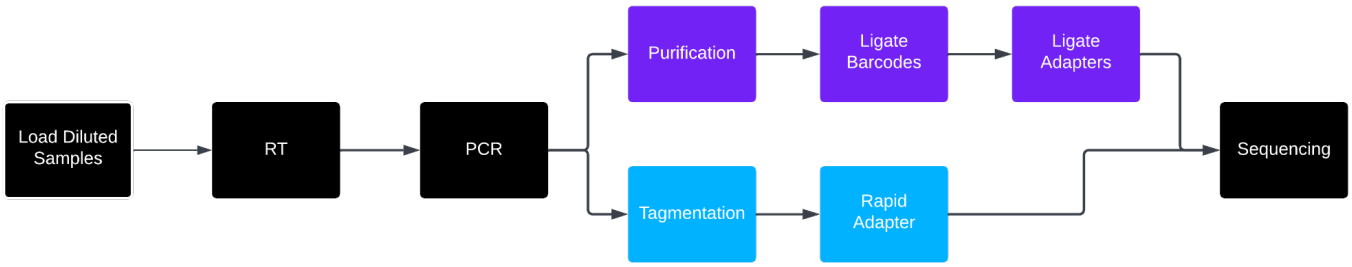
Technical Note: Clear Dx™ SARS-CoV-2 WGS v3.0

Background

Continued evolution of the SARS-CoV-2 virus presents challenges to amplicon-based nucleic acid-based assays, including whole genome sequencing (WGS). In response to the Omicron variant which emerged in the US in late 2021, Clear Labs developed an optimized primer formulation based on a newer reagent chemistry ("Midnight Native," or Clear Dx SARS-CoV-2 WGS v2.0) that showed significant improvement over the prior reagent chemistry (ARTIC v3). The emergence of later variants such as BA.4 and BA.5 has continued to impact assay performance. To ensure the highest quality performance and results, Clear Labs is responding with an updated reagent formulation (Clear Dx SARS-CoV-2 WGS v3.0) that provides benefits in addition to primer dropout. This document describes the changes to the product and the performance relative to the currently deployed product.

Figure 1: Clear Dx SARS-CoV-2 WGS assay workflow



Chemistry Overview

The updated chemistry of Clear Dx SARS-CoV-2 WGS v3.0 has a faster time to sequencing than v2.0. This time savings is a result of how barcodes and adapters are attached to the sequencing reads. Figure 1 compares the v2.0 protocol, in purple, to the v3.0 protocol, in blue. Prior to sample loading, we recommend the same dilution protocol used in the v2.0 protocol: samples with Ct 15 and lower Covid-19 samples should be diluted 1,000-fold, samples with Ct 15 to 18 should be diluted 100-fold, and samples with Ct 18 to 23 should be diluted 10-fold. The RNA is then reverse transcribed to cDNA and amplified using our reformulated Midnight primer set. In the v3.0 workflow (blue), the next step is fragmentation with a transposon complex that cuts PCR amplicons and attaches a barcoded sequence. After a sequencing adapter is attached to the barcoded DNA, the sample is ready for sequencing, for a preparation savings of four hours over v2.0 chemistry.

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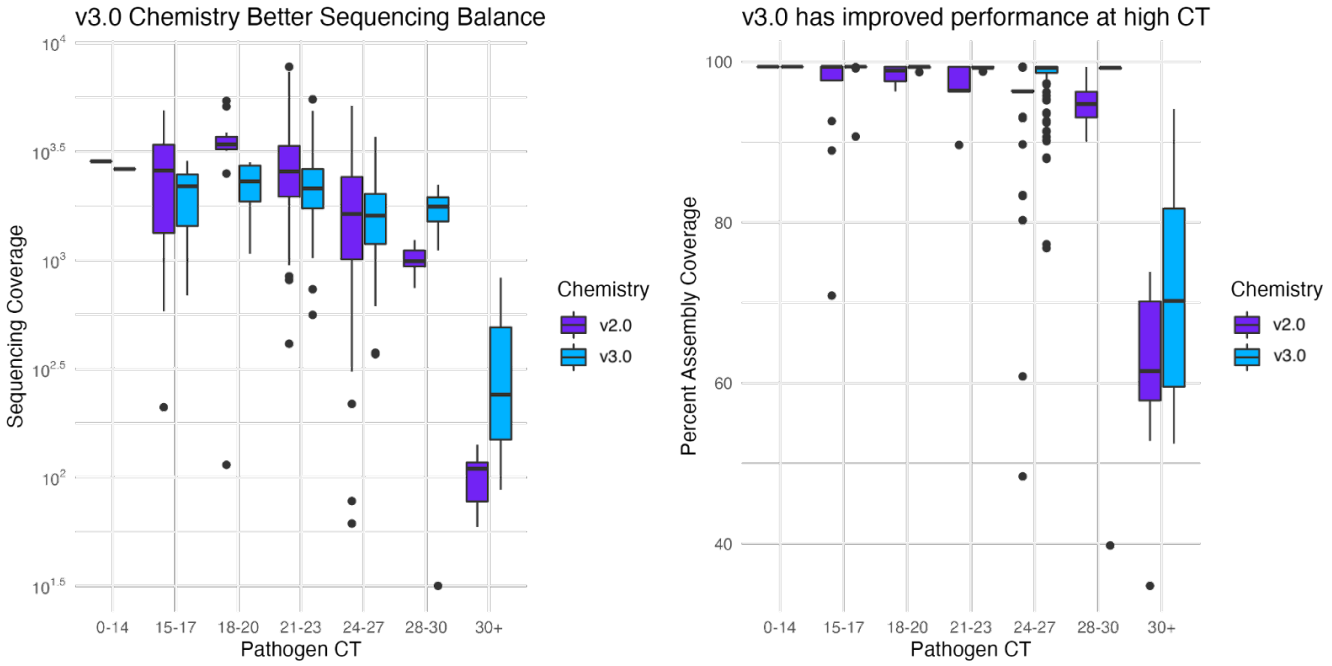
Figure 2: Sequencing coverage (left) and assembly coverage (right) of SARS-CoV-2 samples, each processed on both v2.0 and v3.0



Better Coverage in Low Ct Samples

Clear Dx SARS-CoV-2 WGS v3.0 results in better coverage in samples with lower Ct compared to v2.0. To demonstrate this, 240 samples of various Ct were sequenced under both v2.0 and v3.0 chemistries, including BA.2, BA.4 and BA.5 variants. For low Ct bins (Ct 15-27) processed in v3.0, sequencing coverage had a median depth of approximately 2200X ($10^{3.35}$). For v2.0, sequencing coverage was higher at 2500X ($10^{3.4}$). While the median sequencing coverage may be slightly lower in v3.0 for low Ct samples, average coverage was sufficient for both chemistries. For Ct bins higher than 28, the v3.0 samples achieved a higher sequencing coverage compared to v2.0 and also had a higher assembly coverage. Assembly coverage improves with v3.0 chemistry across all Ct bins (Figure 3, right), due in part to the additional primer spike-ins, improved sequencing balance and longer sequencing hours.

Sequencing time and minimum depth for consensus calls

With the v3.0 release of Clear Dx, the sequencing time has been increased from 8 hours to 12 hours to deliver more sequencing data. Correspondingly, the stringency of the minimum depth requirement for consensus-calling from 20x to 30x. Thus, consensus genome bases in v3.0 genomes have more reads supporting those consensus calls than genomes produced in previous versions of the pipeline. Positions where the 30x depth requirement is not met are masked as “N” in the consensus genome.

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Figure 3: Comparing sequencing coverage between v2.0 (purple) and v3.0 (blue) in representative samples of Omicron subvariants BA.2.12.1, BA.4, and BA.5.

Coverage Across the Genome: Updated Primers and Variable Amplicon Depth

We developed an improved Midnight primer formulation to address the dropout observed in amplicon P21 in Omicron samples. A comparison of v2.0 chemistry using the new Midnight primer formulation versus v3.0 chemistry with the original Midnight primer formulation shows increased performance of many amplicons, especially recovery of P21. This, along with the previously mentioned even sequencing coverage, results in a boost to assembly coverage in the v3.0 chemistry. Figure 3 illustrates these improvements on three different SARS-CoV-2

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samples (BA.2.12.1, BA.4 and BA.5) processed in both v2.0 and v3.0 chemistry. The tagmentation chemistry results in sequencing reads that may begin or end within the middle of amplicon, and thus are shorter than the previous chemistry. Tagmentation also results in read depths that vary across an amplicon. In contrast, the v2.0 chemistry created reads that spanned an entire amplicon, resulting in consistent read depth for the length of an amplicon.

Summary

With the chemistry improvements in v3.0, SARS-CoV-2, less time is needed for sequencing preparation, and more time is devoted to generating sequencing data. This quicker sample preparation time allows for loading of a second batch of samples within 8 hours. Additionally, sequencing coverage is more uniformly balanced between samples with lower and higher Ct values, and several amplicon dropouts caused by Omicron subvariants have been fixed.

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