Accurate Surveillance of SARS-CoV-2 Variants of Concern with an Assay that is Resilient to Genomic Variation

ABSTRACT

SARS-CoV-2 surveillance efforts are facilitated by technologies that extract the viral genome from the patient sample background. Effective surveillance tools should be able to detect variants that have deviated from the original virus strain. The SARS-CoV-2 NGS Assay—RUO is a hybridization capture-based assay featuring the Twist SARS-CoV-2 Research Panel and Biotia COVID-DX (v1.6) software. Here, we highlight the ability of this assay to detect the SARS-CoV-2 Delta variant in clinical samples, which emerged over a year after the panel's design.

INTRODUCTION

Since its emergence in Wuhan, China, in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has continued to spread globally, resulting in over 218 million infections and over 4.5 million deaths worldwide (at the time of writing; Dong et al., 2020). As a result of persistent transmission, there are now several mutations that can be found in highly circulating SARS-CoV-2 variants, including B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), and B.1.617.2 (Delta). These variants have emerged and rapidly changed the viral dynamics, thus threatening ongoing public health efforts to limit the transmission of SARS-CoV-2. The Delta variant, for example, was first reported in May 2021 in the USA. As of September 2021, it currently accounts for 99% of all sequenced samples (CDC, 2021). The development of next-generation sequencing (NGS) protocols and the rapid sharing of viral genomic data via the Global Initiative on Sharing All Influenza Data (GISAID) has enabled the tracking of SARS-CoV-2 evolution in real-time.

In parallel, the development of target enrichment approaches, including multiplex tiling PCR and hybridization capture, has facilitated these surveillance efforts by reducing the cost of sequencing. Notably, the reliable surveillance of SARS-CoV-2 requires an assay that can detect new variants as they arise. Multiplex tiling PCR is sensitive and straightforward but prone to dropouts in coverage from mismatches between short primers and their target sequences. Primer-dimer formation can similarly cause dropouts (Itokawa et al., 2020). Thus, primer sets for amplicon sequencing protocols require frequent updating to ensure continued coverage of mutated regions. The ARTIC Network recently updated the widely used ARTIC primer set a third time to address reported coverage dropouts observed when sequencing the Beta and Delta variants (ARTIC Network, 2021). Comparatively, hybridization capture is much more tolerant to mismatches and, by contrast, is more suitable for the surveillance of an evolving virus. Because hybridization capture probes are much longer than PCR primers, they can tolerate mismatches of up to 10-20% between bait probes and their targets.

Twist and Biotia launched the SARS-CoV-2 NGS Assay—RUO in November 2020. The assay combines the Twist SARS-CoV-2 Research Panel with Biotia COVID-DX software to offer an end-to-end solution for NGS-based detection and characterization of SARS-CoV-2 variants (Nagy-Szakal et al., 2021). In addition to being able to detect single-digit copies of the SARS-CoV-2 genome, the assay displayed tolerance to mutations, accurately identifying the three SNP and one INDEL mutations that distinguish the Australia/VIC01/2020 isolate (MT007544.1) from the original Wuhan isolate (MN908947.3) (Twist Bioscience, 2021). In this application note, we demonstrate that the SARS-CoV-2 NGS Assay—RUO is robust to mutations in the SARS-CoV-2 genome and can accurately detect the Delta variant that emerged in December 2020, after Twist had designed the assay. This application note highlights:

1. Detection and characterization of the SARS-CoV-2 Delta variant in two clinical samples.
2. Coverage of >99% of the genome at 5X or greater after enrichment.

WORKFLOW

The SARS-CoV-2 NGS Assay—RUO was performed as described in the SARS-CoV-2 NGS Assay—RUO Protocol (Illustrated in Figure 1). Briefly:

- RNA was isolated from nasopharyngeal specimens collected in New York City during the first two weeks of June 2021 and converted into cDNA
- Sequencing libraries were generated using the Twist Library Preparation Kit.
- Target enrichment was performed using the Twist SARS-CoV-2 Research Panel.
- Enriched libraries were sequenced on an Illumina NextSeq 550 sequencer.
- The sequencing data were analyzed using Biotia COVID-DX (v1.6), a cloud-based software optimized for use with the SARS-CoV-2 NGS Assay—RUO.

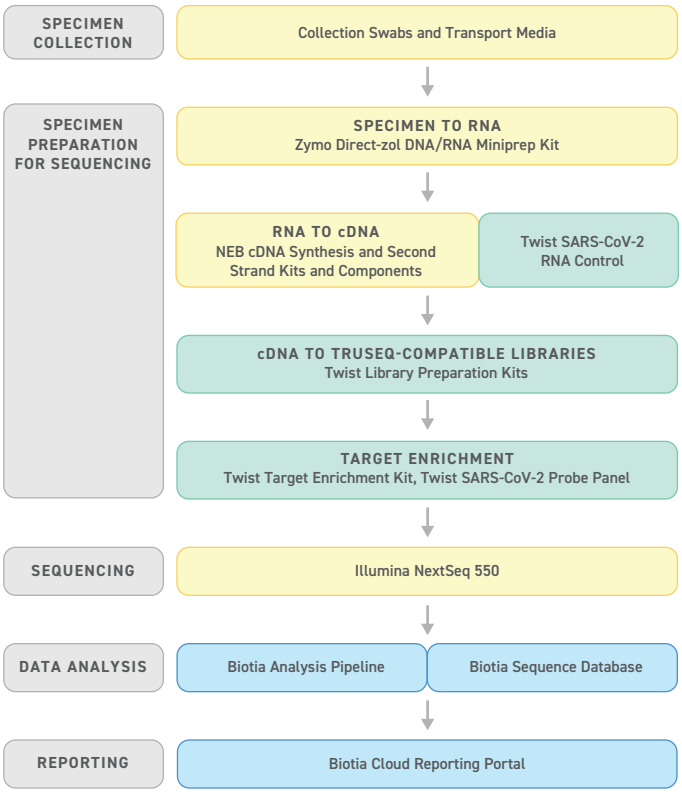

Figure 1: Workflow Overview Diagram. Components for the SARS-CoV-2 NGS Assay are specified as included by Twist (green) or Biotia (blue), and required but not provided (yellow). General sample and reagent processing steps are listed above (grey).

METRIC	SAMPLE 1	SAMPLE 2	CONTROL (MN908947.3)
Total Filtered Reads	923,937	476,901	7,517,102
Mean Coverage across SARS-CoV-2 Genome	1,636	189	198
Coverage at 1X	>99%	>99%	>99%
Coverage at 5X	>99%	>99%	>99%
Coverage at 10X	>99%	>99%	>99%

Table 1: Assay performance metrics. The sequencing and capture performance for both samples, determined using Picard CollectHsMetrics, Callable Loci, and FastQC.

NUCLEOTIDE VARIANT	AMINO ACID VARIANT	SAMPLE 1	SAMPLE 2
C21618G	p.T19R	Y	Y
G21987A	p.G142D	Y	Y
GAGTTCA22028G	p.E156_R158delinsG	Y	Y
C22227T	p.A222V	Y	N
T22917G	p.L452R	Y	Y
C22995A	p.T478K	Y	Y
A23403G	p.D614G	Y	Y
C23604G	p.P681R	Y	Y
G24410A	p.D950N	Y	Y
A24783G	p.N1074S	Y	N
G24928T	V1122V	N	Y
G25352T	p.V1264L	Y	N

Table 2: Detected S gene variants. (Y: Yes, detected; N: No, Not detected)

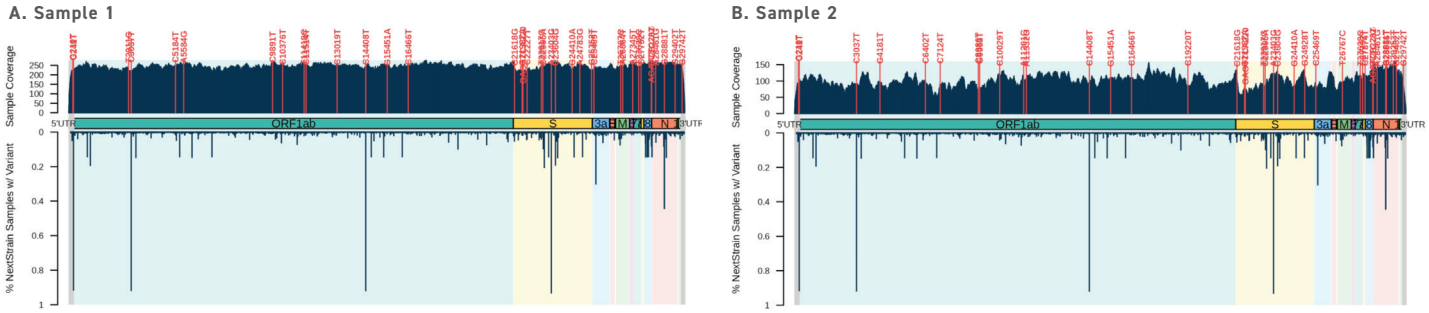

Figure 2: Detection of SARS-CoV-2 lineage B.1.627.2 (Delta) in two nasopharyngeal samples (A. Sample 1; B. Sample 2). The depth of sequencing recovered across the genome is plotted, and the detected mutations are labeled in the top panels. The prevalence of each substitution across variants worldwide, as reported on NextStrain as of March 7, 2021, is shown in the bottom panels.

RESULTS

First, the sequencing and capture performance of the SARS-CoV-2 NGS Assay—RUO was evaluated (Table 1). The assay recovered greater than 99% coverage for both samples at 5X or greater. The mean coverage for sample 1 was 1636, whereas the mean coverage for sample 2 was 189.

The Delta variant possesses the following mutations in the spike (S) protein: T19R, G142D, 156-158del, R158G, L452R, T478K, D614G, P681R, and D950N. **The SARS-CoV-2 NGS Assay—RUO detected all of these mutations in both clinical specimens,** as summarized in Table 2. The assay also detected three additional mutations in sample 1 associated with some Delta isolates (Connor et al., 2021): A222V, N1074S, and V1264L. A total of 37 mutations were detected across the entire SARS-CoV-2 genome in each sample (labeled in Figure 2).

DISCUSSION

This application note shows that targeted sequencing by hybridization capture using the SARS-CoV-2 NGS Assay—RUO can detect the SARS-CoV-2 Delta variant, which emerged after the launch of the SARS-CoV-2 Research Panel. These data highlight the tolerance of Twist hybrid capture probes to mismatches. This high mismatch tolerance indicates the probe panel will remain effective even as new SARS-CoV-2 variants emerge.

The results presented in this application note are corroborated by Rehn and colleagues' side-by-side comparison of commercial bait capture panels (Rehn et al., 2021). Their study shows that the Twist SARS-CoV-2 Research Panel offers higher sensitivity and capture efficiency for SARS-CoV-2 genomes than analogous offerings from Illumina (Respiratory Panel v2) and Daicel Arbor Biosciences (myBaits SARS-CoV-2 Panel). The Twist SARS-CoV-2 Research Panel also required an order of magnitude fewer reads than these other products, highlighting the panel's cost-effectiveness.

Biotia's COVID-DX (v1.6) software generated the presented data. The software provides a user-friendly analytical pipeline optimized for use with the SARS-CoV-2 NGS Assay—RUO. This study shows that the software allows users to model sequencing coverage and call genetic variants in SARS-CoV-2 samples. In addition, researchers can use the software to generate phylogenetic trees comparing the sequenced sample to existing GISAID sequences (data not shown).

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