Fully Automated, Rapid Whole Genome Sequencing of SARS-CoV-2

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BACKGROUND

Effective genomic surveillance during a pandemic such as COVID-19 requires rapid, cost-effective and high-throughput solutions to identify and subsequently track variants with higher transmissibility, virulence, vaccine-escape variants or even novel variants whose functional consequences have yet to be elucidated. Whole genome sequencing (WGS) is able to provide more comprehensive information and is more useful in resolving such variants compared with other approaches. However, manual WGS workflows are time-consuming, labor-intensive and require specialized expertise for analysis which severely limits the practical application of WGS in public health.

METHODS

We have developed a fully automated WGS workflow on Clear Dx platform that can process extracted RNA from up to 32 clinical or environmental SARS-CoV-2 samples in one run, all the way through sequencing and bioinformatic assembly of genomic sequences, with no human intervention required after the start of the run. The Clear Dx platform (shown in Fig. 1) is custom-built with thermocyclers, nanopore sequencer and other workflow accessories housed inside a Hamilton STAR liquid handling robot and is controlled using customized instrument software. The automated WGS workflow (shown in Fig. 2) leverages the tiled two-pool ARTIC v3 primer panel to capture and amplify targeted regions from the cDNA of the respective RNA transcripts, which are further barcoded and pooled. They are then subjected to library preparation steps customized for Oxford Nanopore sequencing technology and sequenced on the GridION sequencer. Real-time basecalling is done using a built-in data processing toolkit called Guppy, while barcode classification of sequencing reads and assembly of the consensus genome sequences are done using an optimized ARTIC bioinformatic pipeline.

Clear Dx WGS vs Manual ARTIC v3

We have optimized several aspects of the ARTIC v3 assay, the fastest sequencing SARS-CoV-2 WGS protocol developed thus far and integrated the workflow steps to provide the best end-to-end automated solution.

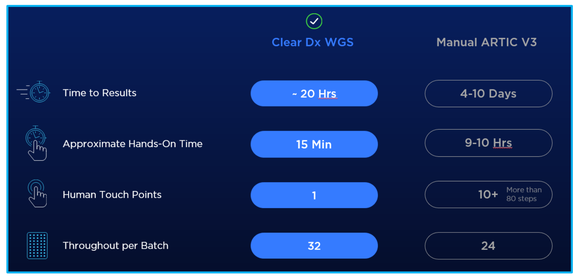
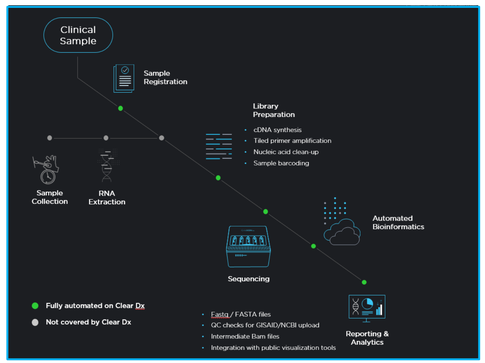


Figure 1. Clear Dx WGS Platform

Figure 2. Clear Dx WGS Workflow



RESULTS

1. We performed a study to characterize the genome coverage performance of our Clear Dx WGS for different genome copies. Stock solution of synthetic SARS-CoV-2 RNA controls (MN908947.3 from Twist Biosciences) at 1,000, 000 copies/µl was serially diluted in MB grade water and the resultant RNA samples (N=128) were processed through our automated platform in 4 separate runs. As shown in the table below, Clear Dx WGS assay produces
   - > 90% of max. genome coverage even for 5 copies/µl
   - > 98% of max. genome coverage for 50 copies/µl
   - Even sequencing depth & % on-target reads across genome copies

SARS-CoV-2 Genome copies	Mean % Genome coverage	Mean Seq. depth	% On-target reads
10,000 cp/µL	92.23 % (99.71 %)	2110 x	90.48 %
1000 cp/µL	92.31 % (99.79 %)	1506 x	90.03 %
100 cp/µL	91.75 % (99.19 %)	2160 x	90.75 %
50 cp/µL	91.04 % (98.42 %)	1535 x	90.66 %
10 cp/µL	88.21 % (95.36 %)	1513 x	90.15 %
5 cp/µL	87.87 % (95.00 %)	1454 x	90.07 %

Since Twist synthetic SARS-CoV-2 RNA controls have breaks after every ~5kb, the max. achievable genome coverage is 92.5%, due to amplicons in those break regions cannot amplify. The numbers in parentheses in column 2 are % coverage after removing those affected amplicons.

2. We performed a similar study with **113 real-world COVID-19 NP/OP swab samples** procured from several of our partner clinical labs. 12 water samples were also processed as no template controls (NTC). RNA extraction was performed on KingFisher Flex automated extraction instrument using MagMax Viral/Pathogen II RNA extraction kits. The extracted RNA was first quantified using TaqPath SARS-CoV-2 qPCR assay. Based on the Ct values for N gene, the RNA was pre-diluted such that all samples have a final RNA conc. equivalent to Ct of 23. The results, as shown in Fig 3., suggest that the Clear Dx WGS assay produces ~ 90% mean genome coverage and above for up for clinical samples up to Ct of 32-33.

3. We investigated the ability of Clear DX WGS assay for identifying the different SARS-CoV-2 variants through recognition of different signature mutations of the variants used by the Pangolin lineage. Fig. 4 illustrates some of the signature deletions of UK variant B.1.1.7 observed in the assembled SARS-CoV-2 sequence of a real-world clinical B.1.1.7 sample processed on our platform. Fig 5. shows the surveillance data of the Variants of Concern (VOC), namely B.1.1.7, B.1.427, B.1.429 & P.1 across all our PHL customer sites over 8000 clinical samples processed in the first 11 weeks of 2021. The data clearly demonstrates the surge of B.1.1.7 across the country in that time frame.

Figure 3. Pilot Study with Real-World Clinical Samples

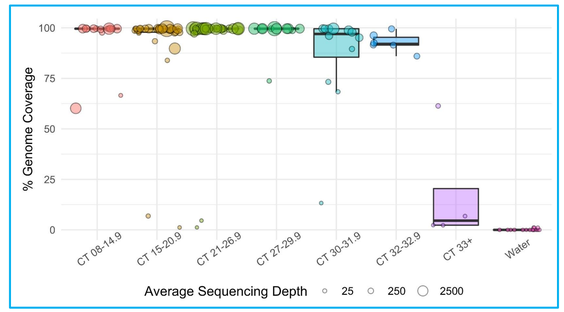


Figure 4. Critical Deletions in the B.1.1.7 Variant

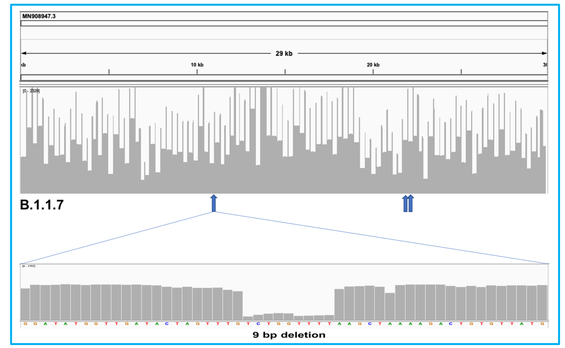
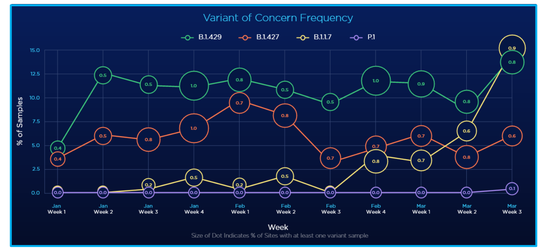


Figure 5. Surveillance of VOC Across PHL Customers



CONCLUSION

The Clear Dx fully automated WGS platform, with is rapid sample-to-assembled genome sequencing, provides an efficient tool to meet critical national public health needs. This platform enables state and county public health labs to track new variants, monitor transmission patterns, and contribute to insights that can help with resolving infection clusters and identifying superspreader events in near real-time. Additionally, it reduces the personnel needs and sample batching requirements for wet lab and bioinformatic analysis in public health labs and allows them to focus on the most critical epidemiological analysis in a timely manner.

For More Information

For more information regarding the Clear Dx platform or the Clear Dx WGS assay, please contact us at inquiries@clearlabs.com

Disclaimer

The Clear Dx instrument is for Research Use Only (RUO) and is not intended for diagnostic purposes. The Clear Labs diagnostic screening assay for SARS-CoV-2, also run on this platform, is approved by the FDA under an emergency use authorization (EUA).

Acknowledgements / Sources

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