Key Summary of Published Article

SARS-CoV-2
Improved Whole-Genome Sequencing

Can SARS-CoV-2 whole-genome sequencing (WGS) throughput be improved for surveillance?

Background

Genomic surveillance of SARS-CoV-2 helps inform public health decisions by monitoring for new and existing variants. Current WGS methods are limited by workflow complexity and difficulty scaling to very high throughput; viral mutations at primer binding sites can also affect sequencing. An optimized WGS method could provide improved surveillance of SARS-CoV-2.

Methods and results

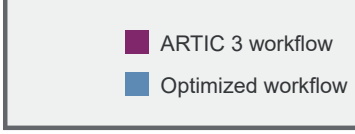
Optimizing the WGS workflow

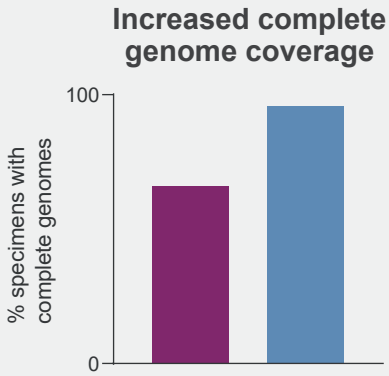
ARTIC v3 workflow +

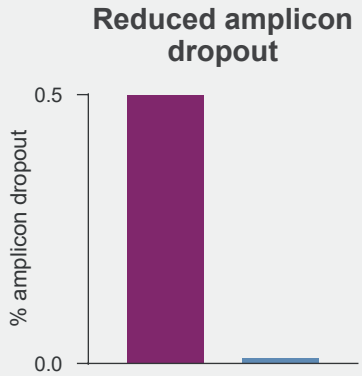
- Robotic liquid handlers
- Enhance clean-up steps
- Touchdown PCR and primer pool optimization

Effects of the optimized workflow

>2,600
Specimens per run







The high throughput and performance of this optimized SARS-CoV-2 WGS method makes it suitable for large-scale surveillance of SARS-CoV-2.

[1] Rosenthal SH, Gerasimova A, Ruiz-Vega R, et al. Development and validation of a high throughput SARS-CoV-2 whole genome sequencing workflow in a clinical laboratory. *Sci Rep*. 2022;12(1):2054. doi:10.1038/s41598-022-06091-00

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Key Summary of Published Article

SARS-CoV-2
Improved Whole-Genome Sequencing

Article Title: Development and Validation of a High Throughput SARS-CoV-2 Whole Genome Sequencing Workflow in a Clinical Laboratory

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Citation: Rosenthal SH, Gerasimova A, Ruiz-Vega R, et al. *Sci Rep*. 2022;12(1):2054. doi:10.1038/s41598-022-06091-0

Background

- New SARS-CoV-2 variants are monitored through genetic surveillance, which often relies on next-generation sequencing (NGS) technologies for whole-genome sequencing (WGS) of SARS-CoV-2.[1-3]
- However, workflow complexity can greatly limit scalability. For example, viral mutations can interfere with certain NGS methods (eg, amplicon-based strategies) and cause loss of information (eg, amplicon dropout).
- **Objective:** To improve SARS-CoV-2 surveillance, the investigators developed an optimized, automated, high-throughput workflow for SARS-CoV-2 WGS that overcomes limitations of workflow scalability.

Methods

- Remnant SARS-CoV-2 RNA from clinical specimens submitted to Quest Diagnostics from February through August 2021 were used to develop the workflow.
- cDNA libraries were prepared using a 2-step PCR with modified ARTIC v3 primers. Optimizations included
  - Integration of robotic liquid handlers
  - Enhanced clean-up steps to increase coverage
  - Touchdown PCR and primer-pool optimization to improve amplicon balance and reduce amplicon dropout
- NGS was performed using an Illumina NovaSeq 6000.
- Consensus sequences were assembled relative to the MN908947.3 reference genome using an in-house bioinformatics pipeline.
- Analytical validation studies were conducted using clinical specimens collected from March to April 2021.

Results

- The 2-step PCR method yielded reduced 973X (SD, 719; CV, 73.9%) coverage, which was reduced compared to standard ARTIC v3 (1,390X [SD, 658; CV, 47.3%]) but still adequate for whole-genome assembly.
- Amplicon dropout was reduced to 0.01% from 0.50%.
- Amplicon balance was improved 2- to 5-fold for low-performing amplicons.
- In analytical validation studies of the optimized workflow on 1,711 unique clinical samples, high precision (100% inter- and intra-assay precision) and accuracy (100% positive percent agreement and 100% negative percent agreement) were demonstrated.
- After implementing the optimized workflow, trends in relative variant prevalence continued to be consistent with those reported by the CDC.

Conclusions

- With the optimization of key methodological processes, the investigators developed an automated, high-throughput workflow for SARS-CoV-2 WGS that facilitates real-time epidemiologic surveillance.

References

1. Boehm E, Kronig I, Neher RA, et al. Novel SARS-CoV-2 variants: the pandemics within the pandemic. *Clin Microbiol Infect*. 2021;27(8):1109-1117. doi:10.1016/j.cmi.2021.05.022
2. Tao K, Tzou PL, Nouhin J, et al. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nat Rev Genet*. 2021;22(12):757-773. doi:10.1038/s41576-021-00408-x
3. Harvey WT, Carabelli AM, Jackson B, et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat Rev Microbiol*. 2021;19(7):409-424. doi:10.1038/s41579-021-00573-0