CUSTOMER SPOTLIGHT

National SARS-CoV-2 Pandemic Surveillance at Labcorp

Pathogen Surveillance at Scale Using Molecular Inversion Probes
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The SARS-CoV-2 pandemic has underscored the need for infrastructure that can be used to identify and track pathogen variation. To date, amplicon-based capture has been the standard method adopted for viral whole genome sequencing surveillance work. However, monitoring strain variation can prove challenging as it requires continual primer redesign and time-consuming protocol re-validation. Molecular inversion probe (MIP) capture is a fundamentally different technology that mitigates the need for primer redesign because of its redundant amplicon tiling approach, while also offering even greater workflow simplicity.

In this webinar, Scott Parker discussed Labcorp's surveillance findings, how he has led his labs through the pandemic, and how a small internal R&D project using Molecular Loop's MIP-based LoopCap™ technology scaled into a tool that's currently one of the frontline mechanisms for tracking the pandemic nationally.

Note: the content in this customer spotlight comes from Scott Parker's presentation during the August 31st GenomeWeb webinar, with some quotes lightly edited for clarity.

Whole Genome SARS-CoV-2 Sequencing

HIGH THROUGHPUT GENOMICS FOR OUTBREAK SURVEILLANCE

"In March 2020, [Labcorp] announced a collaboration with Pacific Biosciences to use viral whole genome sequencing to provide a retrospective view of the outbreak. We had access to a very wide geographic distribution of patient samples and zipcode level information about specimen collection and location.

The goal was to sequence positive samples with a Ct value of less than 27, knowing that higher Cts would be quite challenging.

By early December 2020, we successfully sequenced 7,000 samples as part of a pilot surveillance study. Also in December, Labcorp was awarded a CDC contract with the goal of conducting a large-scale genomic survey of the virus, using a random set of samples collected from across the United States.

By sequencing ~85,000 samples to date, [August 31, 2021], Labcorp has provided important baseline information for national and state-level surveillance."

Long Read Sequencing

DIFFICULTIES WITH LABCORP'S AMPLICON SEQUENCING

"...the majority of the [1.2 kb amplicon] assay was done manually with the use of multichannel and repeat pipettes so it was quite the tedious and long process."

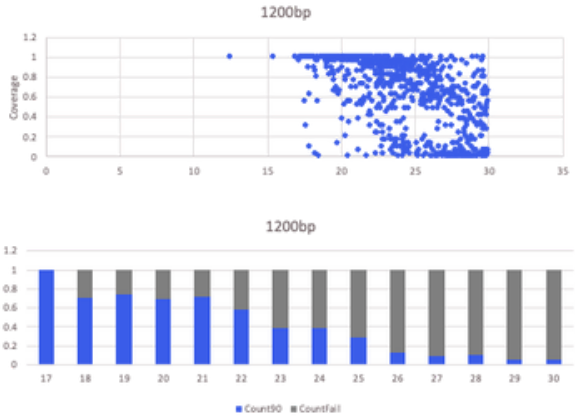


Figure 2, Long amplicon yield versus Ct

"As you can see from this typical graph showing yield versus Ct from one of our development runs, genome yield in blue here, and coverage drops with increasing Ct.

These results are typical and represent a major drawback of this amplicon approach."

"The [1.2 kb amplicon] sequencing protocol that we initially used was developed over many months in collaboration with Pacific Biosciences...Some of the steps in the process were automated using a Hamilton STAR system, but the majority of the assay was done manually with the use of multi-channels and repeat pipettes, so it was quite the tedious and long process.

The 1.2 kb amplicon approach, it just wasn't optimal. It had several drawbacks. As you can see from this typical graph showing yield versus Ct from one of our development runs (Figure 2), genome yield, shown in blue, and coverage drops with increasing Ct. These results are typical and represent a major drawback of this amplicon approach.

Even though we were selecting samples with Cts less than 27 for production runs, we start to lose ~20% coverage between Cts 22-23, and then of course samples of Cts with 27, we see about a 15% genome yield and thus, a higher failure rate."

"On the plate level, typical genome yield ranged anywhere from 35% to 55% meaning this was the percentage of samples with at least 90% genome coverage, and coverage is obviously key for data integrity and that is important to the CDC.

So after many months with this failure rate...we decided to look at alternate sequencing workflows to potentially rescue genome completion."

MOLECULAR LOOP TECHNOLGOY

MOLECULAR INVERSION PROBE INTRODUCTION

INITIAL EVALUATION OF MOLECULAR INVERSION PROBE (MIP) TECHNOLOGY

"Our initial evaluation using the Molecular Loop [MIP-based] SARS-CoV-2 targeted panel was done in February 2021 on the MiSeq System. 96 samples with a wide range of Cts were loaded onto a flow cell and sequenced at a final concentration of 10 pM. Genome yield at the Ct level was about 80% for Cts roughly 27 to 28, and 50% at Cts 31 through 32.

Which is really quite impressive relative to the performance we were seeing with the previous assay."

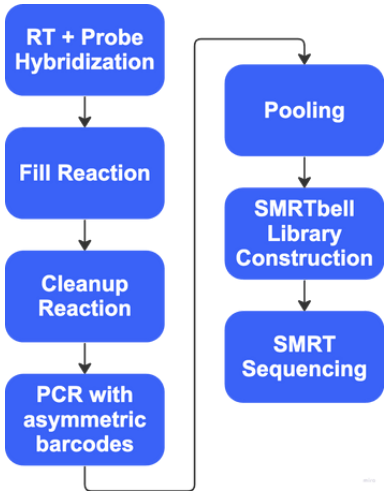


Figure 3, Molecular Loop SARS-CoV-2 workflow at Labcorp

TECHNOLOGY AND WORKFLOW OVERVIEW

"Genome capture is enabled by approximately 1000 MIPs, and these probes create highly redundant, overlapping amplicons across the entire SARS-CoV-2 genome.

The workflow is really simple!

It begins with a one-step RT, where viral RNA is converted into cDNA. Immediately followed by a 16-hour hybridization. A fill in reaction in the presence of dNTPs, ligase and polymerase, helps create that circular loop. The template and the non-circularized probes are removed via a cleanup reaction, and then PCR is carried out using asymmetric barcodes with M13 tags. And finally, amplicons are pooled, and sequenced on the Sequel II instrument."

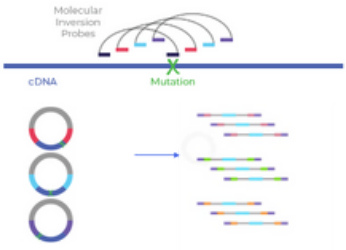


Figure 4, Illustration of Molecular Loop molecular inversion probes capturing a segment of the genome

Molecular Loop Technology Evaluation

OPTIMIZATION FOR PACBIO SEQUENCING

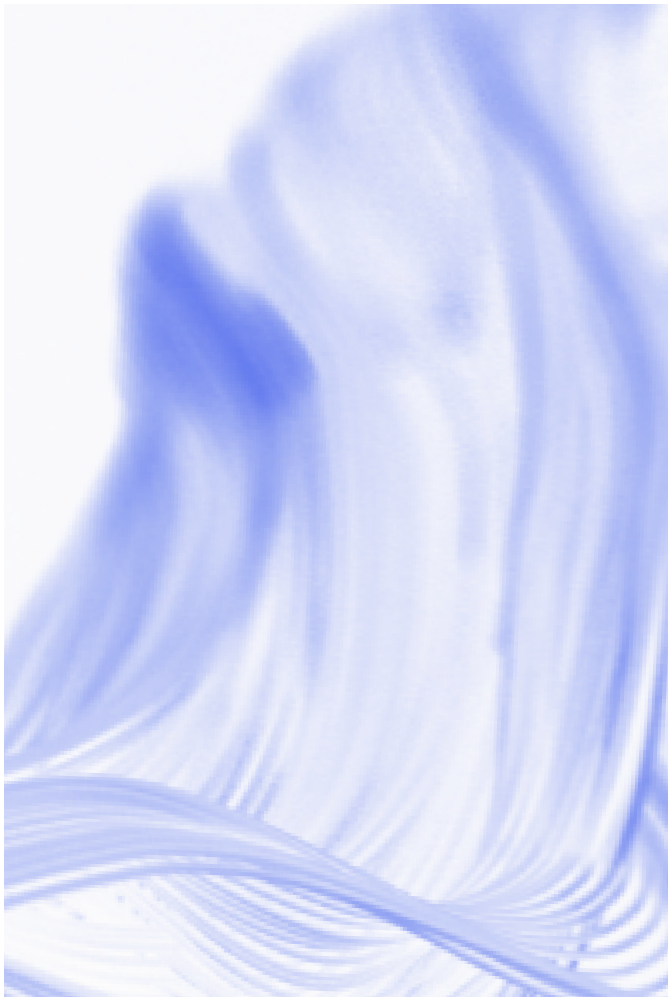
"Optimizing the Molecular Loop for PacBio sequencing didn't require a lot of effort.

The [old ML] backbone...had to go under slight modification just to accommodate our M13 asymmetric barcode approach.

[When evaluating on the PacBio instrument], we tested a new [ML] backbone as well. Both backbone sequences were variants intended for the PacBio workflow. [We then] did some other things like optimize the PCR cycle number, which is an important driver of the final dynamic range of probe representation in the library, and since we don't get a lot of reads from SMRT sequencing relative to other NGS assays, maximizing that uniformity was pretty crucial.

We have 768 previously reported samples sequenced under three different assay condition and...

both designs from Molecular Loop resulted in superior coverage relative to the amplicon assay. "



	Genome	Lineage	Fail
Old ML backbone	82.81%	7.29%	9.90%
New ML backbone	74.74%	11.20%	14.06%
1200 bp Amplicon	36.33%	44.27%	19.40%

Table 1, 768 SARS-CoV-2 samples sequenced under 3 different assay conditions. Each condition shows the percentage of samples that returned full genome results, linage designation results, or failed to return a SARS-CoV-2 result.

Molecular Loop Technology Evaluation

GENOME YIELD COMPARISONS

"Again, as you can see with the old [amplicon] method, here [Figure 5], we get a precipitous decline in the ability of a sample greater than 23 Ct to produce a genome with good coverage.

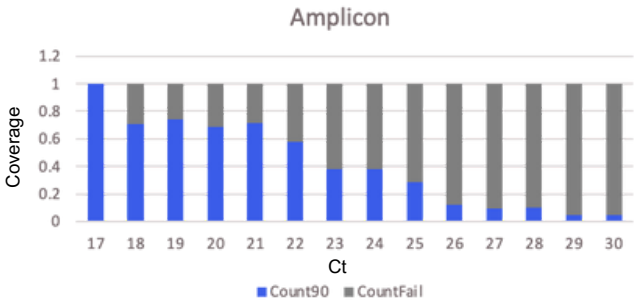


Figure 5, genome yield versus Ct, amplicon method

Conversely, using Molecular Loop's chemistry on the same set of samples, you can see we're getting...50% genome yield at Ct 29. Which is really good!"

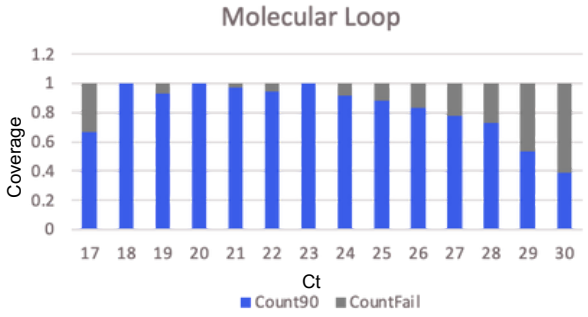


Figure 6, genome yield versus Ct, Molecular Loop

"So, we were quite pleased with the outcome of these data, and of course, we were ready to implement this process into our operation.

We also compared 7X versus 22X probe tiling. Theoretically, the 22X tiling should equalize yield across the index primers... [As expected], the 22X tiling has better base coverage, which makes sense since you have roughly 3 times the number of MIPs tiled at a given nucleotide.

Subsequent repeat experiments produced similar data. Plans were underway to present scientific leadership at Labcorp with these results and formulate a rapid implementation plan.

Labcorp was able to generate a successful clinical validation of the Molecular Loop assay with a >95% concordance for all strains at ≥90% coverage of the SARS-CoV-2 genome."

Labcorp's SARS-CoV-2 Datasets

- One of the most important aspects of the Labcorp dataset is that it's based on random sampling [~500 samples/state].
- The dominant clade...throughout our sampling [in 2021 was] 20A.
- Sublineages AY1, AY2, and AY3... collectively make up roughly 97% of our [2021] data.

Advantages of Molecular Loop

USING MIP TECHNOLOGY AT SCALE

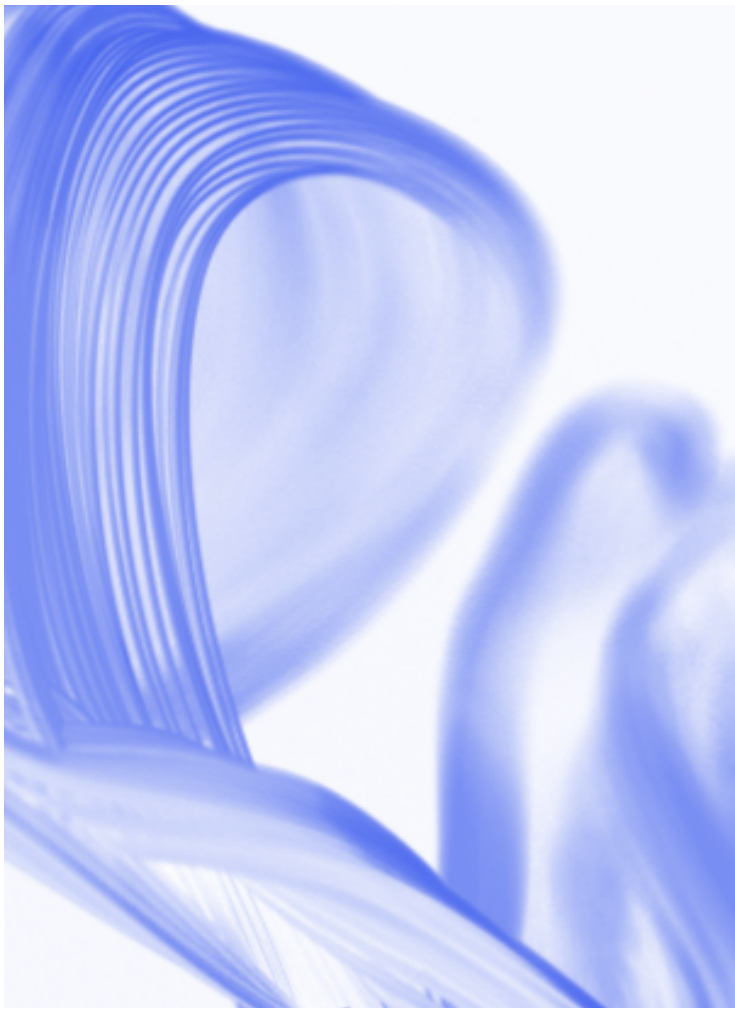
"The Molecular Loop wet lab workflow is much more user-friendly than our old amplicon approach.

The previous process involved splitting and stamping out source plates into daughter plates with the use of multichannel pipettes, which could have introduced the potential for errors in sample processing.

Adopting a single tube process allowed us to streamline and implement rather quickly with fewer pipetting steps as these reagents were add only with no reagent or sample transfers.

We were also able to reduce lab consumables by approximately 50%.

Since reagents are provided ready-to-use, we no longer had to spend time making mastermixes and were able to devote these time-savings to other duties. The sequential addition of color-coded reagents was a big hit with our technologists, enabling them to visualize the workflow without the need for beta-checks to ensure the proper reagents were added."



Benefits of the Molecular Loop Workflow

- 4-step, single tube process for faster throughput
- Low likelihood for error with fewer touchpoints
- Automation-friendly
- Easy to implement and train laboratory staff
- Sequential addition of color-coded reagents
- No normalization step
- More reads!

Viral sequencing doesn't have to be complicated.

molecular loop™

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