Revolutionizing Viral Surveillance: Leveraging LoopCap™, a MIP-Based Technology, for Comprehensive Viral Genome Coverage and Strain Variability Resilience

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Introduction

Viral surveillance necessitates reliable and adaptable methodologies to detect and characterize viral variants across diverse and constantly mutating genomes. Molecular Loop Biosciences' LoopCap™ technology offers a unique approach to viral surveillance by employing dense tiling of advanced molecular inversion probes (MIPs) to detect emerging variants via next-generation sequencing (NGS) without allelic dropouts common to amplicon-based methods **(Figure 1)**.

LoopCap technology is highly scalable, and our unique probe structure allows us to combine target capture and library preparation into a single, streamlined workflow, making it easy to implement for any laboratory looking to establish a NGS-based viral surveillance program. Here, we demonstrate how the unique features of LoopCap technology make it an ideal choice for any viral surveillance application.

Methods

To demonstrate the broad applicability of LoopCap in viral surveillance, we designed whole genome panels for RNA viruses SARS-CoV-2 (SC2), Pan-Respiratory (SC2, influenza A and B, respiratory syncytial virus (RSV) A and B), Marburg virus, and Ebola virus, and DNA viruses Mpox virus (MPV) and adenovirus. We utilized the inherent flexibility of LoopCap for probe design and tiling density to optimize each panel for coverage, specificity, and adaptability to evolving viral landscapes. Designs were tailored for short-read Illumina® or long-read PacBio® sequencing, with an average of 7.5 or 22.5 probes per target base. Multiple iterations of the SC2 panel, as well as the pan-respiratory and MPV panels, were tested internally with commercially-sourced reference materials. Genome coverage was calculated as the percentage of target bases with ≥1X or ≥5X coverage after downsampling to 200k reads.

The SC2 panel adapted for the PacBio instrument was used for routine viral surveillance over the last two years at Laboratory Corporation of America (Labcorp). Labcorp received emergency use authorization (EUA) for their LoopCap-based VirSeq SARS-CoV-2 NGS Test in June 2022, *FDA EUA220054*. Labcorp uses this test for routine SC2 surveillance and for sequences submitted to the CDC.

Sequences reported to the CDC must satisfy the following requirements: 1) Mean of median amplicon coverage >10X (median coverage is computed in 29 separate ~1 kb genomic windows and the mean of these is taken); 2) genome coverage >90% (a callable position must have ≥4X circular consensus sequencing (CCS) coverage); 3) ≤1 ambiguous base ('N') in each 6 bp sliding window of the S gene (since isolated 'N's are rare and instead typically occur in stretches of >1 bp, this usually corresponds to S gene coverage of ≥99%).

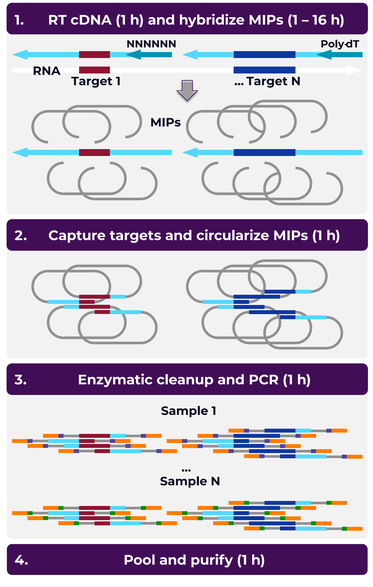

Figure 1. LoopCap workflow. 1) For RNA viruses, reverse transcriptase (RT) synthesizes cDNA from RNA. The resulting cDNA is then used as a target for the annealing of the molecular inversion probes (MIPs). DNA viruses move directly to the annealing step. **2)** After binding to the target (c)DNA, the gap between the two probe ends is filled in and circularized to form a closed "loop". **3)** Unbound and uncircularized probes remain linear and are removed during the enzymatic clean-up. The circularized molecules are then enriched, and sample indices and sequencing adapters are attached, using PCR. **4)** Samples are pooled and purified, resulting in a sequencer-ready library.

Results

The LoopCap viral panel designs demonstrated high genome coverage for all targets *in silico*, ranging from 96.57% (H1N1) to 99.98% (MPV). *In vitro* performance was also robust, with coverage ranging from 93.39% (H3N2) to 99.56% (MPV) for ≥1X, and 87.47% (RSV A) to 99.06% (MPV) for ≥5X **(Table 1)**.

				% of bases at ≥1X		% of bases at ≥5X
Target	**Tiling Density**	**Fill Size**	**Platform**	***In Silico* (design stats)**	***In Vitro*, controls**	
SARS-CoV-2 (Wuhan)	7.5x	225 bp	Illumina	99.69%	98.15%	97.70%
SARS-CoV-2 (Wuhan)	22.5x	675 bp	PacBio	99.69%	98.41%	97.42%
SARS-CoV-2 (XBB)	22.5x	225 bp	Illumina	99.78%	98.91%	98.73%
Panviral – SARS-CoV-2 (BA5)	7.5x	225 bp	Illumina	99.74%	98.62%	98.55%
Panviral – influenza A H1N1	7.5x	225 bp	Illumina	96.57%	94.83%	93.33%
Panviral – influenza A H3N2	7.5x	225 bp	Illumina	96.69%	93.39%	89.25%
Panviral – influenza B	7.5x	225 bp	Illumina	97.01%	93.92%	89.43%
Panviral - RSV A	7.5x	225 bp	Illumina	99.63%	95.76%	87.47%
Panviral - RSV B	7.5x	225 bp	Illumina	99.65%	96.99%	91.34%
Mpox virus	7.5x	225 bp	Illumina	99.97%	99.56%	99.06%
Mpox virus	22.5x	675 bp	PacBio	99.98%	–	–
Adenovirus (HAdV-D111)	7.5x	225 bp	Illumina	99.82%	–	–
Adenovirus (HAdV-D111)	22.5x	675 bp	PacBio	99.78%	–	–
Marburg virus	7.5x	225 bp	Illumina	99.70%	–	–
Marburg virus	22.5x	675 bp	PacBio	99.70%	–	–
Ebola virus	7.5x	225 bp	Illumina	99.77%	–	–
Ebola virus	22.5x	675 bp	PacBio	99.63%	–	–

Table 1. LoopCap infectious disease panel descriptions and *in silico* and *in vitro* coverage statistics.

In routine, real-world testing at Labcorp, there have been no dropouts due to variants of concern (VOC) in the sequences reported. Since its implementation, the VirSeq SARS-CoV-2 NGS Test has consistently and rapidly captured all COVID-19 surges in the U.S. and has reported ~567K high-quality SARS-CoV-2 genome sequences to the CDC as of October 2023.

The robustness of the genomic coverage across the SC2 VOCs observed at Labcorp, as well as representative sample batches selected from different time periods of the SC2 pandemic (Delta, BA.1, and XBB1.9/XBB.1.16) are detailed in **Figure 2**.

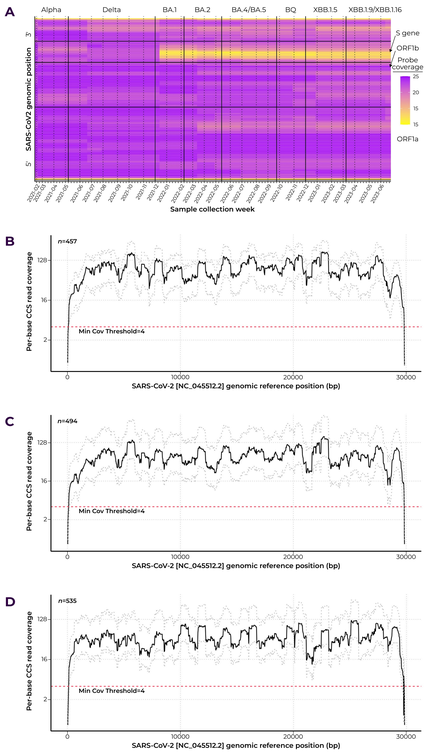

Figure 2. Robustness of whole genome LoopCap-based SC2 sequencing. A) Heat map showing the genome-wide probe coverage of the most common lineage in circulation for each collection week with genomic positions shown 5' (bottom) to 3' (top). Probes were considered failures if a deletion, insertion, or SNP was detected in either probe arm. Large genic regions (ORF1a, ORF1b, S) are indicated by horizontal lines and are labeled on the right. Results are stratified by sample collection week with vertical bars separating the major waves of the pandemic with the causal variant shown above. Waves are demarcated using the collection week when the causal variants first reached 5% prevalence.

B-D) Log2 per-base CCS coverage of sample batches selected from different time periods of the SC2 pandemic (**B**=Delta, **C**=BA.1, **D**=XBB.1.9/XBB.1.16). In all panels, the black line indicates the median coverage, while the grey dotted lines above and below indicate the upper and lower quartiles, respectively. The horizontal red dashed line represents a CCS coverage threshold of 4, which is the minimum required for a based to be called in the consensus SC2 genome. The number of samples in each batch is indicated at the top left of each panel.

Conclusion

Densely tiled LoopCap panels for whole genome viral surveillance, combined with state-of-the art MIP capture chemistry and a streamlined workflow, achieve comprehensive genome coverage with exceptional resilience against strain variability and allele dropout, as evidenced by the real-world success of our SC2 panel throughout the pandemic and emergence of new variant strains.

Our study also demonstrates the versatility of LoopCap technology, as we engineered whole genome panels for a wide range of viruses, with variable fill lengths and tiling densities, for two different sequencing platforms. Panels for which we were able to obtain reference materials showed exceptional *in vitro* performance.

LoopCap technology represents an adaptable, scalable, and robust method for NGS-based viral surveillance. With its resilience and flexibility, LoopCap has the potential to be a transformative tool for detecting and characterizing viral variants in the face of a dynamic viral landscape.

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