PILLAR BIOSCIENCES

Making precision medicine accessible

Pillar Biosciences designs targeted NGS panels that deliver fast and accurate results for key regions of interest in the most actionable genes while minimizing false negatives.

Pillar Biosciences provides an end-to-end comprehensive NGS solution

ampPD™
Intelligent primer design platform

SLIMamp®
Library preparation

Sequencing

PiVAT™
Bioinformatics pipeline

Precision testing should deliver rapid and accurate insights.

Simple NGS library prep workflow

Maintain control of samples and results with single-tube, tiled amplification that can be performed in-house by any NGS lab

Sensitive and robust chemistry

Achieve variant detection as low as 1% VAF† without UIDs‡ and 0.1% with UIDs even with limited DNA input or poor sample quality

† VAF, variant allele frequency
‡ UID, unique ID; also known as unique molecular ID (UMI)

Reduced fully-loaded lab costs

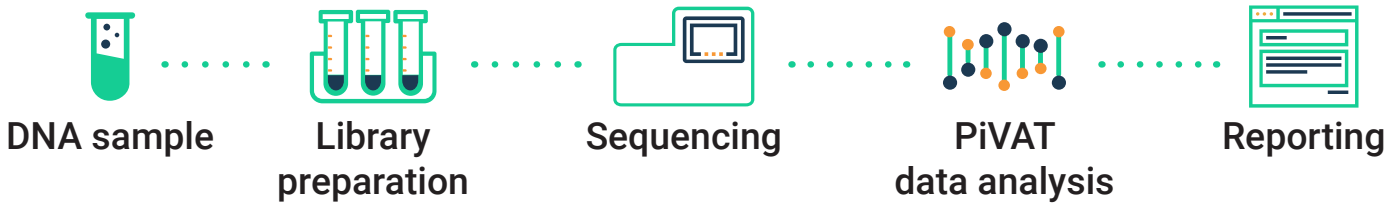
Improve lab efficiency with quicker turnaround time & reduced “no calls”, repeat testing, and difficult interpretation decisions

Pillar NGS assays deliver confident results

Attributes	Advantages
Simple, single-tube workflow	Tech friendly; reduces handling errors; easily automatable
Rapid turn-around time (<8 hours; minimal hands-on time)	Sample to sequencer in one day; maximizes tech utilization; increases NGS throughput
Low DNA input (as low as 1ng)	Fewer “no calls” from limited or degraded samples
Low limit of detection (as low as 1% VAF without UIDs and 0.1% with UIDs)	Obtain results from rare variants without the complexity of UIDs
High sensitivity	High precision even with limited DNA input near the limit of detection
High on-target rates (>90%)	Effectively utilizes sequencing real estate
High coverage uniformity (>90%)	Reduces sequencing depth; cost-effective sequencing

Simple, one-day workflow

Pillar NGS panels follow a simple workflow with a DNA-to-sequencer time of less than 8 hours. The enrichment chemistry is technician-friendly and performed in a single tube, so there is no need to pool amplicons and risk errors from sample handling. The workflow is extremely flexible and fully automatable.



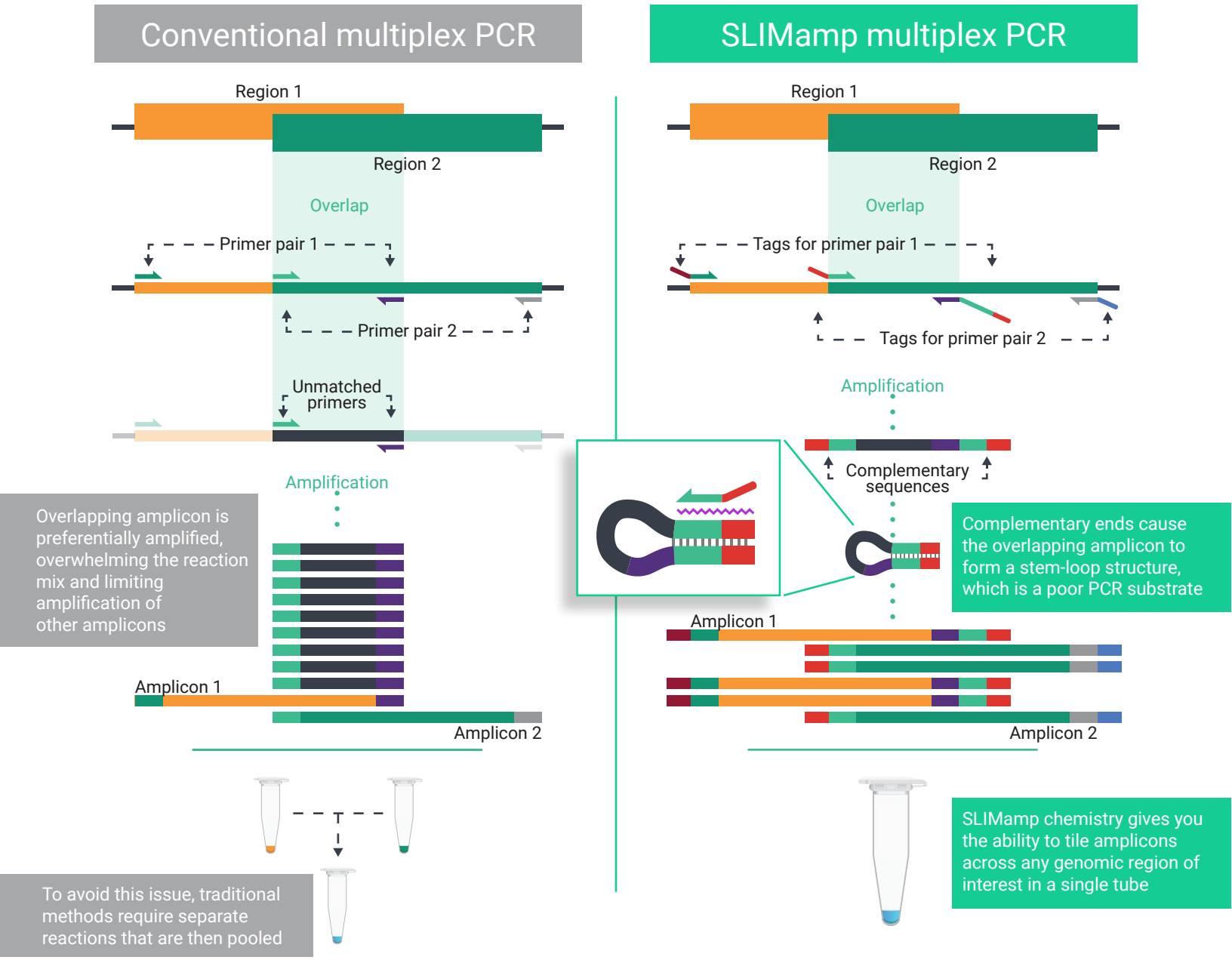
Gene-specific PCR & cleanup · Indexing PCR & cleanup · Quantitation & normalization · Load sequencer

Workflow advantages

- Single-tube amplification
- Minimal hands-on time
- Sample to sequencer in less than 8 hours
- Fully automatable workflow

SLIMamp enrichment chemistry

Stem-**L**oop **I**nhibition-**M**ediated **amp**lification (SLIMamp) is a proprietary tiled multiplex PCR-based enrichment chemistry that enables multiplex PCR to amplify overlapping regions in a single tube. SLIMamp library preparation is efficient and delivers high mapping and on-target rates.



Advantages of SLIMamp chemistry

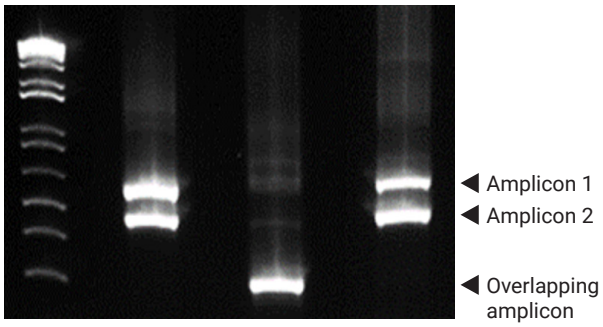
- Overlapping multiplex PCR
- Single-tube amplification
- Tile amplicons across regions of interest
- Lower DNA inputs with a single-tube workflow

SLIMamp performance

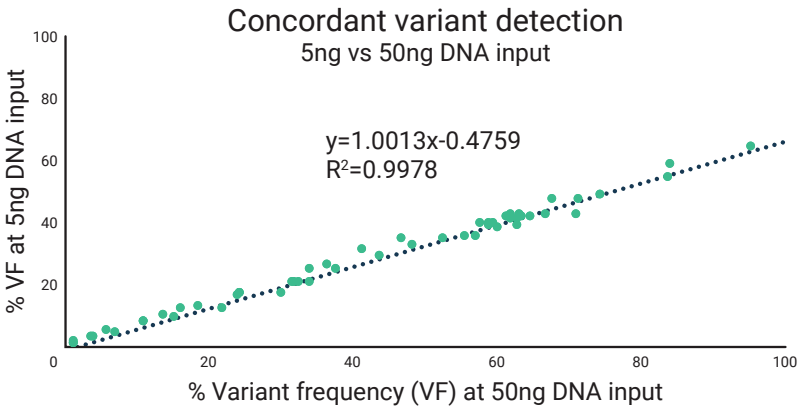
Single-tube, preferrential target amplification

Two overlapping regions of interest were amplified by multiplex PCR using conventional chemistry with primer pairs vs. SLIMamp chemistry with tagged primer pairs.

- Lane 2 shows a mix of two single-plex PCR reactions (amplicon 1 - 538bp; amplicon 2 - 401bp)
- Lane 3 shows the product of a conventional two-plex PCR of the two regions of interest using primer pairs for both regions of interest; **the single 236bp product is the unwanted overlapping amplicon**
- Lane 4 shows the products of SLIMamp two-plex PCR using tagged primer pairs to both regions of interest; **the two amplicons match the controls in lane 2, demonstrating the ability of SLIMamp to preferrentially amplify targets of interest over unwanted overlapping amplicons**



Lane 1 100bp ladder | **Lane 2** Mix of amplicons 1 & 2 | **Lane 3** Conventional 2-plex w/ primer pairs | **Lane 4** SLIMamp 2-plex w/ tagged primer pairs



Sensitive variant detection

ONCO/Reveal™ panels are highly precise even with low DNA inputs, demonstrating high concordance (R^2 >99%) between input amounts across a wide range of variant allele frequencies.

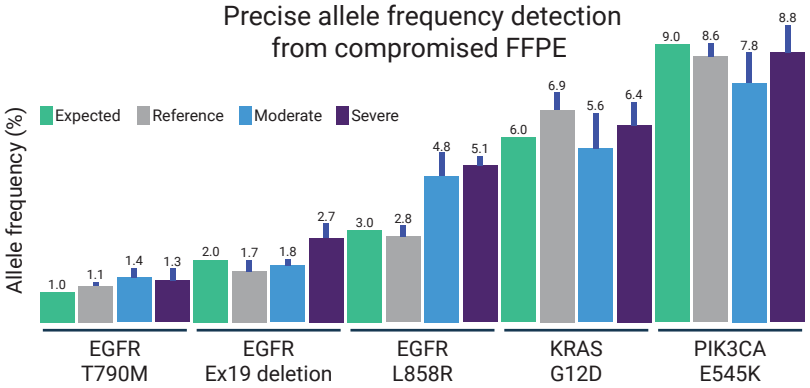
In the figure to the left, variant frequencies were detected by the ONCO/Reveal Lung and Colon Cancer Panel in 15 patient samples (6 NSCLC & 9 colon cancer) diluted to 5ng & 50ng. Assays and analyses were performed by Dartmouth Hitchcock and presented at AMP 2016.

Robust variant detection

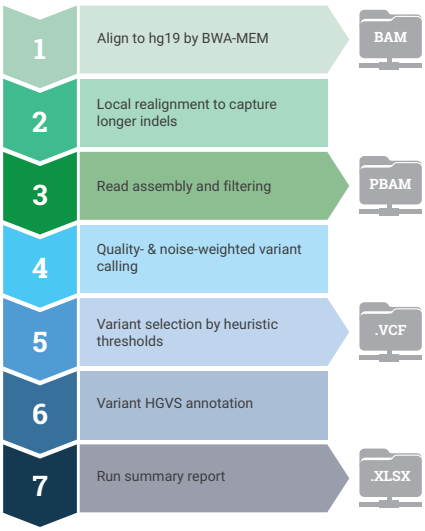
ONCO/Reveal panels are accurate, precise, repeatable and sensitive to near the limit of detection regardless of FFPE quality.

In the figure to the right, formalin-compromised reference standards (Horizon Discovery) were evaluated using the ONCO/Reveal Lung and Colon Cancer Panel, which demonstrated accurate results from degraded samples.

N=10; error bars=standard deviation



PiVAT bioinformatics pipeline



The Pillar Variant Analysis Toolkit (PiVAT) complements SLIMamp-based NGS assays for accurate and confident variant calling. The PiVAT software package is configurable to be deployed in the cloud or locally and processes data using the standard FASTQ file format.

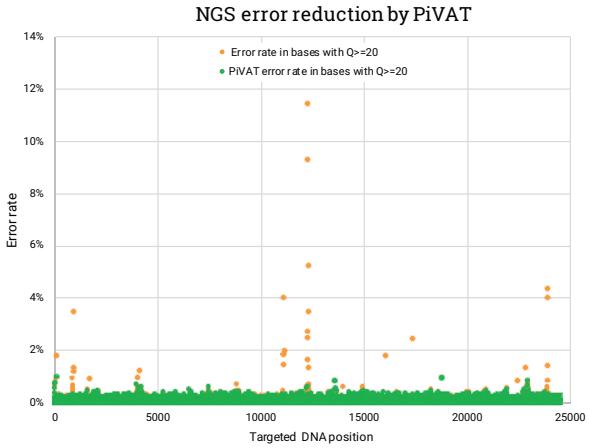
Through techniques such as local realignment and *de novo* read assembly, longer indels are detectable and errors suppressed that affect other enrichment and analysis techniques.

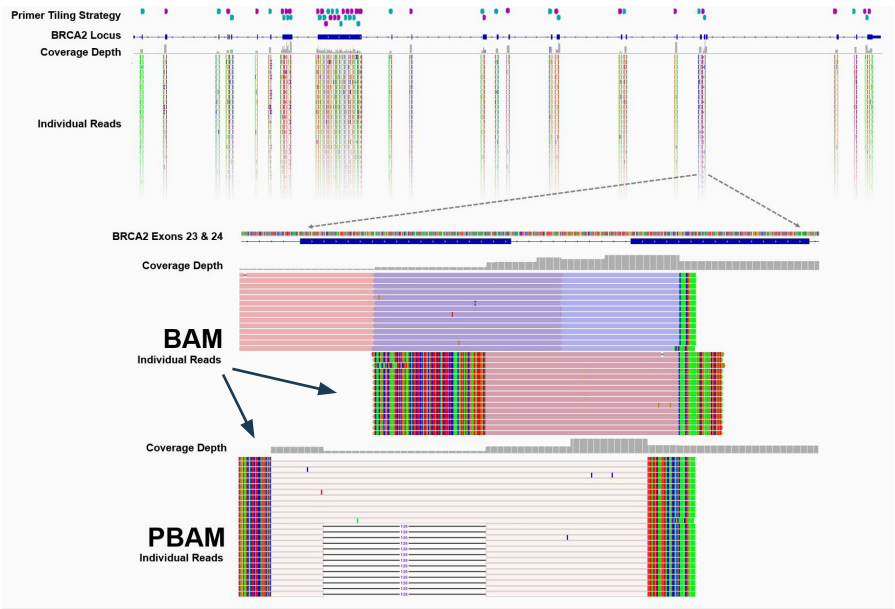
PiVAT provides both a standard Binary Alignment Map file (BAM) as well as a PiVAT version called PBAM. Both are used interchangeably in standard bioinformatic pipelines.

PiVAT effectively suppresses errors

After initial mapping and local re-alignment, PiVAT assembles the **overlapping paired-end (PE) reads**, retains base quality information and filters out reads without the proper amplicon structure. PiVAT takes advantage of overlapping PE reads in Pillar somatic mutation panels and, along with a quality- and noise-weighted calling algorithm, can call variants **as low as 1% allele frequency**.

The graph to the left illustrates the ONCO/Reveal Multi-Cancer target sequence of 25kb on the x-axis and its error rate shown as a function of coordinate positions. The frequency of ordinary recurring errors (Q scores of 20 and above) is shown in orange, and the much lower PiVAT error rate is shown in green.





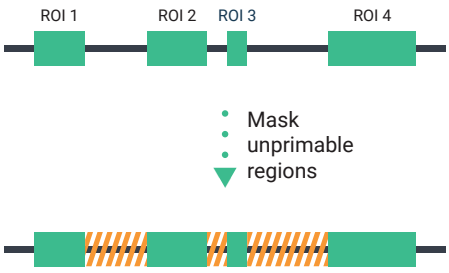
Use local aligment to detect large deletion events

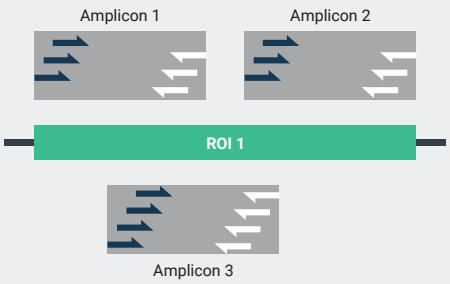
A reference sample with a known 126bp deletion in the BRCA2 gene was analyzed by the ONCO/Reveal BRCA1 & BRCA2 Panel, and both the BAM and PBAM files are shown in the IGV plot to the left. The deletion is indicated by the arrows in the figure.

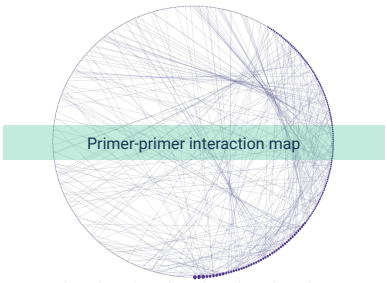
ampPD intelligent primer design

The proprietary ampPD primer design platform is used to build all of Pillar's standard and custom NGS panels by selecting the best primers for each and every amplicon. ampPD proactively masks all regions where primers cannot bind, automatically identifies all potential primer-dimers, and evaluates all options for primer placement. ampPD then selects the best primers for each and every amplicon. **ampPD rapidly and accurate designs primers to allow your project to progress quickly.**

Target preparation	Primer drafting and tiling	Primer screening and pooling
Perform tunable masking for multi-copy regions, repeats, and variants	Exhaustively calculate all primer tiling solutions for each ROI	Calculate potential amplifiable dimer pairs via cluster-based computing
Delineate regions of interest (ROI) and collapse proximal ROIs	Select primers that are optimized for FFPE samples	Compute single pool with the lowest dimer potential







Learn more at
pillar-biosciences.com

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Pillar Biosciences, Inc.
12 Michigan Dr
Natick, MA 01760
(508) 655-3027
info@pillar-biosciences.com