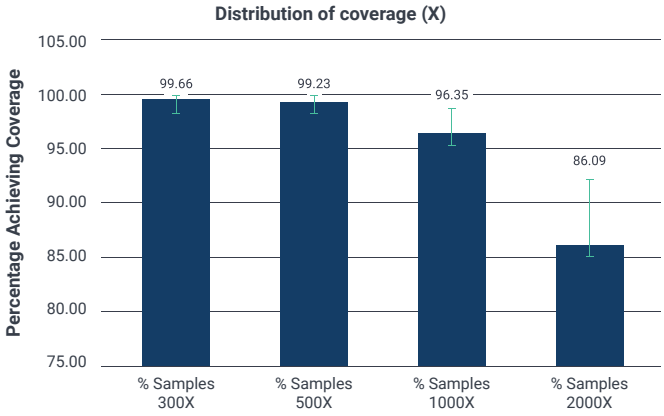

Figure 2 Distribution of coverage. SLIMamp has highly uniform coverage.

The group looked at the analytical accuracy of SLIMamp using GIAB NA12878, a DNA line used to validate all of their NGS assays. **(Figure 3)** shows the variant allele frequency (VAF) percentage for each of the genes with identified somatic variants. All variants were well defined as somatic mutations for each variant type with 100% concordance between 136 clinically determined variants. This data shows that the assay can accurately detect variants from both intra-run and inter-run replicates with strong coverage correlation and reproducibility.

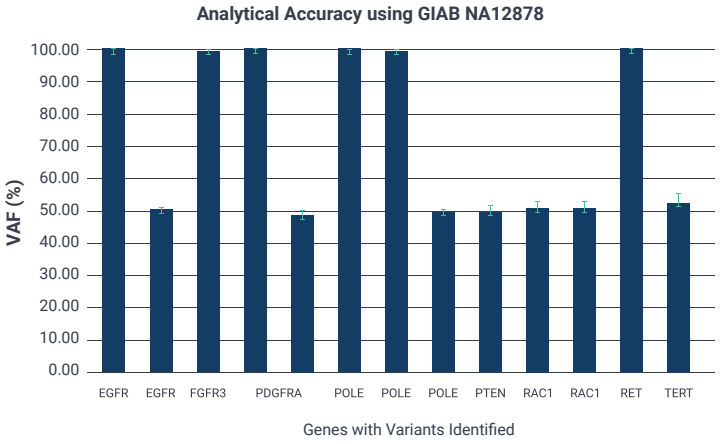

Figure 3 Analytical accuracy of SLIMamp using the reference line GIAB NA12878. For each reference gene, SLIMamp found an associated somatic mutation.

To find the lower limit of DNA input, the group looked at the ability for the assay to find minor allele frequencies (MAF) using decreasing amounts of DNA (20ng, 10ng, and 2.5ng) for each sample. The assay identified actionable variants in each tumor sample with high confidence and sensitivity **(Figure 4)**. Researchers also looked at the limits for detecting VAFs using serial dilutions (20%, 10%, 5%, and 2.5%). They found actionable variants in tumors with VAFs as low as 2.5%. Lastly, the researcher compared their current standard NGS pipeline to the new SLIMamp-powered panel. When compared to SoftGenetic's NextGene, the Pillar assay was comparable in determining variant allelic frequencies **(Figure 5)**.

In light of these validation results, Columbia University Irving Medical Center acquired New York approval for use of the CSTP in clinical cases.

Following introduction into clinical use, the CSTP has been instrumental in providing optional patient care. Dr. Barua provided two examples in his CSCO 2020 talk.

The first patient was a 93-year-old female with a clinical history of invasive adenocarcinoma. DNA (<5ng/ul) was extracted from FFPE and analyzed using the CSTP, and the team identified an activating EGFR exon 19 deletion mutation.

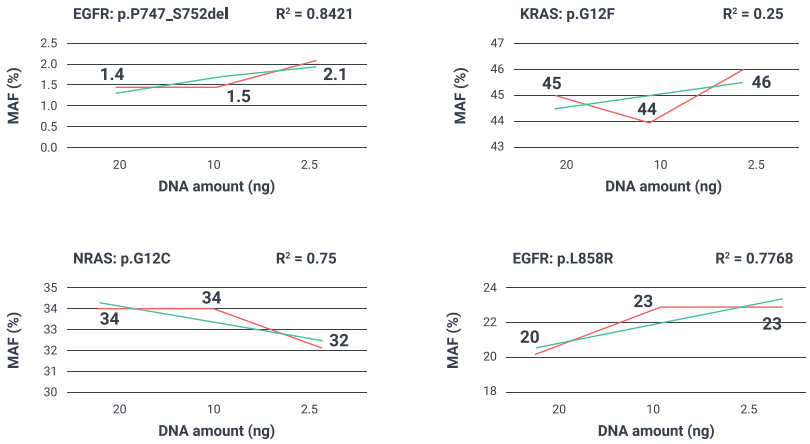

Figure 4 Validation of DNA input limits. SLIMamp found actionable alterations for each serial dilution across four mutations.

The second patient was a 60-year-old male with a history of cholangiocarcinoma. DNA (<1ng/ul) was extracted from FFPE, and the group found activating IDH1 mutations at codon 132 with a variant allele frequency of 8.9% using the Pillar assay.

These data demonstrate that the Comprehensive Solid Tumor Panel, powered by SLIMamp chemistry, is a sensitive and robust assay to detect solid tumor-associated variants.

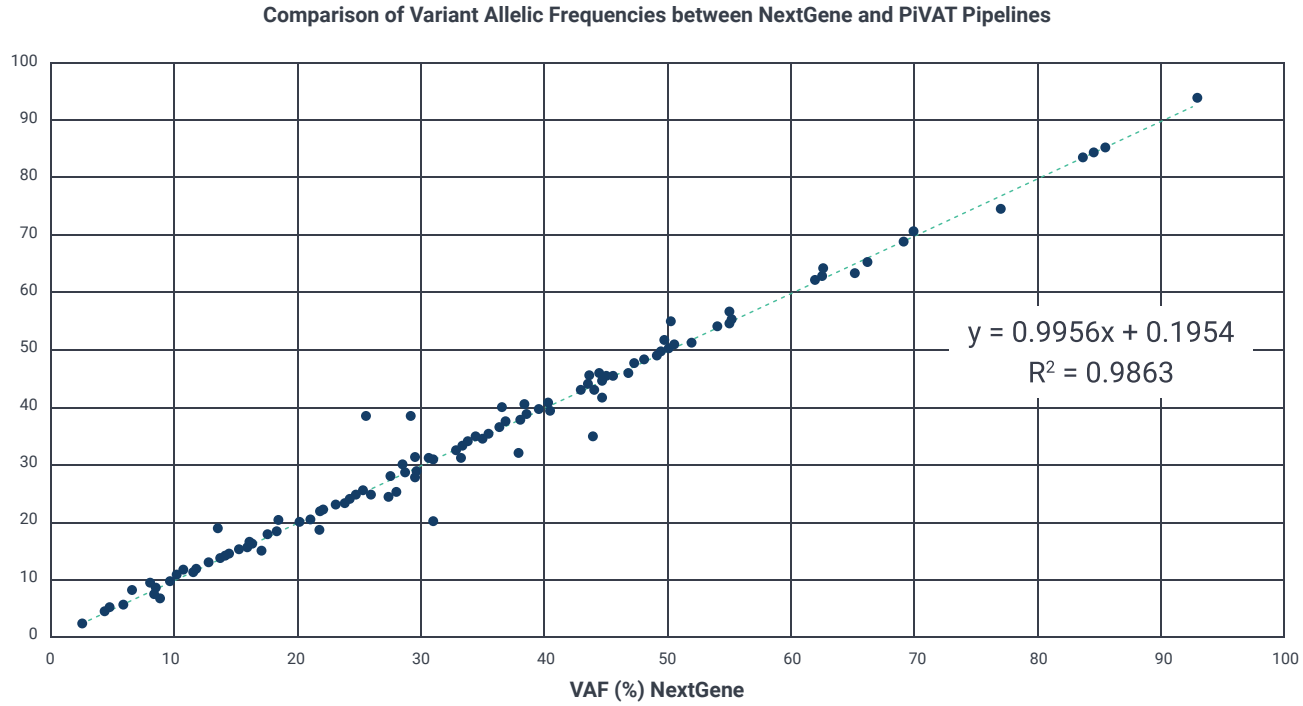

Figure 5 Comparison of SLIMamp-based PIVAT pipeline with NextGene pipeline. SLIMamp has comparable VAF detection with established pipelines.

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Pillar Biosciences, Inc.
9 Strathmore Rd Natick, MA 01760
(800) 514-9307
pillar-biosciences.com