TECHNICAL NOTE

Improved performance using the Illumina COVIDSeq™ Assay (96 samples)

The ARTIC v4 primer pool provides improved genomic coverage for SARS-CoV-2 variants

illumina®

For Research Use Only. Not for use in diagnostic procedures.

Accessible COVID-19 surveillance

The COVID-19 pandemic has raged across the world for more than a year.[1] The emergence of the delta variant and possibly other new SARS-CoV-2 variants that are more contagious or deadly have raised concerns about public health efforts, certain diagnostic tests, and vaccines developed to combat the pandemic.[2] This highlights the need for genomic surveillance to identify and monitor new SARS-CoV-2 variants. For labs who want to perform surveillance, but face financial barriers of capital expense for high-throughput sequencing systems and large-format library prep kits, we offer the Illumina COVIDSeq Assay (96 samples). This low- to mid-throughput amplicon-based next-generation sequencing (NGS) assay accommodates a low number of samples to enable smaller clinical research labs to identify and track the emergence and prevalence of new SARS-CoV-2 variants and lineages.

The Illumina COVIDSeq Assay (96 samples) includes a primer pool based on the validated, publicly available ARTIC multiplex PCR protocol to detect and characterize SARS-CoV-2 RNA (ARTIC v3). A modified, optimized primer pool (ARTIC v4) has been designed to improve the performance of the Illumina COVIDSeq Assay (96 samples) for SARS-CoV-2 detection and variant calling. This technical note demonstrates the improved analytical sensitivity of virus detection and increased viral genome coverage of the ARTIC v4 pool when used with the Illumina COVIDSeq Assay (96 samples) for COVID-19 surveillance.

Experimental design

Sample preparation

Four contrived samples were prepared by spiking 200 viral copies of Twist Synthetic SARS-CoV-2 RNA Controls (Table 1) representative of different COVID variants into a background of universal human reference (UHR) RNA (Thermo Fisher Scientific, Catalog no. QS0639). RNA extracted from nasopharyngeal (NP) swab samples determined to be COVID-positive by qPCR (Ct values ≤ 30) were also included for evaluation.

Table 1: Contrived SARS-CoV-2 samples

Twist Synthetic RNA Control	Pangolin nomenclature[a]	WHO label[b]	Twist Catalog no.
15	B.1.1.7	Alpha	103909
16	B.1.351	Beta	104043
17	P.1	Gamma	104044
23	B.1.617.2	Delta	104533

a. Pangolin (Phylogenetic Assignment of Named Global Outbreak Lineages) was developed to implement the dynamic nomenclature of SARS-CoV-2 lineages

b. The World Health Organization (WHO) recommends using letters of the Greek alphabet for discussion of SARS-CoV-2 variants by nonscientific audiences

Library preparation

Libraries were prepared in parallel for all samples using the Illumina COVIDSeq Assay (96 samples) with the ARTIC v3 primer pool (included with the kit) and the ARTIC v4 primer pool (available as an accessory product).

Sequencing

Prepared libraries were sequenced at read lengths of 2 × 74 bp and 2 × 150 bp on the NextSeq™ 550Dx instrument using the NextSeq 500/550 Mid-Output v2.5 Kit (150 or 300 cycles). The ARTIC v4 primer pool has been validated with the COVIDSeq Test (RUO) and COVIDSeq Assay (96 samples) on all Illumina sequencing systems from the iSeq 100 to the NovaSeq 6000 System; no significant differences in performance were seen between platforms (data not shown).

Analysis with DRAGEN™ COVID Lineage App

FASTQ sequencing files from the NextSeq 550Dx instrument were input to the Illumina DRAGEN COVID Lineage App v3.5.4 in BaseSpace™ Sequence Hub for alignment to a SARS-CoV-2 reference genome. Starting from FASTQ files, the app performs mapping/alignment, variant calling, and consensus sequence generation. Lineage and clade (phylogenetic) calls are made by accessing and comparing the FASTA consensus to the latest version of Pangolin.

For Research Use Only. Not for use in diagnostic procedures.

Results

Improved performance in contrived samples

The performance of the ARTIC v4 primer pool was evaluated with contrived samples prepared with Twist Synthetic SARS-CoV-2 RNA Controls. Evaluation of viral genome coverage showed improved performance with ARTIC v4 primers across the contrived samples, as measured by the percent of non-N bases (a quantitative measurement of the percent of the genome captured) and median coverage (Figure 1). Importantly, ARTIC v4 primers showed more uniform coverage within the spike protein locus, a critical region of the SARS-CoV-2 genome[3-5] (Figure 2).

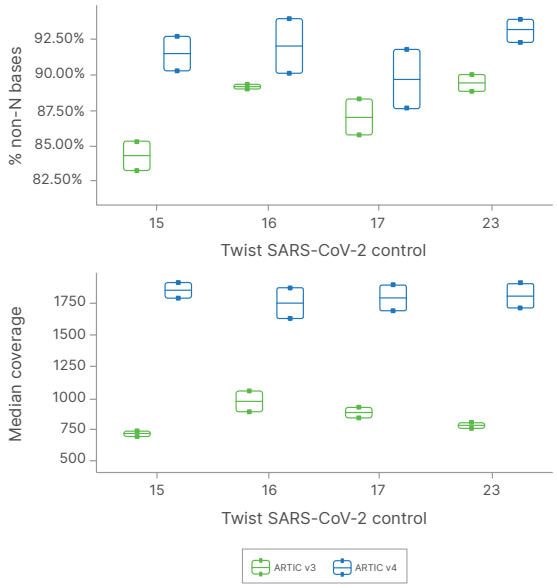

Figure 1: Improved SARS-CoV-2 genome coverage—ARTIC v4 primers showed improved viral genome coverage across Twist Synthetic RNA controls, as measured by % non-N bases and median coverage. Note, contrived controls are expected to have lower genomic coverage than true viral samples.

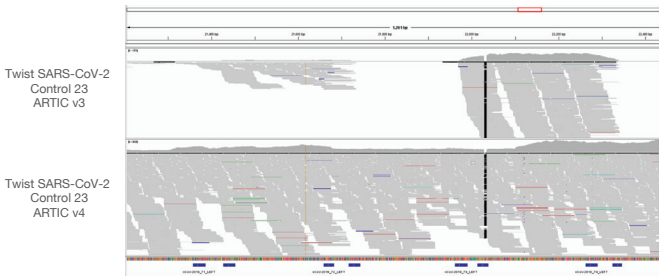

Figure 2: Improved coverage in spike protein loci—ARTIC v4 primers provided more uniform coverage across the spike protein locus in Twist SARS-CoV-2 Control 23.

Improved performance in COVID-positive NP swab samples

The performance of the ARTIC v4 primer pool was evaluated using COVID-positive NP swab samples. SARS-CoV-2 viral targets were detected at similar levels across NP samples (data not shown). Viral genome coverage was improved with ARTIC v4 primers across NP samples (Figure 3).

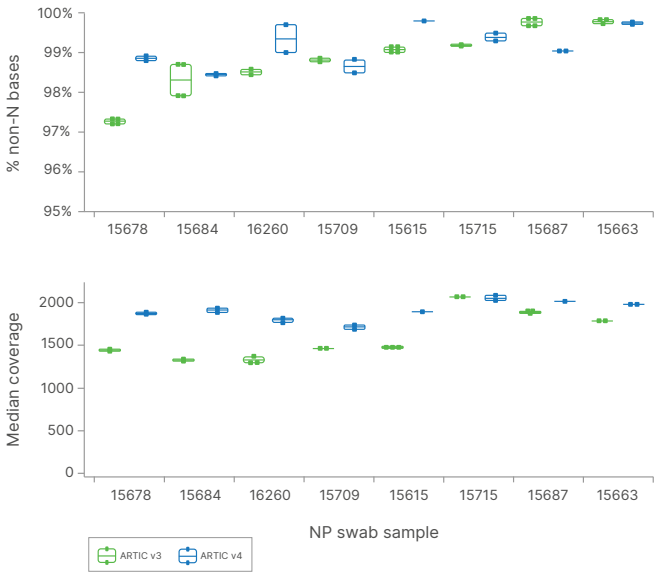

Figure 3: Improved SARS-CoV-2 genome coverage in NP swab samples—ARTIC v4 primers showed improved viral genome coverage across NP samples, as measured by % non-N bases and median coverage.

Viral genome coverage was evaluated in B.1.617.2 (Delta) variant COVID-positive NP swab samples. ARTIC v4 primers showed more uniform coverage as compared to ARTIC v3, particularly in the spike protein locus (Figure 4).

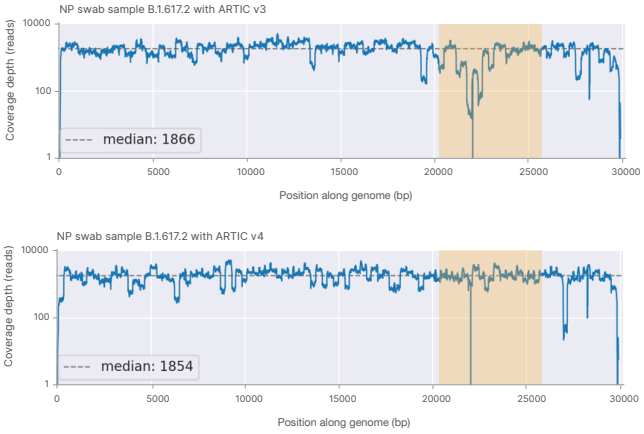

Figure 4: Improved genome coverage in B.1.617.2 (Delta) variant SARS-CoV-2—ARTIC v4 primers (bottom) showed more uniform viral genome coverage across the spike protein locus (highlighted region). Note, sharp coverage drop seen with both primer pools is not a drop-out but a true 6-base deletion.

Improved performance in high and low titer NP swab samples

The ARTIC v4 primer pool was evaluated for viral genome coverage in B.1.617.2 (Delta) variant COVID-positive NP swab samples with high viral titer (qPCR Ct values ≤ 27). ARTIC v4 primers showed improved performance at read lengths of 2 × 75 bp and 2 × 150 bp across a range of read depths (Figure 5A and Figure 5B). Improved performance was also seen with ARTIC v4 primers in B.1.617.2 (Delta) variant COVID-positive NP swab samples with low viral titer (qPCR Ct values ≥ 30) (Figure 5C).

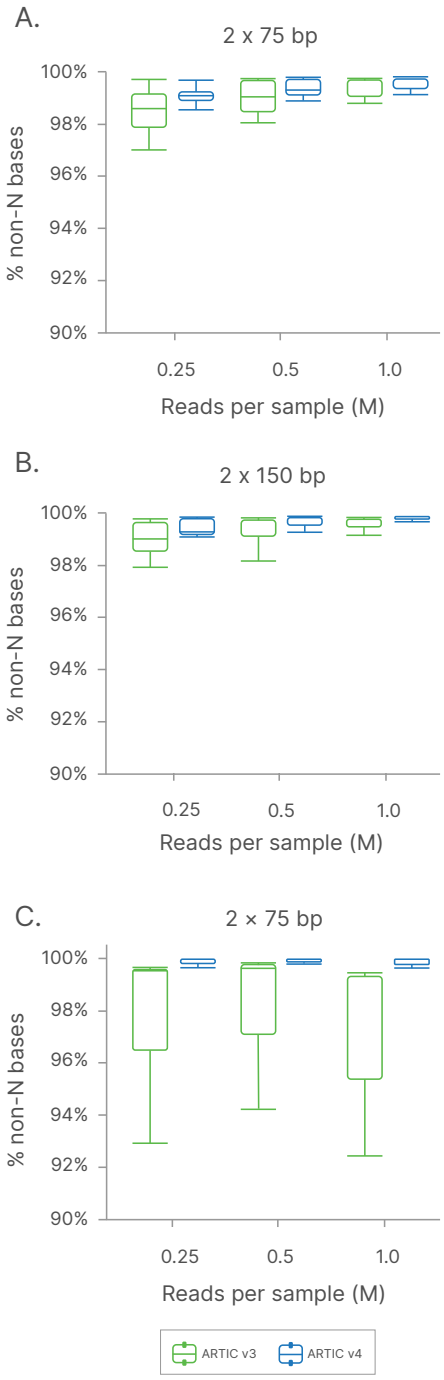

Figure 5: SARS-CoV-2 genome coverage in high and low titer COVID-positive NP swab samples—ARTIC v4 primers showed improved viral genome coverage in high titer COVID-positive NP samples at both (A) 2 × 75 bp and (B) 2 × 150 bp read lengths and (C) low titer COVID-positive samples.

For Research Use Only. Not for use in diagnostic procedures.

Summary

The emergence and spread of new SARS-CoV-2 variants during the COVID-19 pandemic highlights the need for sequencing-based viral surveillance. The Illumina COVIDSeq Assay (96 samples) accommodates a low number of samples to enable smaller clinical research labs to identify and track the emergence and prevalence of new SARS-CoV-2 variants and lineages. While the ARTIC v3 primer pool included in the kit performs well for SARS-CoV-2 detection and genotyping, the ARTIC v4 primer pool (available as an accessory product) provides increased viral genome coverage, particularly in the spike protein locus, indicating that the ARTIC v4 primer pool may be preferred by labs that need full genome coverage for detailed SARS-CoV-2 sequencing.

Learn more

Illumina COVIDSeq Assay (96 samples), www.illumina.com/products/by-type/clinical-research-products/covidseq-assay.html

References

1. World Health Organization. WHO Director-General's statement on IHR Emergency Committee on Novel Coronavirus (2019-nCoV). 30 January 2020.
2. Baric, RS. Emergence of a highly fit SARS-CoV-2 variant. *N Engl J Med*. 2020;383:2684–2686.
3. McCarthy KR, Rennick LJ, Nambulli S, et al. Recurrent deletions in the SARS-CoV-2 spike glycoprotein drive antibody escape. *Science*. 2021; doi:10.1126/science.abf6950.
4. Addetia A, Xie H, Roychoudhury P, et al. Identification of multiple large deletions in ORF7a resulting in in-frame gene fusions in clinical SARS-CoV-2 isolates. *J Clin Virol*. 2020; 129:104523.
5. Rosenthal SH, Kagan RM, Gerasimova A, et al. Identification of eight SARS-CoV-2 ORF7a deletion variants in 2,726 clinical specimens. *bioRxiv*. 2020; doi.org/10.1101/2020.12.10.418855.

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

© 2021 Illumina, Inc. All rights reserved. All trademarks are the property of Illumina, Inc. or their respective owners. For specific trademark information, see www.illumina.com/company/legal.html.
M-GL-00408 v1.0