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APPLICATION NOTE

Sequencing seasonally circulating influenza viruses with the MiSeq™ i100 Series

Accurate detection of influenza A and B viruses using targeted sequencing

Accurate detection and genomic characterization of influenza A and B strains for viral surveillance

Flexible enrichment-based library preparation solutions to meet user needs

Fast results with an end-to-end sequencing workflow on the MiSeq i100 Series and DRAGEN™ secondary analysis

Introduction

Influenza viruses are a common cause of acute respiratory infections that can result in significant morbidity and mortality worldwide.[1] Influenza A and B viruses have genomes comprised of eight negative-sense, single-stranded RNA segments. Of note, hemagglutinin (HA) and neuraminidase (NA) genome segments are important for immunity and are the basis for subtype classification.[2] Mutations in HA and NA gene products are important targets of immune response, including escaping host antibodies, developing drug resistance, and increasing virulence.[2] Therefore, continuous monitoring in the form of robust and timely viral surveillance is needed to inform vaccine composition, assess pandemic potential, and guide public health responses.[3,4]

Historically, influenza surveillance heavily depended on virus isolation and amplification using cell culture methods before genetic characterization. While effective, this method required significant delays in time to results and could bias viral genome sequences due to adaptation during culture.[5] In contrast, next-generation sequencing (NGS) provides rapid, unbiased genomic profiling, improving the speed and resolution of influenza virus detection and characterization, enabling a "sequence-first" approach directly from clinical research specimens.[6] Targeted NGS methods, including enrichment-based sequencing, provide highly accurate variant information while significantly reducing turnaround time, resulting in real-time genomic surveillance of influenza viruses to support precise, timely public health responses.

This application note demonstrates the detection and characterization of influenza A and B viruses in contrived samples and real-world nasal swab specimens using a flexible NGS workflow that includes Illumina enrichment-based library preparation, the MiSeq i100 Series, and onboard DRAGEN secondary analysis, and can be completed in under 24 hours (Figure 1).

Methods

Samples

A total of 39 samples were tested, including 12 contrived samples and 27 deidentified, influenza-positive remnant nasopharyngeal swab specimens (Table 1). Deidentified remnant clinical samples and associated RT-PCR results were provided by Aegis Labs (Nashville, TN, USA). Contrived samples were prepared by spiking 10–10,000

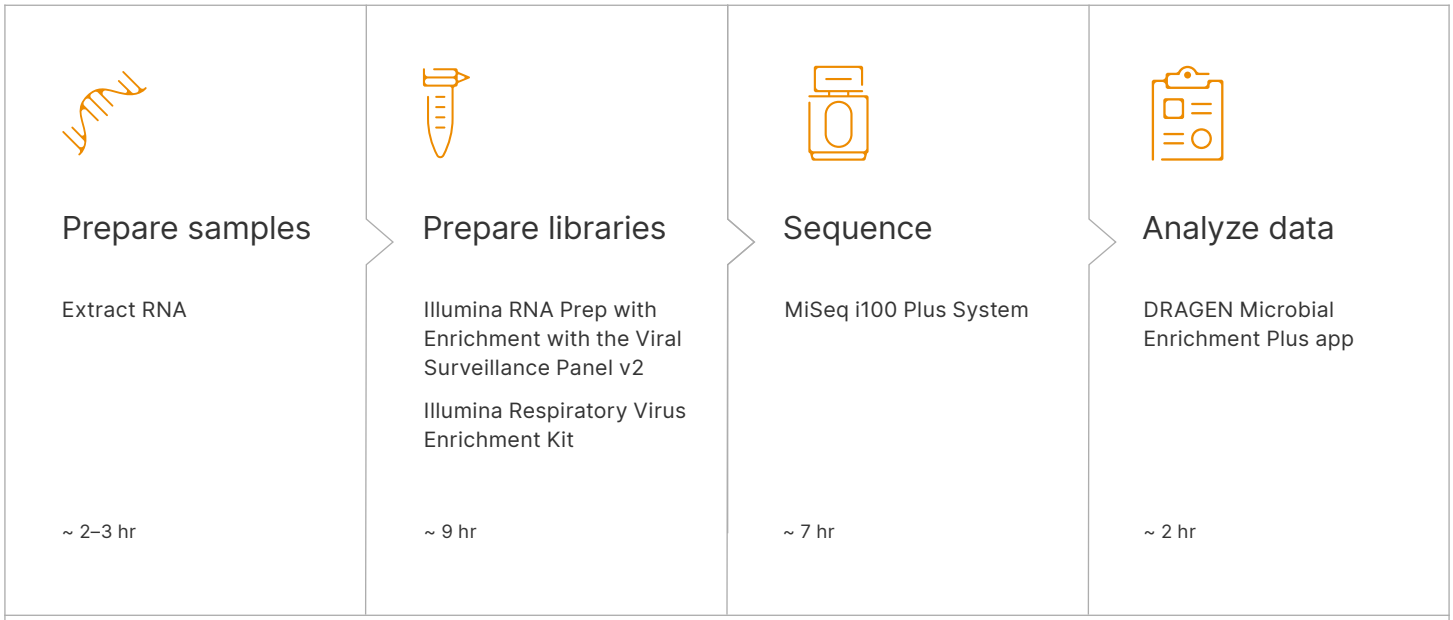

Figure 1: Comprehensive NGS workflow for influenza virus sequencing
Combine Illumina library preparation using a target enrichment approach with sequencing on the MiSeq i100 Series and DRAGEN secondary analysis for accurate detection and genomic characterization of influenza A and B viruses.

copies of influenza A (H1N1, H3N2) or influenza B genomic RNA into 10 ng of Universal Human Reference RNA (UHRR) (Agilent Technologies, Catalog no. 740000-41) (Table 2 and Table 3).

The nasal swab samples underwent diagnostic molecular testing for influenza A (H1N1, H3N2) and influenza B. Nucleic acids were extracted using the QIAsymphony SP Instrument (QIAGEN, Catalog no. 9001301), according to the manufacturer's instructions.

Table 1: Samples assayed for performance evaluation

Sample type	Expected detection	No. of samples
Contrived samples (genomic RNA in UHRR)	Influenza A (H1N1)	4[a]
	Influenza A (H3N2)	4[a]
	Influenza B (Yamagata lineage)	4[a]
Total no. of contrived samples		**12**
Clinical influenza-positive nasopharyngeal swab specimens	Influenza A (H1N1)	7
	Influenza A (H3N2)	11
	Influenza B (Victoria lineage)	9
Total no. of swab specimens		**27**
Total no. of samples		**39**

a. Each contrived sample is a titration of 10, 100, 1000, and 10,000 viral copies spiked into UHRR for a limit of detection study; each titration has a single replicate for a total of four samples per influenza type.

Table 2: Viral copy numbers for contrived samples

Copies/reaction	Copies/µl
10	1.2
100	12
1000	118
10,000	1175

Table 3: Influenza strains used for contrived samples

Type	Strain	Vendor	Catalog no.
Influenza A (H1N1)	A/PR/8/34	ATCC	VR-95DQ
Influenza A (H2N3)	A/Hong Kong/8/68	ATCC	VR-1679D
Influenza B (Yamagata lineage)	B/Florida /4/2006	ATCC	VR-1804DQ

Library preparation

Sequencing-ready libraries were prepared from samples using either the Illumina Respiratory Virus Enrichment Kit (Illumina, Catalog no. 20100469) or Illumina RNA Prep with Enrichment (L) Tagmentation (Illumina, Catalog no. 20040536) and the Viral Surveillance Panel v2 Kit (Illumina, Catalog no. 20108081). Libraries prepared with Illumina RNA Prep with Enrichment were sequenced before enrichment (termed "shotgun libraries") for comparison.

Sequencing

Enriched libraries were sequenced on the MiSeq i100 Plus System (Illumina, Catalog no. 20115695) using a 25M flow cell with a run configuration of 2 × 151 bp. Shotgun libraries were sequenced on the NextSeq™ 550 System (Illumina, Catalog no. SY-415-1002) with a run configuration of 2 × 151 bp for comparison.

Data analysis

FASTQ data sets were downsampled to 500,000 clusters or 2M paired-end (PE) reads. FASTQ files were analyzed using the DRAGEN Microbial Enrichment Plus app either onboard the MiSeq i100 Plus System or in BaseSpace™ Sequence Hub with Viral Surveillance Panel v2 or Illumina Respiratory Virus Enrichment Kit references. Shotgun libraries were analyzed with the DRAGEN Microbial Enrichment Plus app and Viral Surveillance Panel v2 references. Statistical analyses and data visualization were performed using GraphPad Prism 10 and JMP 18.

Results

Sequencing metrics

Enriched libraries were sequenced across two runs on the MiSeq i100 Plus System. All runs generated high-quality data with > 94% of reads passing filter (PF). The total number of PE reads PF exceeded 64M reads per run, supporting high-confidence downstream analysis. For both runs, the combined instrument run and analysis time was under 10 hours (Table 3). This demonstrates that the MiSeq i100 Plus System offers speed and efficiency to provide timely results important for public health monitoring and response.

Genome coverage across varying viral genome concentrations

Evaluation of genome coverage, median sequencing depth, and reads per kilobase per million mapped reads (RPKM) values showed the exceptional performance of both the Illumina Respiratory Virus Enrichment Kit, and Illumina RNA Prep with Enrichment with the Viral Surveillance Panel v2 in detecting influenza subtypes down to 100 viral copies (Figure 2).

Influenza A and B detection in nasopharyngeal swab specimens

To evaluate performance in real-world samples, influenza-positive nasopharyngeal swab specimens were processed using enrichment and shotgun sequencing approaches. Shotgun libraries sequenced on the NextSeq 550 System demonstrated lower genome coverage, particularly in samples with lower viral abundance (as indicated by higher qRT-PCR Ct values), relative to enrichment workflows on the MiSeq i100 Plus System (Figure 3).

Both the Illumina Respiratory Virus Enrichment Kit, and Illumina RNA Prep with Enrichment with the Viral Surveillance Panel v2 successfully detected influenza A (H1N1) with 71% of nasal swab samples achieving > 95% callable bases and maintaining strong coverage in samples with lower viral loads (Figure 3A). Similar performance was observed for influenza A H3N2 with 82% of samples achieving > 95% callable bases (Figure 3B) and for influenza B with 77% of samples achieving > 95% callable bases (Figure 3C). Representative nasal swab samples with low Ct values and high viral abundance demonstrated comprehensive genome coverage with both library preparation kits (Figure 4).

Table 4: Sequencing metrics for the MiSeq i100 Series[a]

Run	Library type	No. of samples	Sequencing run time	Analysis time with DRAGEN Microbial Enrichment Plus[b]	Total no. of PE reads PF	% PF
1	Viral Surveillance Panel v2	30	7 hr 11 min	1 hr 41 min	67,382,396	95.25%
2	Illumina Respiratory Virus Enrichment Kit	30	7 hr 12 min	2 hr 18 min	67,083,162	94.36%

a. Metrics for shotgun libraries are not included in this table.
b. Analysis was performed onboard the MiSeq i100 Plus System.
c. Analysis was performed in BaseSpace Sequence Hub.

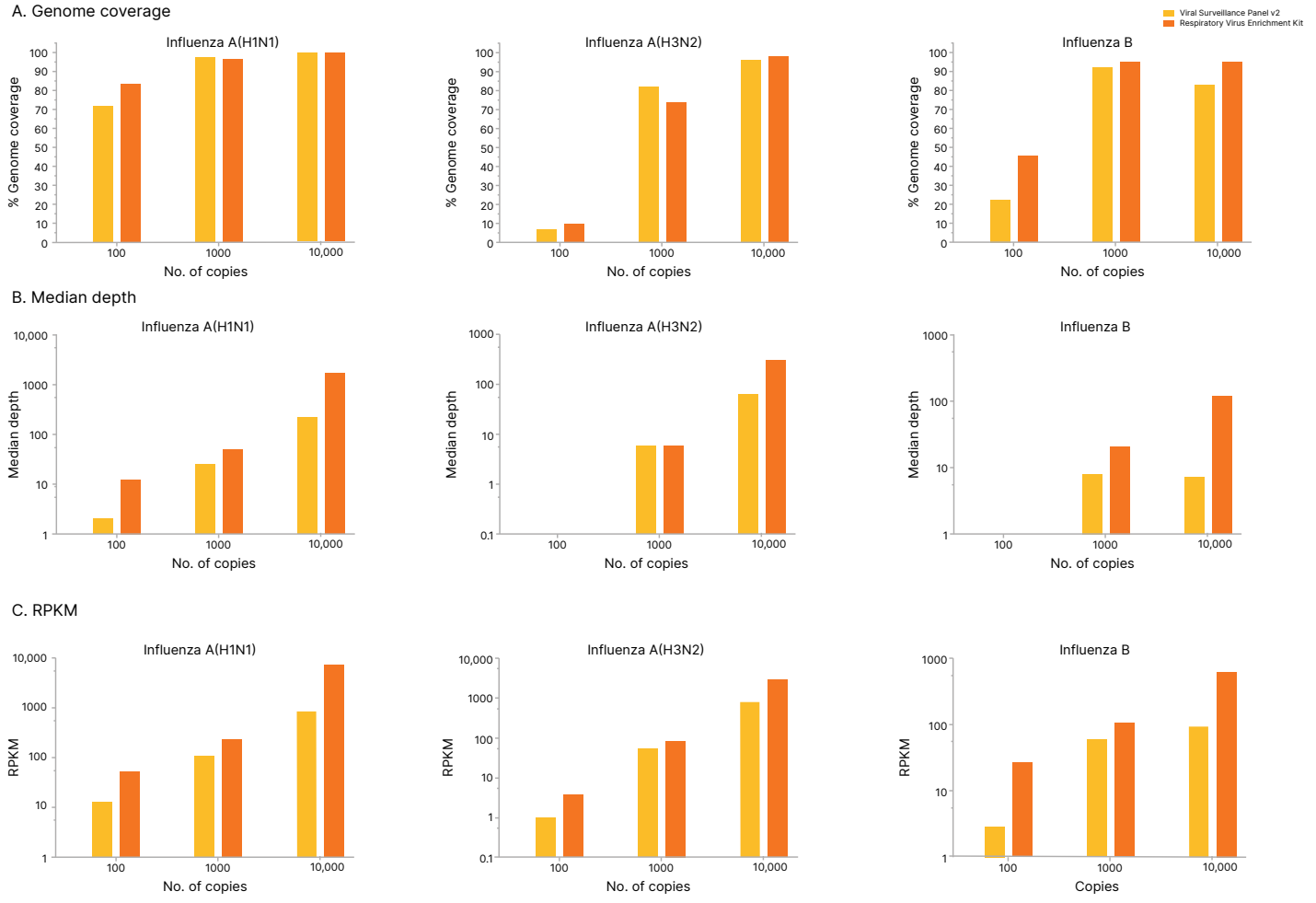

Figure 2: Illumina enrichment panels demonstrate exceptional detection of viral genomes

(A) Genome coverage, (B) median depth, and (C) reads per kilobase per million mapped reads (RPKM) were evaluated for influenza A (H1N1), influenza A (H3N2), and influenza B for 100, 1000, and 10,000 viral copy numbers. The Viral Surveillance Panel v2 (yellow bars) and Illumina Respiratory Virus Enrichment Kit (orange bars) successfully detected influenza subtypes down to 100 copies.

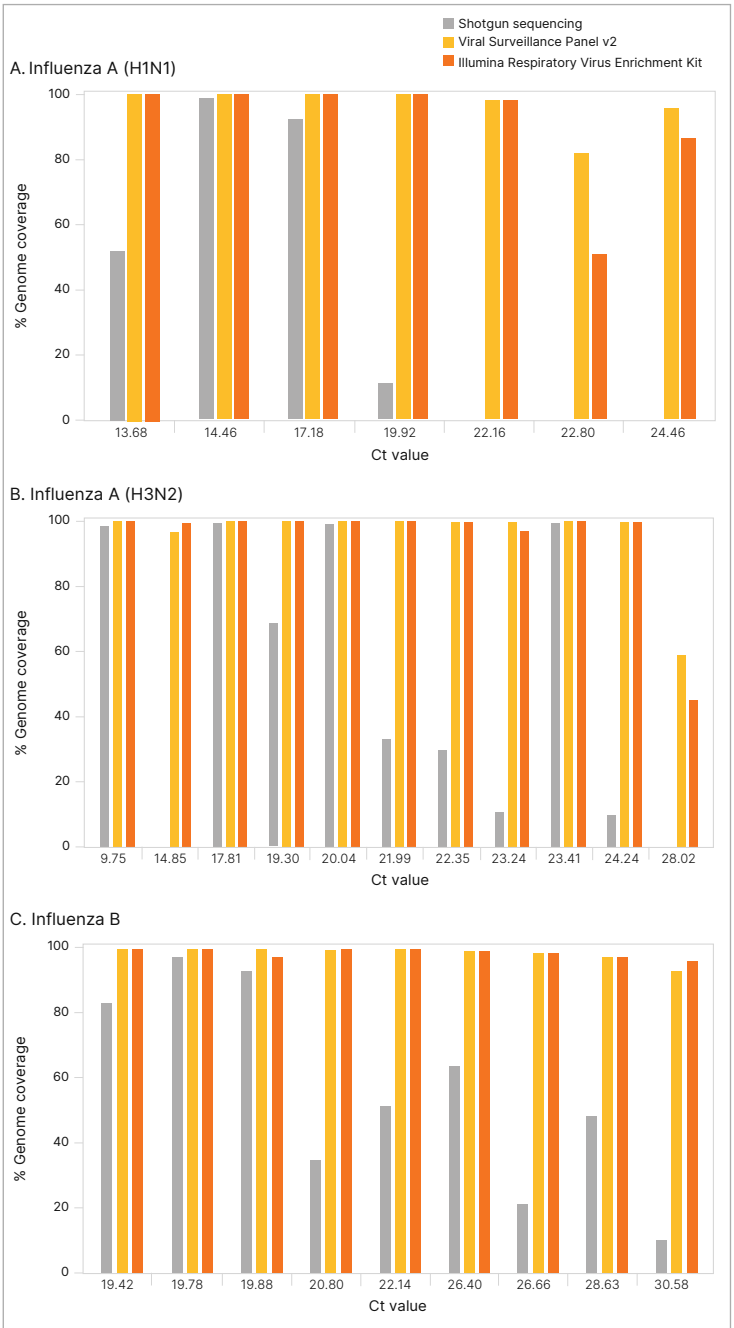

Figure 3: Detection of Influenza subtypes in nasopharyngeal swab specimens

The Illumina Respiratory Virus Enrichment Kit (yellow bars) and Illumina RNA Prep with Enrichment with the Viral Surveillance Panel v2 (orange bars) successfully detected (A) influenza A H1N1, (B) influenza A H3N2, and (C) influenza B in clinical specimens across a range of viral abundance (indicated by qRT-PCR Ct value). Genome coverage was maintained across most viral abundance levels with reduced coverage for the lowest viral abundance specimens. Data was compared to shotgun sequencing (gray bars).

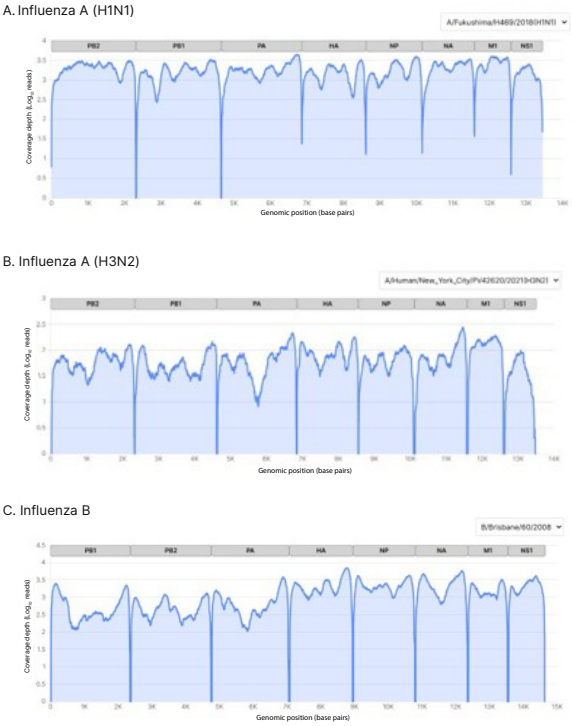

Figure 4: Genome coverage of Influenza subtype segments in nasopharyngeal swab specimens

The Illumina Respiratory Virus Enrichment Kit and Illumina RNA Prep with Enrichment with the Viral Surveillance Panel v2 both showed comprehensive genome coverage for all segments, including HA and NA for (A) influenza A H1N1, (B) influenza A H3N2, and (C) influenza B in representative clinical specimens with low Ct values and high viral abundance.

Summary

The MiSeq i100 Series combined with high-quality library preparation using the Illumina Respiratory Virus Enrichment Kit, or Illumina RNA Prep with Enrichment with the Viral Surveillance Panel v2 demonstrated targeted sequencing capabilities for influenza detection and subtype characterization. Both enrichment-based methods yielded full genome coverage of Influenza strains with less susceptibility to known challenges in Influenza amplification. This application note demonstrates that the MiSeq i100 Series is part of a flexible, efficient NGS workflow for Influenza characterization that delivers fast results to meet user needs.

Learn more

MiSeq i100 Series

Viral Surveillance Panel v2

Illumina Respiratory Virus Enrichment Kit

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