TECHNICAL NOTE

# LoopCap TECHNOLOGY

## ELEGANT, HIGH-PERFORMANCE TARGETED NGS

Targeted next-generation sequencing (NGS) allows for the focused interrogation of sequence space. Molecular Loop's novel LoopCap™ technology offers the depth of coverage required for your application at the scale needed by your organization. The workflow has been refined over a decade to achieve the data quality of hybridization capture-based enrichment with a workflow simpler than amplicon sequencing. With robust performance across biological inputs and flexible panel size and design, your imagination is the limit.

molecular loop

# Technology

## Probe design

LoopCap™ is a unique targeted NGS sample prep technology that utilizes advanced molecular inversion probes (MIPs). The use of MIPs in targeted NGS applications was first described in 2007 by Molecular Loop's Co-Founder and CEO, Greg Porreca.[1] The technology has been refined and optimized to maximize workflow simplicity, without sacrificing data quality. As shown in Figure 1, the LoopCap 4-step NGS workflow removes the need for conventional library prep and generates a targeted, sequencer-ready library of molecules representing genomic regions of interest in a highly redundant manner.

LoopCap design offers:

- Variable fill-in length for optimized paired-end sequencing on both short- or long-read sequencing platforms.
- Platform-specific amplification primers with unique dual sequencing indices (UDIs) to mitigate the impacts of index hopping.
- Unique molecular barcodes for use in low-input and high-sensitivity applications such as variant calling from cf/ctDNA.

## The power of redundancy

While the design of the ligation and extension arms enable exquisite specificity, the real power of LoopCap lies in the high and uniform coverage derived from redundant, dual-stranded probe tiling. Target bases are covered by multiple overlapping probes, each hybridizing to a unique sequence in the target region. This redundancy mitigates the potential impacts of new or uncharacterized variants that may lead to uneven coverage or allelic dropout in PCR- and hybrid capture-based methods. As an example, Molecular Loop's SARS-COV-2 Research Panel enables identification of all WHO variants of concern[2] without the need for iteration.[3]

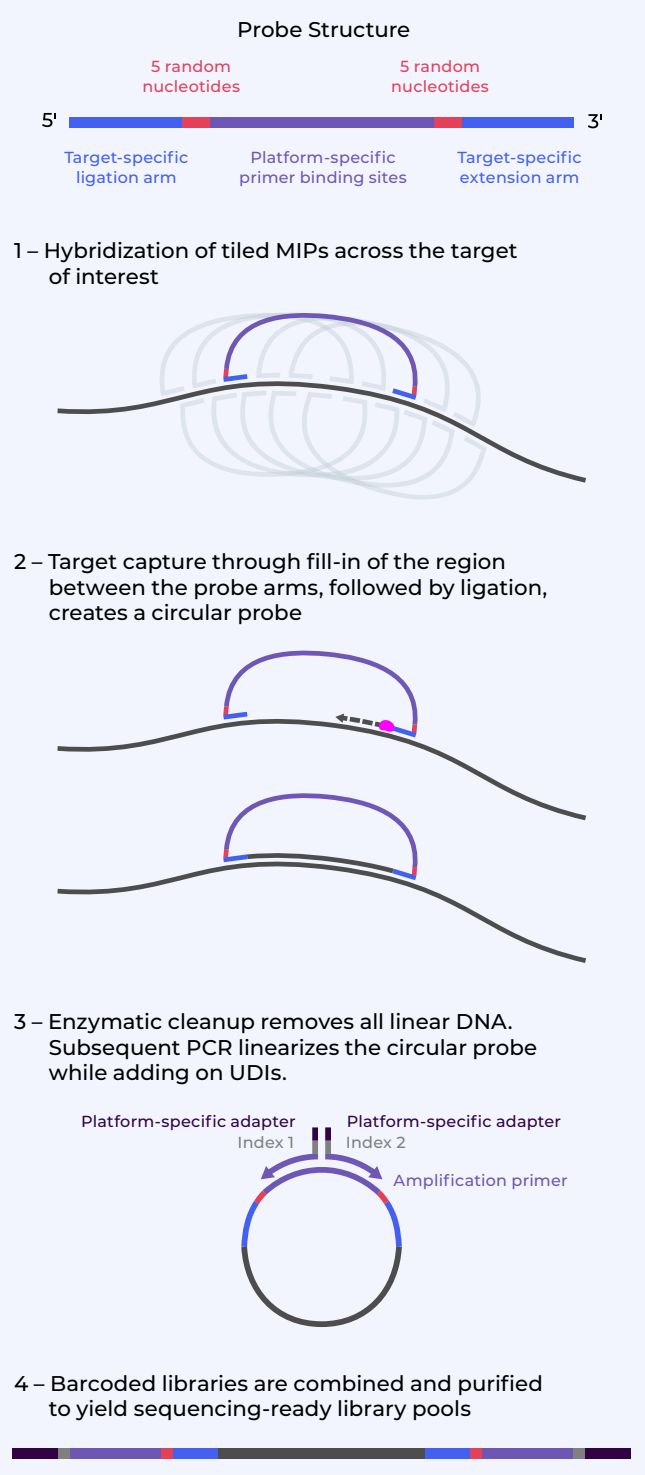



**Figure 1. Molecular Loop NGS target enrichment workflow.** The ligation arm and extension arm of each probe are designed to target a genomic region of interest, with the distance between the arms customizable for short- or long-read applications. All designs ensure that the region of interest is covered by multiple probes, thereby creating a robust system for variant detection. For DNA applications, both strands are targeted.

## Why choose Molecular Loop assays?

### Robust performance

- Redundant tiling ensures high and uniform coverage, protects against allelic dropout, and enables accurate and sensitive variant calling.
- Sequencing bias is reduced by uniquely positioned probes that produce reads with different start sites.

### Elegant workflow

- Sequencing-ready library pools are generated in four easy steps without transfers between reagent tubes or sample-level cleanups (Figure 2).
- Uniquely colored reagents ensure fail-safe reagent addition enabling in-process QC.
- Entire workflow requires only 75 min of hands-on time and may be completed in a single workday with a 4 hr hybridization.

### Scalability

- LoopCap™ technology supports panel sizes much larger than traditionally supported in amplicon-based protocols, enabling coverage of hundreds of genes and thousands of variants in a single tube.
- Simple workflow is easy to implement in automated, high-throughput pipelines.
- Hundreds of UDIs support highly multiplexed sequencing.

### Flexible panel design

- Tiling level may be adjusted based on input, application, experimental goals, and sequencing economy.
- Genomic content may be added or removed without impacting performance.
- Library insert size optimized for short- and long-read sequencers, applications, and sample input.

## Applications

LoopCap™ technology is ideally suited for a range of applications in medicine, infectious disease management, and agriculture.

The technology is compatible with a wide range of inputs and sample types including saliva, blood, cf/ctDNA, fresh/frozen and FFPE tissue, and viral RNA. As such, it enables:

- Carrier screening
- Hereditary and somatic oncology research
- Molecular characterization of rare and complex diseases
- Pathogen characterization and surveillance
- Genotyping-by-sequencing

Custom panels and applications are supported by design experts with a broad background in molecular biology, clinical sequencing, and bioinformatics. Custom panels are developed in a highly collaborative manner and are performance-tested prior to delivery.

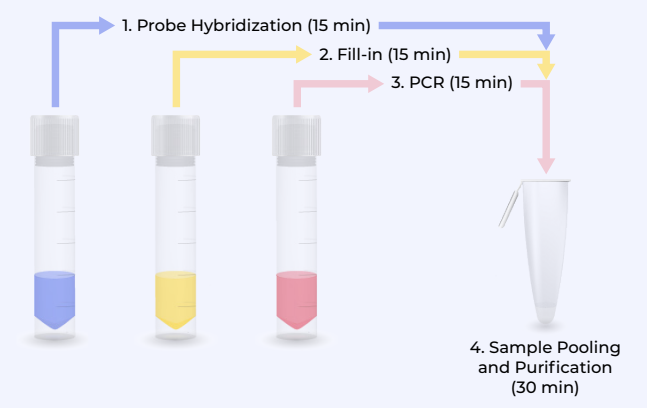
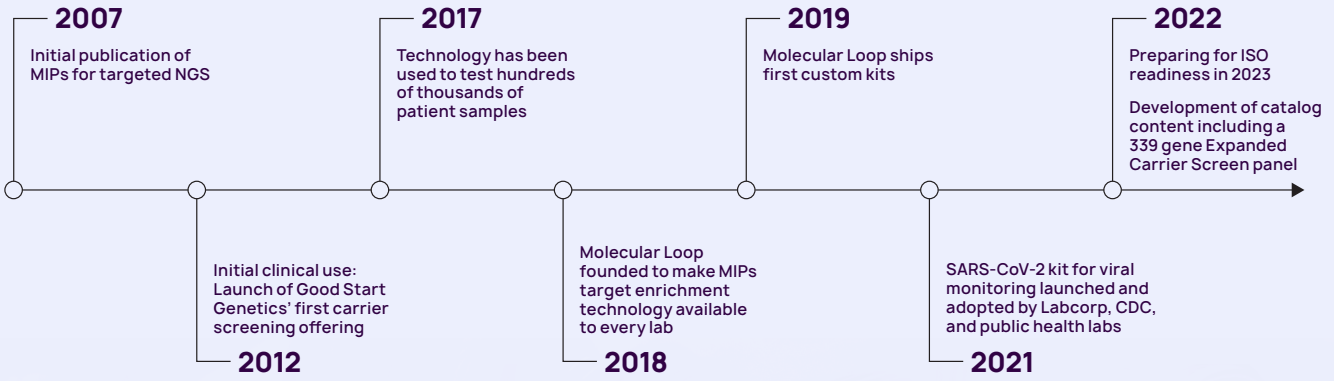



**Figure 2. Elegant 4-step workflow.** Individual samples are processed separately through steps 1 – 3. Barcoded libraries are pooled before the final cleanup, to yield a sequencing-ready library pool in just 75 min of hands-on time. For RNA samples, reverse transcription is performed concomitantly with the hybridization step.

## No-compromise solutions refined over the course of a decade

Our team includes pioneers in the use of MIP technology in high-throughput clinical NGS pipelines. Our mission is to support sophisticated NGS applications with elegant solutions. To this end, we specialize in the development of production-grade chemistry that simplifies your workflow, reduces cost and time, significantly increases throughput, and—most importantly—delivers high-quality results.




## References

1. Porreca G, Zhang K, Li, J. et al. *Nat Methods* 2007; 4: 931 – 936. doi: 10.1038/nmeth1110.
2. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants. Accessed 29 January 2023.
3. https://molecularloop.com/sars-cov-2-research-panel. Accessed 29 January 2023.

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