

Illumina Microbial Amplicon Prep— Influenza A/B

Streamlined library preparation solution for influenza virus research and public health surveillance

- Enables rapid, unbiased, whole-genome coverage of influenza A and B virus strains
- Accommodates RNA inputs from a range of sample types and viral loads
- Simplifies whole-genome analysis and variant interpretation



Introduction

Influenza A and B virus strains can cause seasonal, pandemic, and zoonotic respiratory disease outbreaks, posing a global threat to human and animal health. The COVID-19 pandemic demonstrated the utility of next-generation sequencing (NGS)-based pathogen genomic surveillance, enabling researchers to track emerging variants and guide the public health response. Similar amplicon-based approaches have been applied to sequence and characterize influenza viruses.^{1,2} However, influenza viruses exhibit high genomic variability, making them challenging to sequence accurately. Additionally, many available sequencing protocols are not streamlined and can produce variable sequencing results.

The Illumina Microbial Amplicon Prep—Influenza A/B kit is based on the proven Illumina COVIDSeq™ Assay and enables accurate, rapid, unbiased sequencing and characterization of influenza viruses. Integration with the easy-to-use DRAGEN™ Targeted Microbial App simplifies data analysis, making variant identification and viral subtyping accessible to laboratories without bioinformatics expertise. As part of an integrated, scalable workflow (Figure 1) the Illumina Microbial Amplicon Prep—Influenza A/B kit enables researchers to expand their genomic surveillance efforts to protect the health of their communities.

Streamlined workflow

Illumina Microbial Amplicon Prep—Influenza A/B is a highly multiplexed RT-PCR-based library preparation solution for rapid, unbiased influenza virus sequencing and characterization (Table 1). This kit, based on the robust Illumina COVIDSeq Assay workflow, includes steps for isolation of genetic material through sequencing and data analysis for detection and characterization of influenza virus strains (Figure 1).

Library preparation

Illumina Microbial Amplicon Prep—Influenza A/B kit includes reagents necessary for reverse transcription of RNA, PCR amplification, bead-linked transposomes for amplicon tagmentation, and indexed adapters to generate sequencing libraries from whole-genome amplicons (Figure 2). The kit is compatible with RNA extracted using commercially available kits.

Table 1: Total turnaround time and hands-on time

No. of samples	Turnaround time	Hands-on time
8 samples	8 hours	1.9 hours
48 samples	9.3 hours	3.2 hours

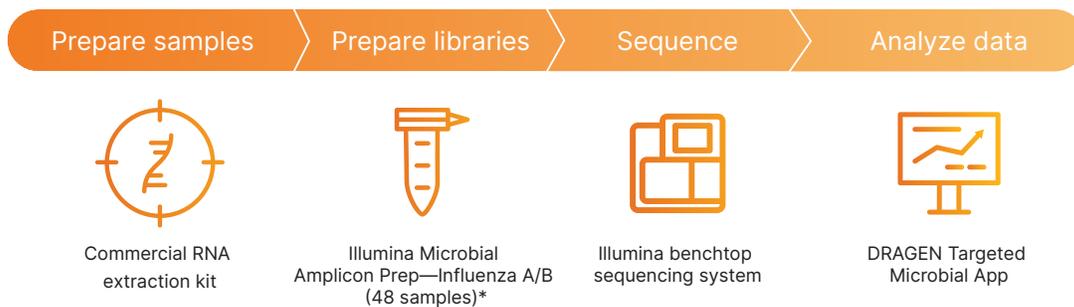


Figure 1: Illumina Microbial Amplicon Prep—Influenza A/B workflow—In a streamlined workflow, influenza virus libraries are prepared using the Illumina Microbial Amplicon Prep—Influenza A/B kit, sequenced on any Illumina benchtop sequencing system, and analyzed in the DRAGEN Targeted Microbial App for detection, variant calling, and strain typing. *Kit provides all reagents necessary for library preparation, including a universal influenza A/B virus primer pool, RT-PCR reagents, and unique dual indexes. RNA extraction reagents are not included.

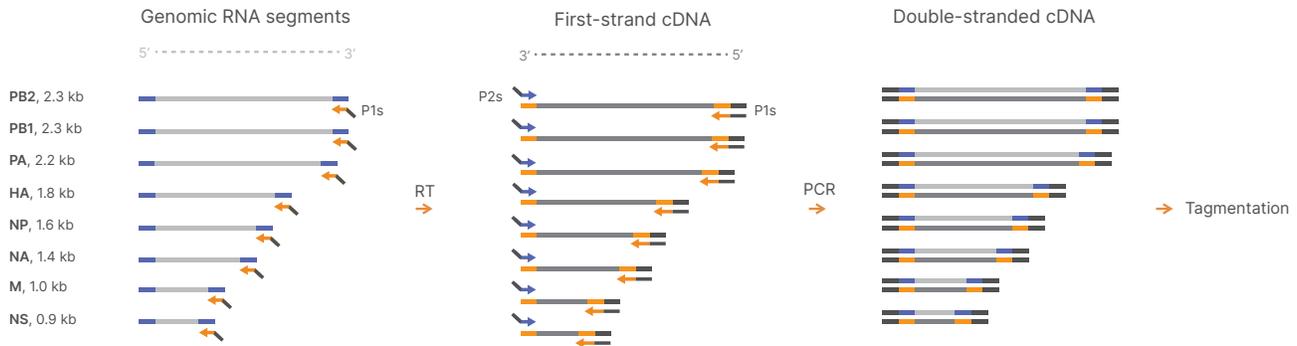


Figure 2: Universal influenza A/B virus whole-genome RT-PCR approach to generate amplicons ready for tagmentation—The influenza A/B primer pool contains 16 primers designed to hybridize to the 3' ends of the first and second complementary DNA (cDNA) strands, enabling both full-length cDNA synthesis of each genomic RNA segment and subsequent PCR amplification to produce complete amplicons ready for tagmentation and library completion. Blue and orange lines represent the conserved noncoding regions of the genomic RNA segment. Black lines represent the additional DNA sequences added by the universal primers. Light gray lines correspond to the genomic RNAs or second strand cDNA while the dark gray lines correspond to the first-strand cDNA.

Sequencing

Prepared libraries can be sequenced on any Illumina sequencing system. However, the 48-sample configuration of Illumina Microbial Amplicon Prep—Influenza A/B makes it ideal for benchtop platforms, including the iSeq™ 100, MiniSeq™, MiSeq™, NextSeq™ 500, NextSeq 550, NextSeq 1000, and NextSeq 2000 Systems.

Data analysis

The Illumina DRAGEN Targeted Microbial App is freely available in BaseSpace™ Sequence Hub. The easy-to-use app aligns reads to reference genomes, calls variants, and generates a consensus genome sequence representing the population of nucleic acid species in the sample (Figure 3). When available, external curated databases are accessed for additional lineage analysis.

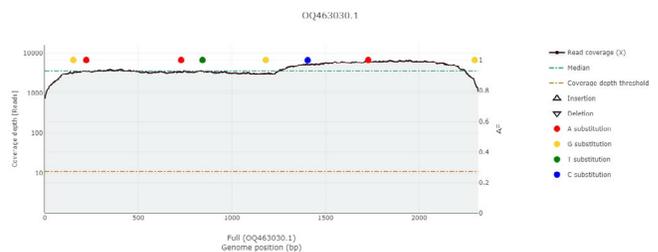


Figure 3: Coverage plot of the influenza A virus generated using the DRAGEN Targeted Microbial App—The coverage plot outputs from the DRAGEN Targeted Microbial App are interactive, with the ability to toggle between log-scale, include or exclude the median coverage lines, and/or the substitution/deletion information. For segmented viruses, such as influenza A and B viruses, each segment is shown in a separate coverage plot. Each circle represents a SNP that varies from the reference genome. Each color indicates whether it is A,G, T, or C substitutions. Insertions and deletions are also indicated by triangles. Interactive plots can be downloaded as PNG files as shown here.

Excellent coverage across influenza A/B virus genomes

Illumina Microbial Amplicon Prep—Influenza A/B is designed to provide extensive sequence coverage across both influenza A and B virus genomes. This kit includes a pool of 16 well-tested, established universal primers^{3,4} that provide more than 98% coverage for influenza A and B virus strains, including the highly variable Neuraminidase (NA) and Hemagglutinin (HA) genes (Figure 4, Figure 5).⁵

The flexible Illumina Microbial Amplicon Prep—Influenza A/B kit is compatible with RNA samples isolated using standard extraction protocols without the need for modifications. Clinical research samples, including nasal swabs, can vary in quality and viral load. The Illumina Microbial Amplicon Prep—Influenza A/B kit retains excellent performance across virus isolates with Ct values ranging from 21.2 to 31.1 (ie, from high to low virus titers). For optimal results, samples with Ct values less than 30 are recommended.

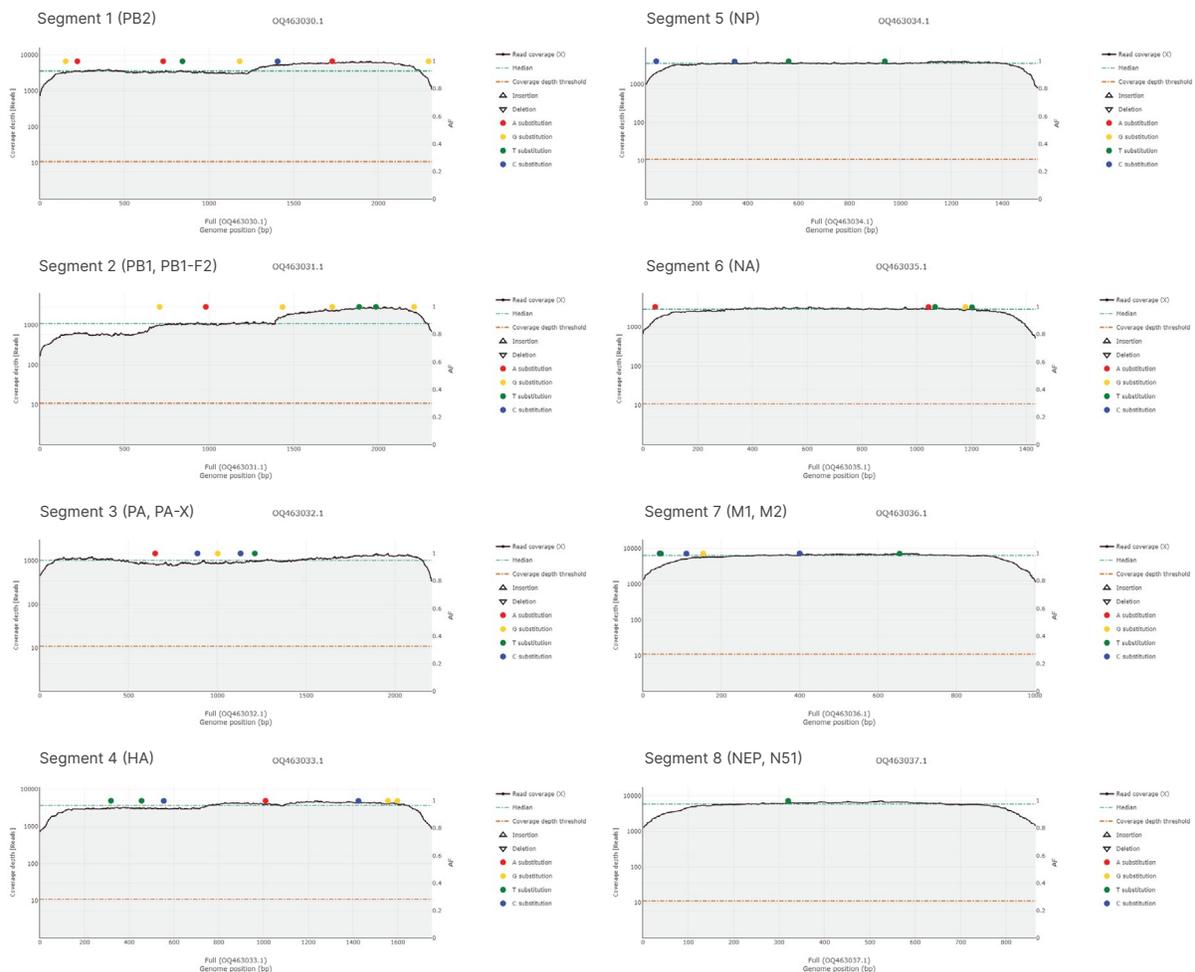


Figure 4: Genomic coverage plots for influenza A virus samples—Representative coverage plots from a H1N1 virus sample (Ct value= 21.2, ~482 copies of viral RNA) from nasopharyngeal swabs showing genome-wide coverage across all eight segments of the influenza A virus. Each graph represents the coverage of each segment of the influenza A virus genome. Reference genome accession IDs are provided in each graph. Dotted lines represent median coverage depth. 1M paired-end reads (2×149 bp) were used for data analysis. Data were analyzed using the DRAGEN Targeted Microbial App.

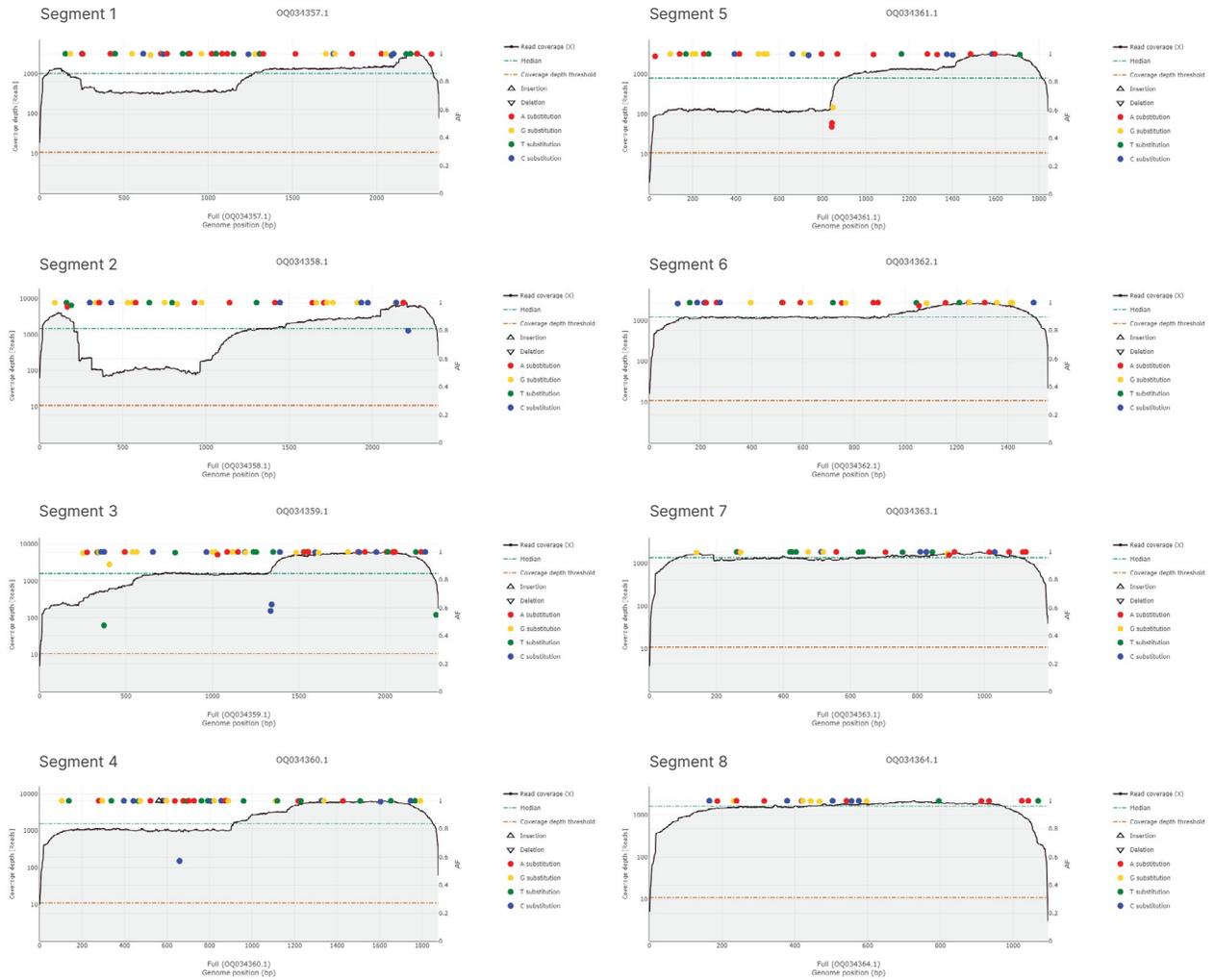


Figure 5: Genomic coverage plots for influenza B virus samples—Representative coverage plots from an influenza B virus sample (ATCC VR-1735, Ct value = 22.1, ~85K copies of viral RNA) showing genome-wide coverage across all eight segments of the influenza B virus. Each graph represents the coverage of each segment of the influenza B virus genome. Reference genome accession IDs are provided in each graph. Dotted lines represent median coverage depth. 1M paired-end reads (2 × 149 bp) were used for data analysis. Data were analyzed using the DRAGEN Targeted Microbial App.

Summary

The Illumina Microbial Amplicon Prep—Influenza A/B kit enables public health researchers to protect the health of their communities by providing a streamlined solution for influenza virus characterization and surveillance. This flexible assay is compatible with RNA inputs extracted from cultures or nasal swabs and washes with variable viral loads. Integration with innovative NGS platforms delivers exceptional data quality, flexibility, and scalability for highly effective public health surveillance and high-impact influenza virus research.

Learn more

[Illumina Microbial Amplicon Prep—Influenza A/B](#)

[Microbial genomics](#)

Ordering information

Product	Catalog no.
Illumina Microbial Amplicon Prep— Influenza A/B (48 samples)	20106305

References

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