Gain greater **insights** into microbial mysteries

Innovative microbial sequencing solutions

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M-GL-02402 v3.0

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Working together to make an impact in the microbial world

Today more than ever, next-generation sequencing (NGS) is empowering a new level of accuracy and speed in obtaining answers in microbial and infectious disease research. This proven sequencing approach greatly enhances research efforts and offers potential solutions to address One Health issues.*

A broader view, a brighter future

With Illumina technology and support, researchers around the world are rapidly sequencing the genomes of microbes and gaining insights into their behavior, interactions with their hosts and environments, evolution, and circulation within and throughout global populations.

Depending on the microbes of interest, sample type, and the kinds of questions you want answered, different NGS workflows and solutions can be used. The optimal workflow should be determined by what goals need to be achieved.

Illumina NGS solutions are up for the challenge

Single-genome, multipathogen, and discovery workflows can support the full range of microbiology and infectious disease research applications.

Illumina offers powerful library preparation kits, sequencing systems, and corresponding analysis and reporting solutions that enable discoveries and provide insights for a better understanding of the microbes most relevant to your research.

ADVANCE YOUR RESEARCH
Learn how the right suite of NGS tools can help you gain a more complete picture in your research

*One Health: cdc.gov/onehealth.

WORKFLOW APPLICATION

Microbial discovery

NGS isauseful approach forunbiasedanalysis of aprimary sample. Shotgun DNA or RNA sequencing enables analysis of the genetic material present in complex microbial communities, such as environmental samples (eg, wastewater), human microbiomes, and primary samples (eg, sputum, lower respiratory aspirates).

Metagenomics and metatranscriptomics

Illumina DNA Prep

Fast, flexible library prep for microbiome discovery research and surveillance

- Run multiple sample types, input amounts, and methods starting from DNA or cDNA
- Access fast library prep with a total turnaround time of ~3.5 hours
- Apply the DRAGEN™ Metagenomics pipeline to perform taxonomic classification of reads and provide single-sample and aggregate reporting

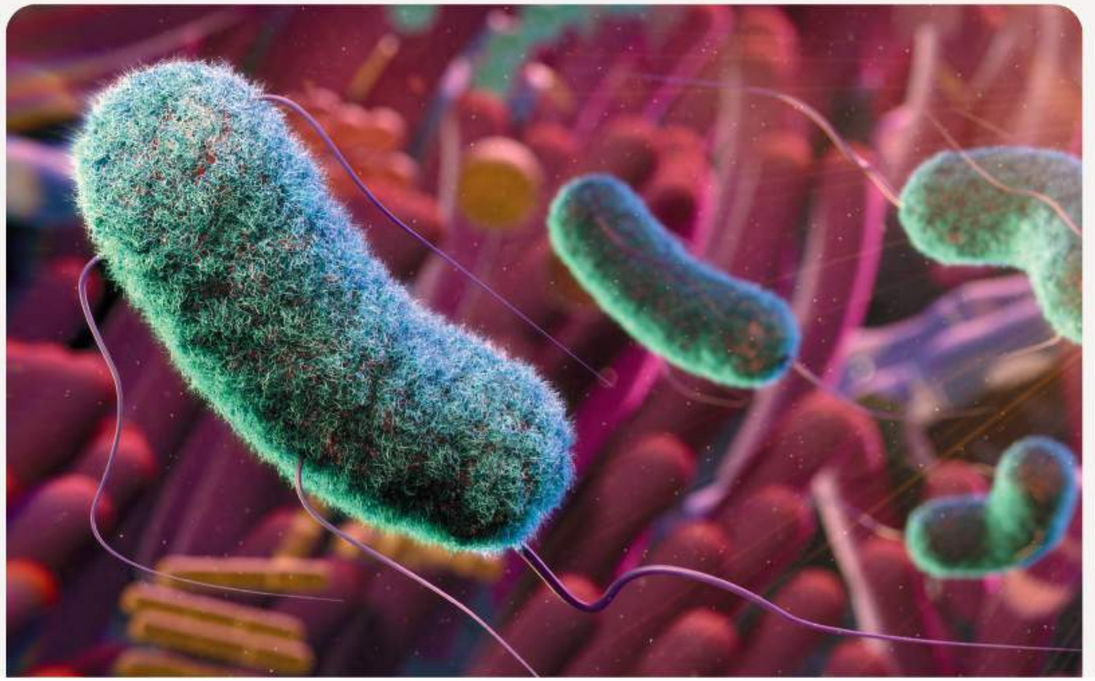
Illumina Stranded Total RNA Prep with Ribo-Zero™ Plus Microbiome

Fast, efficient library prep that provides robust depletion of undesirable host and panbacterial rRNA from complex microbial samples for microbiome analysis

- Eliminate unwanted rRNA from bacteria in complex microbial samples for highly efficient metatranscriptomics research
- Obtain reliable results with an optimized library preparation workflow
- Simplify data analysis and visualization with the BaseSpace™ Microbiome Metatranscriptomics App

UNCOVER MORE
Discover how metatranscriptomics enables community-level gene expression studies

Gut microbiome



WORKFLOW APPLICATION

Single-genome sequencing

Sequencing of a single pathogen is best used in situations when a single, known microbe is being analyzed.† These solutions will help you gain valuable insights in your research as you work to develop effective diagnostic tools, vaccines, and targeted therapies.

MONITOR MORE
Download the single-genome sequencing methods guide to select the right solution for your lab

Small whole-genome sequencing

Illumina DNA Prep

Fast, flexible library preparation kit for characterizing cultured isolates; ideal for investigating foodborne outbreaks and health care–associated infections

- Reduce library preparation time (~3.5 hours) with a low numberof steps and minimal hands-on time
- Obtain robust, consistent results over a wide range of DNA inputs, even at low amounts (1 ng)

Targeted tuberculosis sequencing

Illumina and GenoScreen Deeplex® Myc-TB Combo Kit

Determine tuberculosis (TB) strain and drug-resistance profiles faster than traditional culture-based methods for a more timely response to this critical public health threat

- Produces results in < 48 hours directly from sputum, no culture required
- Provides secure, automated analysis and easy interpretation of results via the Deeplex Myc-TB Web App
- Predicts resistance to 15 anti-TB drugs

Mycobacterium tuberculosis bacteria

Escherichia coli bacteria

† Data on file. Illumina, Inc. 2024 .

WORKFLOW APPLICATION

Single-genome sequencing

Targeted virus characterization

COVIDSeq™ Assay and COVIDSeq Test

Scalable, simple, and affordable solutions for whole-genome analysis of the SARS-CoV-2 virus to aid in COVID surveillance and viral research efforts

- 96- and 3072-sample kits available
- Flexible input for multiple sample types, including nasal swabs and wastewater samples
- Highly accurate virus characterization enabled by the DRAGEN Microbial Amplicon App

Illumina Microbial Amplicon Prep–Influenza A/B

Simple and affordable whole-genome characterization of influenza A and B viruses for surveillance and seasonal vaccine research

- Covers more than 98% of both influenza A and B virus genomes
- Streamlines workflows; nine hours from extracted RNA to sequencing-ready library
- Simplifies whole-genome analysis and variant interpretation with the DRAGEN Microbial Amplicon App

Illumina Microbial Amplicon Prep

Flexible, streamlined NGS library preparation kit that enables various public health surveillance and microbial research applications

- Pairs with lab-designed primers, published primers, or commercially available primer sets
- Simplifies analysis with the DRAGEN Microbial Amplicon App
- Enhances flexibility to accommodate different sample types, including nasal swabs, wastewater, and culture

Influenza virus

WORKFLOW APPLICATION

Multipathogen sequencing

Hybrid–capture enrichment sequencingsolutions areideal whencertain pathogens are suspected to be present or forsurveillance of multiple knownpathogens.Theyallow for targeted or whole-genome sequencing (WGS) of multiple organisms without requiring the high read depth needed for shotgun metagenomic sequencing of unenriched libraries.‡

Broad viral, zoonotic, and environmental surveillance

Viral Surveillance Panel v2

Extensive WGS panel for reliable characterization of the most critical viral public health threats for broad-based surveillance efforts

Conduct WGS of > 200 viruses identified as high risk to public health and > 230 antiviral-resistance variants in influenza virus

Benefit from compatibility with a range of host and environmental sample types

Detect low-abundance viruses that shotgun sequencing would miss‡

Analyze and interpret data with ease using the DRAGEN Microbial Enrichment Plus App

Respiratory Virus Enrichment Kit

Extensive, streamlined workflow offering highly sensitive detection and characterization of common respiratory viruses

Conduct WGS of > 40 respiratory viruses, including influenza A and B, SARS-CoV-2, and many more

Identify targeted viruses and new variants present and track variants from various sample types

Detect low-abundance viruses that shotgun sequencing would miss‡

Analyze and interpret data with the DRAGEN Microbial Enrichment Plus App

ANALYZE MORE
See how targeted microbial sequencing brings more profiling capabilities to your lab

Coronavirus (COVID-19)

‡ Data on file. Illumina, Inc. 2024.

WORKFLOW APPLICATION

Multipathogen sequencing

Flexible, comprehensive syndromic sequencing and antimicrobial resistance surveillance

Respiratory Pathogen ID/AMR Enrichment Panel Kit

Comprehensive panel designed for reliable identification and quantification of respiratory pathogens and characterization of associated antimicrobial resistances for clinical research and surveillance

Identifies > 280 pathogens associated with respiratory tract infections

Characterizes antimicrobial resistance of > 2000 markers associated with 26 antimicrobial classes

Accommodates various sample types

Performs automated analysis powered by the DRAGEN Microbial Enrichment Plus App

Urinary Pathogen ID/AMR Enrichment Panel Kit

The broadest sequencing-based panel for detecting urinary and sexually transmitted pathogens and their associated antimicrobial resistances for clinical research and surveillance

Identifies > 170 pathogens associated with urinary tract and sexually transmitted infections

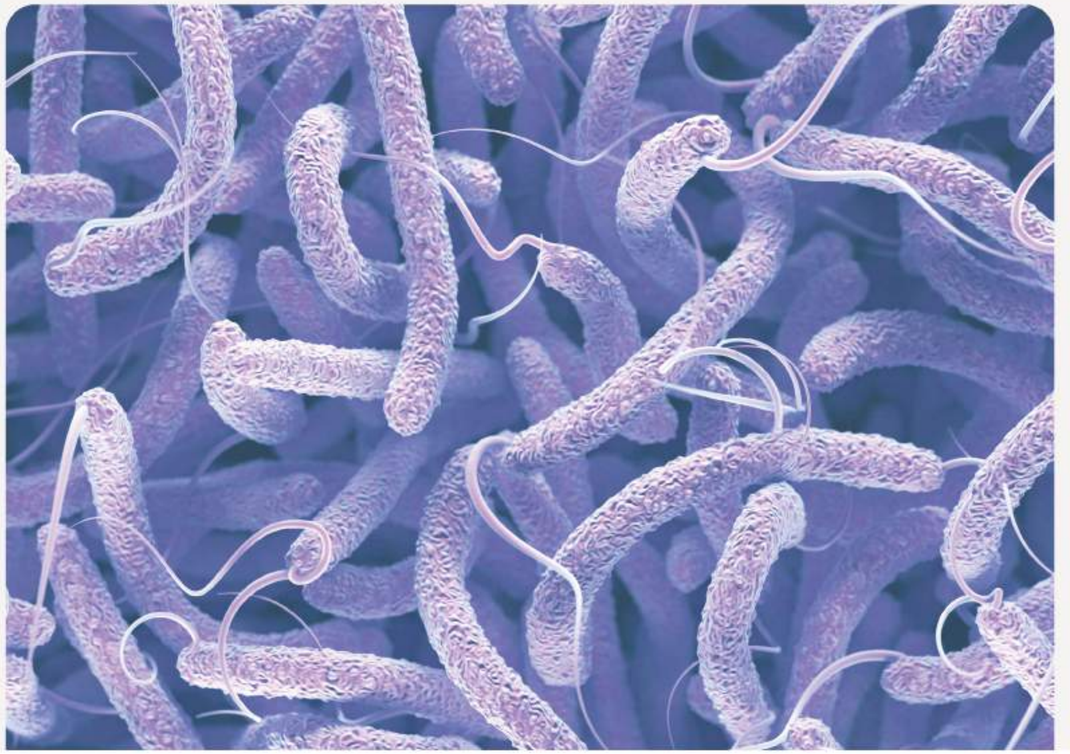
Characterizes antimicrobial resistance of > 3700 markers associated with 18 antimicrobial classes

Accommodates various sample types

Performs automated analysis powered by the DRAGEN Microbial Enrichment Plus App

DETECT MORE
Download the Precision Metagenomics eBook to go deeper into sequencing possibilities

Vibrio cholerae bacteria



SEQUENCING PLATFORM

Introducing the MiSeq™ i100 Series

Impossibly simple sequencing, from setup to data analysis

The MiSeq i100 Series brings sequencing capabilities to users of all levels. Advancements in system design, sequencing chemistry, and data analysis integration deliver operational simplicity, exceptional speed, and proven accuracy.

As part of an end-to-end NGS solution, the MiSeq i100 Series provides same-day results for various applications that have impacts in infectious disease and microbiology. Whether tracking outbreaks, classifying novel microorganisms, or researching microbiomes, the simplicity of the MiSeq i100 Series enables sequencing with confidence and certainty.

SIMPLIFIED RUN SET-UP
Completed in three steps in under 20 minutes. Download the MiSeq i100 Series infographic to learn more

ENHANCED FLEXIBILITY
Multiple reagent kits support a wide range of throughputs and applications. Check out the MiSeq i100 Series virtual overview for details

SEQUENCING PLATFORM

Advanced flexibility

Scalable output enables expanded applications

With the MiSeq i100 Series, you can access 10 reagent configurations across multiple flow cell options and read lengths. You can readily increase sample throughput and perform deeper sequencing for various applications, from sWGS to metagenomics and more.

MiSeq i100 Series System‡

MISEQ i100 SERIES | SEQUENCING SYSTEM

Outputs ranging from 1.5 to 15 Gb
Read lengths from 1 × 100 bp to 2 × 300 bp
5M and 25M flow cells

MiSeq i100 Series Plus System

MISEQ i100 SERIES | PLUS | SEQUENCING SYSTEM

Outputs ranging from 1.5 to 30 Gb
Read lengths from 1 × 100 bp to 2 × 300 bp
5M, 25M, 50M,‡ and 100M‡ flow cells

‡ Available in H2 2025.

SEQUENCING PLATFORMS

Choose the right platform for your lab

Trustedtechnologyand proven performanceempower microbiologists andinfectiousdisease researcherstoexplore further, withconfidence,sothey canoptimize itsdailyworkflows.

EXPLORE MORE
Find additional details on Illumina sequencing platforms




Key specifications	iSeq™ 100 System	MiSeq i100 Series	NextSeq™ 1000 and 2000 Systems	NovaSeq™ X Series
Max output per flow cell	1.2 Gb	30 Gbb	540 Gba	8 Tbc
Run timee (range)	~9.5–19 hr	~4–15 hrd	~8–44 hrd	~17–48 hrd
Max reads per run (single reads)	4M	100M	1.8Ba	26B (single flow cell)c 52B (dual flow cells)c
Max read length	2 × 150 bp	2 × 300 bp	2 × 300 bp	2 × 150 bp
Ideal applications and methodsf				
Small WGS	✓	✓	✓	
Microbial community diversity analysis	✓	✓	✓	
Shotgun metagenomics		✓	✓	✓
Microbial gene expression/ metatranscriptomics		✓	✓	✓
~~Microbial discovery~~		✓	✓	✓
~~Targeted pathogen surveillance~~	✓	✓	✓	

a. Maximum specifications based on a P4 flow cell run. P4 flow cells are available for the NextSeq 2000 System only.
b. Maximum specifications based on a 100M flow cell run; 100M flow cells are available for the MiSeq i100 Plus System only.
c. Specifications based on the Illumina PhiX control library or a TruSeq™ DNA Library created with NA12878 at supported cluster densities. Performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors.
d. Run times include cluster generation, sequencing, and base calling on a NextSeq 1000, or NextSeq 2000 System and calling on a NextSeq 1000 or NextSeq 2000 System. Run times include automated onboard cluster generation, sequencing, automated post-run wash, and base calling on the NovaSeq X Systems.
e. Run times include cluster generation, sequencing, base calling, and quality scoring.
f. Check mark indicates an application or method optimized for the sequencing system.

DATA ANALYSIS

Turn data into meaningful results

Intuitive, efficientIlluminadata analysissolutions are built withthesameexceptional level of accuracy, accessibility, andsecuritystandardsas Illumina sequencing solutions.The apps meet a broad range of microbial research, regardless of bioinformatics experience.

EASY ACCESS
Explore BaseSpace Sequence Hub, a user-friendly genomics cloud-computing platform that helps simplify analysis

Overview of apps

Microbiome Metatranscriptomics App

Performs taxonomic and pathway enrichment analysis on metagenomic samples. Analyzes RNA sequencing libraries derived from microbiological communities using a four-step pipeline.

16S Metagenomics App

Analyzes DNA from amplicon sequencing of prokaryotic 16S small subunit rRNA genes. Provides visuals of taxonomic classification.

SRST2 App

Reports the presence of STs (sequence types) from an MLST database and/or reference genes from a sequence data base of virulence genes, resistance genes, and plasmid replicons.

Deeplex Myc-TB Web App**

Provides secure, automated analysis and easy interpretation of results from the Illumina and GenoScreen Deeplex Myc-TB Combo Kit.

DRAGEN small Whole Genome Sequencing (sWGS)

The DRAGEN sWGS App enables read mapping of single microbial genomes to a reference genome.

DRAGEN Microbial Enrichment Plus App

Delivers easy-to-use, powerful secondary analysis of Illumina infectious disease and microbiology hybrid–capture enrichment panel kits, with workflows for sample QC, viral WGS, pathogen detection and quantification, and antimicrobial resistance (AMR) marker profiling.

DRAGEN Metagenomics Pipeline

Aligns reads from shotgun metagenomic samples to genomic references, classifying to the lowest common level (down to genus level). Provides taxonomic classification for complex microbial samples.

DRAGEN Microbial Amplicon App

Analyzes sequencing libraries generated from Illumina infectious disease and microbiology amplicon panel kits, such as the COVIDSeq Test and Illumina Microbial Amplicon Prep.

DRAGEN

** Not available on BaseSpace Sequence Hub.

Working as one, your lab and Illumina can bring the benefits of NGS-powered microbiology and infectious disease research to everyone

How can we help you?

At Illumina, we offer technology and support to cover integrated NGS workflows—from library preparation to sequencing to data analysis to sharing. With optimized end-to-end solutions, you'll experience unrivaled accuracy, operational simplicity, and fast turnaround times.

Illumina is committed to promoting global health

Beyond product offerings, Illumina offers a wide range of educational resources, support, and training programs to enhance your research. Let's make an impact, together.

Learn how NGS can support your microbiology research goals

NGS is opening new doors in microbial genomics, revealing fresh insight into how microbes impact humans and the environment. With NGS, see the bigger picture by examining the smallest details in the tiniest organisms. Learn more about how NGS can change your approach to microbiology.

Find the right workflow for your microbiology application

Take the guesswork out of your next workflow. The NGS Workflow Finder provides personalized solution recommendations and resources for your microbiology and infectious disease applications.

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We are always available for questions, insights, and conversation.
Visit us at illumina.com.

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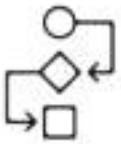
Track emerging infectious diseases with wastewater sur veillance

Broad detectionofpathogenswiththe MiSeq™ i100 Series



Comprehensive workflow includes library preparation, target enrichment, sequencing, and data analysis

Accurate detection and characterization of a wide range of RNA and DNA viral pathogens

Fast, flexible sequencing that delivers same-day results for effective and efficient wastewater surveillance

M-GL-02915 v1.0

Introduction

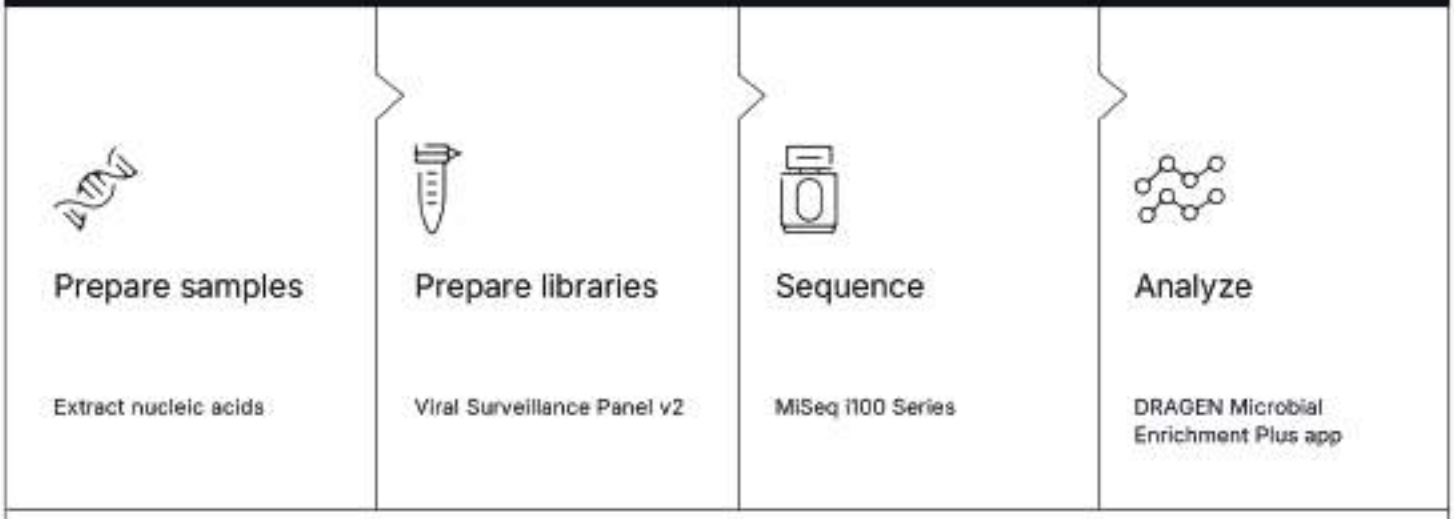
The modern world is witnessing an alarming increase in the threat posed by infectious diseases. Globalization and international travel facilitate the rapid spread of infectious agents across borders.1 Additionally, expanding urbanization and growing population density create ideal conditions for the transmission of infectious agents. Moreover, the emergence of drug-resistant strains of bacteria and viruses poses a significant challenge in the treatment and control of infections.2

Wastewater surveillance is a method for detecting, identifying, tracking, and characterizing potential pathogens to assess the health of a population.3 This method provides data to help monitor outbreaks and serves as an early warning of infectious threats at the community level, as demonstrated through genomic surveillance of SARS-CoV-2.4 By knowing where these threats are, communities can better allocate resources during a public health response. PCR based–methods can provide rapid and relatively low-cost snapshots of the presence or absence of expected pathogens, but their performance for monitoring and detection depends on preexisting knowledge of microorganism sequence variability and of variant determining mutations. Next-generation sequencing (NGS) workflows are relatively more expensive in time and cost, but are more tolerant to mutation, enable variant discovery, and can provide detailed genomic information beyond qualitative detection of pathogens.5–7

Accurate and comprehensive detection of viral pathogens in wastewater depends on both the upfront enrichment of viral genomes by filtration or concentration techniques and NGS library prep enrichment methods to overcome the analytical challenges inherent to studying the relatively small genomes of viruses, the most abundant of which make up only a small proportion of the total genomic material in wastewater.8–10 By increasing the relative abundance of genomic content of interest in the library, enrichment also unlocks the potential for sequencing on benchtop instruments.

This application note demonstrates detection and characterization of viral pathogens in real-world wastewater samples using an NGS workflow that integrates the Illumina Viral Surveillance Panel v2, the MiSeq i100 Series, and onboard DRAGEN™ secondary analysis (Figure 1). The MiSeq i100 Plus System delivers same-day results for efficient wastewater surveillance to enable a rapid public health response.

Figure 1: Comprehensive NGS workflow for wastewater surveillance



Combine Illumina library preparation with fast, simple sequencing on the MiSeq i100 Series and DRAGEN secondary analysis for accurate and efficient wastewater surveillance.

Methods

Samples

Raw wastewater samples were collected from wastewater treatment plants by Wisconsin State Laboratory of Hygiene (WSLH) (n = 12) and from student dormitories by Colorado State University (CSU) (n = 12); both sites are located in the United States. Samples were collected from each site over multiple time points from November 4, 2022 to December 16, 2022. 10–50 ml of sample wastewater was prepared by WSLH by capture and concentration of viruses with Nanotrap Microbiome A Particles (Ceres Nanosciences, Inc., Catalog no. 44202). Nucleic acids were extracted using the Wizard Enviro Total Nucleic Acid Kit (Promega Corporation, Catalog no. A2991). Samples prepared by CSU involved removal of solids via centrifugation at ~2000 × g, followed by capture and concentration of viruses with the CP Select Concentrating Pipette (InnovaPrep, Inc.). Nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Catalog no. 52 9 0 4).

Library preparation

Sequencing-ready libraries were prepared from a maximum volume of 8.5 µl (≥ 100 ng of extracted total nucleic acid (TNA)) input using the Viral Surveillance Panel v2 Kit, Set A (96 samples) (Illumina, Catalog no. 20108081). Libraries were combined into four pools of six libraries each (6-plex) with a loading concentration of 80 pM for sequencing.

Sequencing

Prepared libraries were sequenced on the MiSeq i100 Plus System using a 25M flow cell with a run configuration of 2 × 150 bp. For larger studies, sequencing runs can be scaled up to the NextSeq™ 1000, NextSeq 2000, NovaSeq™ 6000, and NovaSeq X Systems.

Data analysis

After sequencing was complete, data were downsampled to 1M and 4M clusters/fragments per sample using the FASTQ Toolkit App. Downsampled and nondownsampled data was analyzed using the DRAGEN Microbial Enrichment Plus (DME+) app onboard the MiSeq i100 Plus System. The app can also be accessed in the cloud in BaseSpace™ Sequence Hub.

Results

Sequencing metrics

The four wastewater pools were sequenced across four runs on the MiSeq i100 Plus System. All four runs resulted in an average % Q30 greater than 90% and percent reads passing filter (PF) of ~80%, indicating both high-quality reads and consistent instrument loading concentrations. The total number of paired-end (PE) reads obtained exceeds the 50M specification of the flow cell. For all four runs, the combined instrument run time and onboard analysis times were under eight hours (Table 1). This demonstrates that the MiSeq i100 Plus System offers speed and efficiency necessary to provide timely results important for public health monitoring and response.

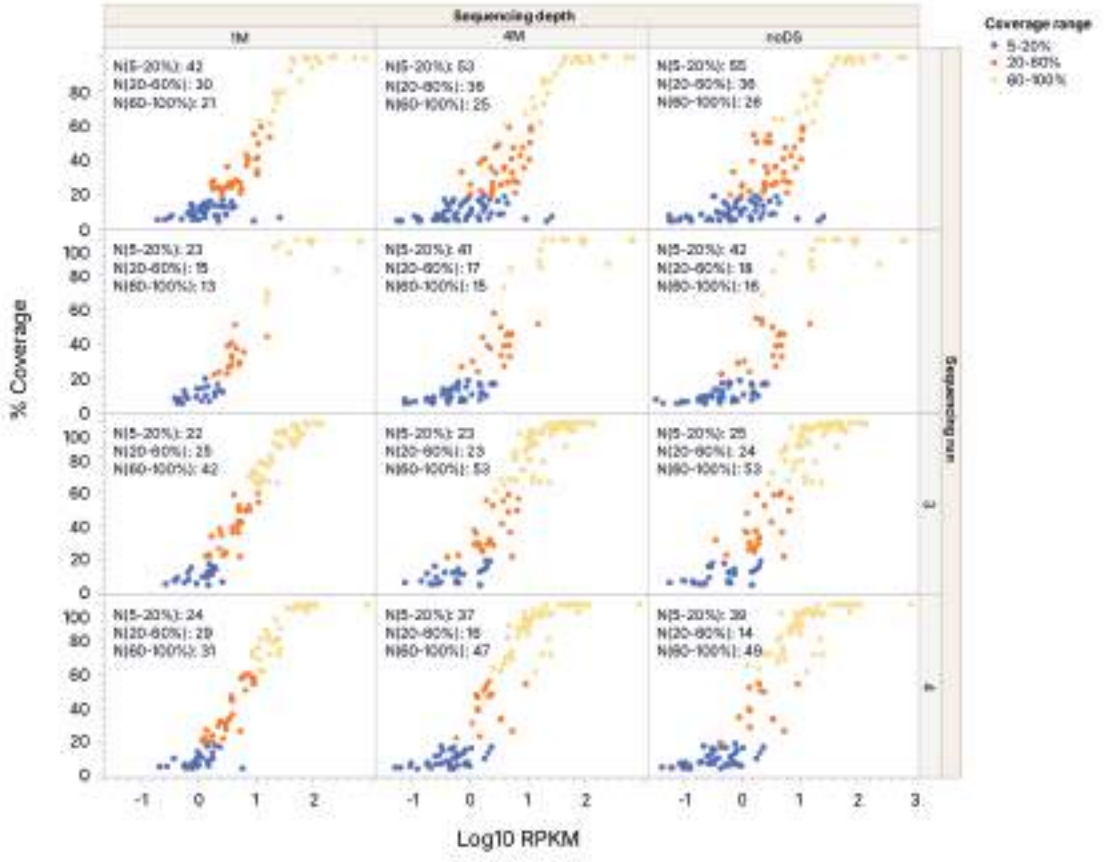
Table 1: Sequencing metrics for the MiSeq i100 Series

MiSeq i100 Plus run	Average % Q30	% PF	Total no. of paired-end reads	Total no. of paired-end reads PF	Run time	Onboard analysis time
Run 1 (CSU)	93.65%	78.49%	79,073,280	62,064,116	7 hr 11 min	18 min
Run 2 (CSU)	93.35%	78 .0 6%	79,073,280	61,723,176	7 hr 12 min	17 min
Run 3 (WSLH)	94.57%	81.62%	79,073,280	64,539,830	7 hr 12 min	19 min
Run 4 (WSLH)	94.38%	81.43%	79,073,280	64,389,944	7 hr 12 min	19 min

Detection of viral genomes

To help establish and compare the amount of sequencing depth needed for effective pathogen detection, analysis of the reads was simulated at various depths. In addition, data normalization was needed for accurate quantification due to the large variation in length of the detected viral genomes. Therefore, the percent of the genomes covered was plotted against the log10 RPKM (Reads Per Kilobase per Million) reads across the four wastewater sample library pools and simulated at three sequencing depths: downsampled to 1M fragments, downsampled to 4M fragments, and full depth (not downsampled or ~5M fragments per sample). RPKM is a common means to normalize NGS data that combines the depth of coverage with the length of the target region. This permits more accurate comparisons across different pathogens, as well as for a single pathogen over time. Coverage of the detected viruses can be segregated into three general ranges, detection (5–20%), surveillance (20–60%), and full genome coverage (60–100%) (Figure 2). Viral pathogens in the lowest range were confidently detected, but lacked sufficient coverage to track viral evolution or accurate variant information. Viral genomes in the mid-range had sufficient coverage for effective molecular characterization and changes in viral concentrations over time. Genomes detected in the highest range were able to be characterized with variant determination. At greater read depths, the total number of viral genomes was higher, but plateaued at around 4M clusters/fragments (8M PE reads) (Figure 2 and Table 2).

Figure 2: Varying genome coverage for viral pathogens detected in wastewater samples



Percent genome coverage was plotted against log10 RPKM over four MiSeq i100 Plus sequencing runs. Data were downsampled to 4M and 1M reads or not downsampled. Genome coverage is segregated into three ranges: detection range (5–20%), surveillance range (20–60%), and full genome coverage range (60–100%). N represents the number of viral genomes detected in each range. Even at a simulated depth of 1M reads per library, coverage of ≥ 60% was achieved for ≥ 22% of detected viral targets in each of the four runs.

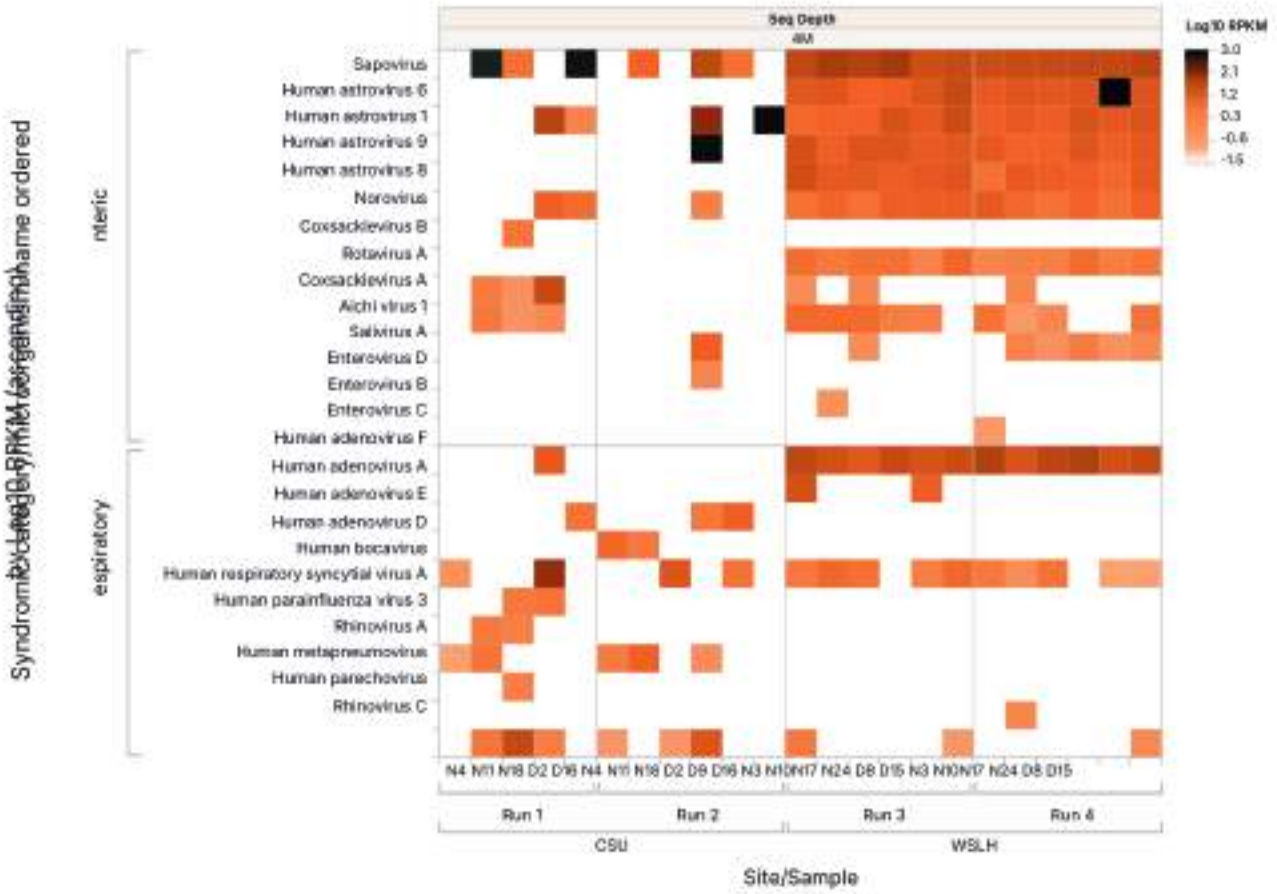
Table 2: Total number of viral genomes detected by site

Site	Genome coverage	Sequencing depth		
		1M reads	4M reads	Full depth
CSU	5–20%	65	94	97
	20 – 60%	45	53	54
	60–100%	34	40	92
WSLH	5–20%	46	60	64
	20 – 60%	54	39	38
	60–100%	73	100	102

Characterization of viral genomes

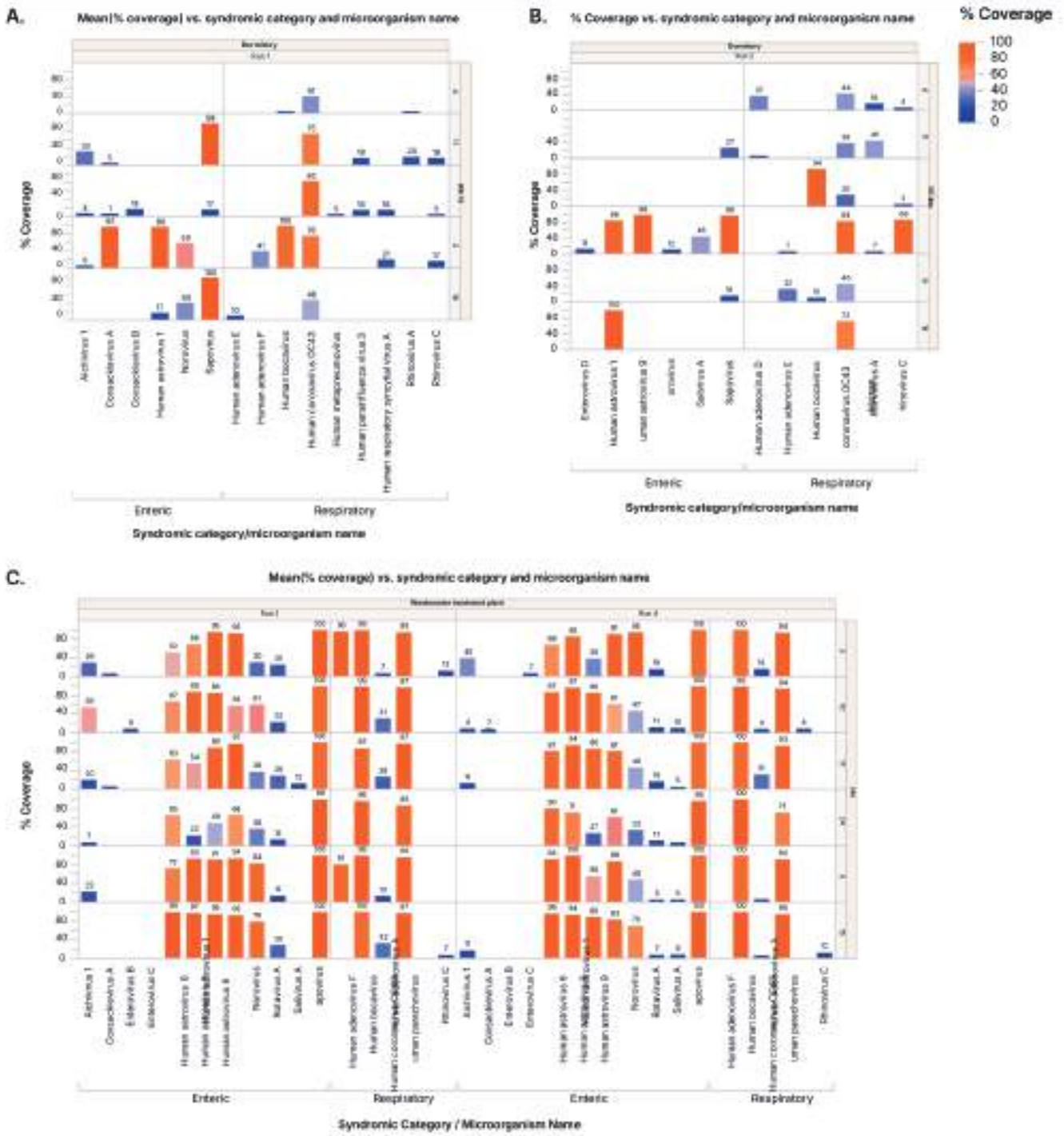
As part of the longitudinal study, detected viral genomes were plotted and arranged with ascending log10 RPKM values in a heat map that showed various levels of detection for enteric and respiratory viruses across collection sites and over time, as measured by relative abundance (Figure 3) and by genome coverage (Figure 4). Plotting genome coverage over time showed increased levels of enteric viruses, including Coxsackie virus A/B, Norovirus, Mamastrovirus (human astrovirus), and Salivirus in December time points (Figure 4), consistent with previous reports detecting high abundance of these and other pathogenic viruses shed in human stool.11

Figure 3: Heat map of viral abundance in wastewater samples



Viral genomes detected from CSU and WSLH samples are arranged with ascending Log10 RPKM values. Viruses corresponding to enteric and respiratory categories are shown in this heat map. WSLH samples (N = 145) showed higher viral genome detection compared to CSU samples (N = 63). Due to the relatively lower number of people contributing to the dorm wastewater samples, fewer pathogen detections are observed compared to samples from the treatment plants. Collection dates in November (N) and December (D) are indicated above each collection site.

Figure 4: Changes in viral pathogens detected over time



Monitoring of viral pathogen levels as part of a longitudinal study showed changes in enteric and respiratory viruses in samples collected from (A) both sites, (B) CSU, and (C) WSLH. Genome coverage is plotted against viruses from enteric and syndromic categories over time (dates indicated on the right side of plot).

Similarly, the respiratory viruses Bocavirus and Rhinovirus A/C showed elevated levels in samples collected in December (Figure 4). These data are consistent with historically observed seasonal increases in transmission of respiratory pathogens in early winter, perhaps accelerated by travel over the Thanksgiving holiday.12

Summar y

The MiSeq i100 Series is part of a fast, comprehensive NGS workflow that enables broad detection of viral pathogens for effective wastewater surveillance as part of public health efforts.

Learn more →

Viral Surveillance Panel v2

MiSeq i100 Series

DRAGEN secondary analysis

References

1. CDC.Disease Patterns in Travelers. cdc.gov/travel/yellowbook/2024/introduction/disease-patterns-in-travelers. Updated May 1, 2023. Accessed February 5, 2025.
2. Reyes R, Ahn R, Thurber K, Burke TF. Urbanization and Infectious Diseases: General Principles, Historical Perspectives, and Contemporary Challenges. Challenges in Infectious Diseases. 2012;123-146. Published 2012 May 19. doi:10.1007/978-1-4614-4496-1_4.
3. CDC. Wastewater Monitoring—How Does It Work? cdc.gov/nwss/pdf/Wastewater-COVID-infographic-h.pdf. Accessed January 24, 2025.
4. Gupta P, Liao S, Ezekiel M, et al. Wastewater Genomic Surveillance Captures Early Detection of Omicron in Utah. *Microbiol Spectr*. 2023;11(3):e0039123. doi:10.1128/spectrum.00391-23.
5. US GAO. Science & Tech Spotlight: Wastewater Surveillance. gao.gov/products/gao-22-105841. Published April 11, 2022. Accessed January 24, 2025.
6. Diamond MB, Keshaviah A, Bento AI, et al. Wastewater surveillance of pathogens can inform public health responses. *Nat Med*. 2022; 28(10):1992-1995. doi:10.1038/s41591-022-01940-x
7. Crits-Christoph A, Kantor RS, Olm MR, et al. Genome Sequencing of Sewage Detects Regionally Prevalent SARS-CoV-2 Variants. mBio. 2021;12(1):e02703-20. Published 2021 Jan 19. doi:10.1128/mBio.02703-20.
8. Dueholm MKD, Nierychlo M, Andersen KS, et al. MiDAS 4: A global catalogue of full-length 16S rRNA gene sequences and taxonomy for studies of bacterial communities in wastewater treatment plants. *Nat Commun*. 2022;13(1):1908. Published 2022 Apr 7. doi:10.1038/s41467-022-29438-7.
9. Kantor RS, Jiang M. Considerations and Opportunities for Probe Capture Enrichment Sequencing of Emerging Viruses from Wastewater. *Environ Sci Technol*. 2024;58(19):8161-8168. doi:10.1021/acs.est.4c02638.
10. Cancela F, Lizasoain A, Panzera Y, et al. Targeted Enrichment Sequencing Utilizing a Respiratory Pathogen Panel for Genomic Wastewater-Based Viral Epidemiology in Uruguay. *Food Environ Virol*. 2025;17(1):14. Published 2025 Jan 9. doi:10.1007/s12560-024-09629-9.
11. Tisza M, Javornik Cregeen S, Avadhanula V, et al. Wastewater sequencing reveals community and variant dynamics of the collective human virome. *Nat Commun*. 2023;14(1):6878. Published 2023 Oct 28. doi:10.1038/s41467-023-42064-1.
12. CDC. Respiratory Illnesses Data Channel. cdc.gov/respiratory-viruses/data/index.html#cdc_data_surveillance_section_2-weekly-national-summary. Updated February 21, 2025. Accessed February 25, 2025.

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M-GL-02915 v1.0

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APPLICATION NOTE

Wastewater surveillance using the Urinary Pathogen ID/AMR Panel

Broad pathogen detection and antimicrobial resistance (AMR) tracking with the MiSeq™ i100 Series

Exceptional breadth and depth of coverage of antimicrobial resistance genes (ARGs) enabled with enrichment with the Urinary Pathogen ID/ AMR Panel

Fast sequencing delivers results in < 12 hr for efficient wastewater surveillance

Simplified analysis with the DRAGEN™ Microbial Enrichment Plus app provides streamlined pathogen detection and characterization

Introduction

Antimicrobial resistance (AMR) has been described as a "silent pandemic."**1** By decreasing the effectiveness of available antimicrobials, AMR has the potential to indiscriminately influence the severity and clinical outcome of infections independent of underlying disease or other risk factors.**1** Expanding urbanization, global travel, and growing population density create ideal conditions for the transmission of infectious agents, and with them, antimicrobial resistance genes (ARGs).**2-3**

Many existing AMR surveillance programs are based on interactions with health care providers and, therefore, cannot effectively capture ongoing transmission of drug-resistant organisms outside of the health care setting. Furthermore, traditional growth-based methods for monitoring drug susceptibility cannot provide genotypic information about the organisms nor the ARGs themselves, or any information about organisms that are not cultivable in the laboratory using standard protocols.**3-4**

Wastewater surveillance includes a laboratory method that can help address these gaps and uncover the circulating "resistome" of a given community, including drug-resistant organisms and associated mobile genetic elements bearing ARGs (eg, plasmids) that may circulate in parallel.**3** Next-generation sequencing (NGS)–based wastewater surveillance of AMR has been shown to correlate with phenotypic resistance data from conventional isolate-based surveillance in various populations and settings.**4** Furthermore, wastewater surveillance can be useful for broad surveillance of known or novel threats that may be transmitted between people, nonhuman populations, such as companion animals or agricultural animals, and their shared environment in a "One Health" approach.**3**

Accurate and comprehensive detection of ARGs and associated bacteria in wastewater depends on the upfront enrichment of relevant genomic content by filtration or concentration techniques. Library preparation enrichment methods can help overcome the sequencing depth otherwise needed to comprehensively profile the resistome in wastewater samples.**5** By increasing the relative abundance of genomic content of interest in the library, enrichment also unlocks the potential for sequencing libraries from a biologically complex sample like wastewater on NGS benchtop instruments.

This application note demonstrates the detection and characterization of ARGs and associated bacterial pathogens in real-world wastewater samples using an NGS workflow that integrates the Urinary Pathogen ID/AMR Enrichment Kit, the MiSeq i100 Plus System, and onboard DRAGEN secondary analysis (Figure 1). The MiSeq i100 Series delivers same-day results for efficient wastewater surveillance with high-resolution data to inform public health response.

Figure 1: Comprehensive NGS workflow for wastewater surveillance

Methods

Samples

Raw wastewater samples were collected from wastewater treatment plants (WWTP) by Wisconsin State Laboratory of Hygiene (WSLH) (n = 27) and from student dormitories by Colorado State University (CSU) (n = 33); both sites are in the United States. Samples were collected from each site over multiple time points from November 4, 2022 to December 16, 2022. Samples from WSLH were prepared from 10–50 ml of sample wastewater by capturing and concentrating the microbes with Nanotrap Microbiome A Particles (Ceres Nanosciences, Inc., Catalog no. 44202). Nucleic acids were extracted using the Wizard Enviro Total Nucleic Acid Kit (Promega Corporation, Catalog no. A2991) according to the manufacturer's instructions. Samples obtained from CSU involved removal of solids via centrifugation at ~2000 × g, followed by capture and concentration of viruses with the CP Select Concentrating Pipette (InnovaPrep, Inc.). Nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Catalog no. 52904) according to the manufacturer's instructions.

Library preparation

Sequencing-ready libraries were prepared from a maximum volume of 30 µl of extracted total nucleic acid (TNA) input using the Urinary Pathogen ID/AMR Enrichment Kit, Set A (RUO) (96 indexes, 96 samples) (Illumina, Catalog no. 20090308). For comparison, shotgun metagenomics libraries were prepared using the same kit, omitting enrichment steps.

Sequencing

Prepared libraries were sequenced on the MiSeq i100 Plus System using a 25M flow cell with a run configuration of 2 × 151 bp.

Data analysis

After sequencing was complete, data were downsampled to 1M (enriched and unenriched) and 4M (unenriched only) clusters/fragments per sample using the FASTQ Toolkit App. Downsampled and nondownsampled data were analyzed using the DRAGEN Microbial Enrichment Plus app in BaseSpace™ Sequence Hub. This app can also be accessed onboard the MiSeq i100 Plus System.

Results

Sequencing metrics

The wastewater libraries were sequenced across three runs on the MiSeq i100 Plus System. Sequencing metrics across the three runs were high with > 94% reads greater than Q30 and of ≥ 87% reads passing filter (PF), indicating both high-quality data and consistent instrument loading concentrations. The total number of paired-end (PE) reads obtained exceeded the 50M specification of the flow cell, resulting in deeper coverage and potentially more confident identification of ARGs and microbes. For all three runs, the combined instrument run time and analysis times were under 10 hours (Table 1).

Table 1: Sequencing metrics for the MiSeq i100 Plus System

Sequencing run	No. of samples	Run time	Analysis time	Total no. PE read PF	% PF	% reads ≥ Q30
Run 1	21	6 hr 48 min	~40–45 min	6 8,795,0 9 6	87.00%	9 6.6 8%
Run 2	21	7 hr 12 min	~40–45 min	68,850,076	87.07%	94.72%
Run 3	18	7 hr 13 min	~40–45 min	70,374,364	8 9.0 0%	94. 20%

Wastewater sample composition

Visual analysis tools within the DRAGEN Microbial Enrichment Plus app facilitate interpretation of enrichment performance at a glance. Sample composition plots show the proportion of targeted, untargeted, ambiguous, and unclassified reads. The percentage of reads in each category represents their proportion relative to the total number of reads in the sample (Figure 2A) or relative to the proportion of reads within the targeted or untargeted categories themselves (Figure 2B). For example, 52.1% of the total reads in the sample are classified as targeted AMR reads, and 33.1% of all the reads in the sample are untargeted bacterial (Figure 2A). In contrast, 98.7% of targeted reads are AMR reads, and 94.4% of all untargeted reads are classified as bacterial reads (Figure 2B).

AMR detection in wastewater samples

The target enrichment design of the Urinary Pathogen ID/AMR panel is highly sensitive, outperforming shotgun metagenomics for identifying genes associated with AMR. The target enrichment approach also allows for greater analytical sensitivity at a lower number of total reads compared to shotgun metagenomics methods (Figure 3). Importantly, the DRAGEN Microbial Enrichment Plus app automatically flags relevant ARGs associated with extended-spectrum β-lactamase (ESBL) and carbapenemase genes. These genes are designated as critical priorities by global health agencies due to their role in multidrug resistance and rapid dissemination.[6-8] This enables users to focus on the most relevant ARGs in large data sets, providing a clear starting point for further analysis.

Analysis of results from sequencing wastewater samples with the Urinary Pathogen ID/AMR panel showed the proportion of AMR genes detected at each collection site, categorized by known association with a family of antimicrobial drugs (Figure 4A). These findings highlight the prevalence of diverse AMR genes in various communities, inclusive of dense human populations (dormitory samples) and a "One Health" sample of a broad community (WWTP samples).

Microbe detection in wastewater samples

Analysis of sequencing results from wastewater samples using the Urinary Pathogen ID/AMR panel revealed the distribution of microbes across two collection sites, including gram-negative bacteria, gram-positive bacteria, and viruses (Figure 5). The majority of reads from both sites were associated with gram-negative bacteria, including typical gut-associated organisms and opportunistic pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Enrichment with the Urinary Pathogen ID/AMR panel also revealed the presence of genomic content from other bacterial and viral organisms. For example, dormitory samples showed a relatively higher proportion of gram-positive bacteria, including *Enterococcus* spp, *Staphylococcus* spp, *Streprococcus* spp, and acid-fast organisms were detected across both sites, although dormitory samples showed a higher proportion (Figure 5).

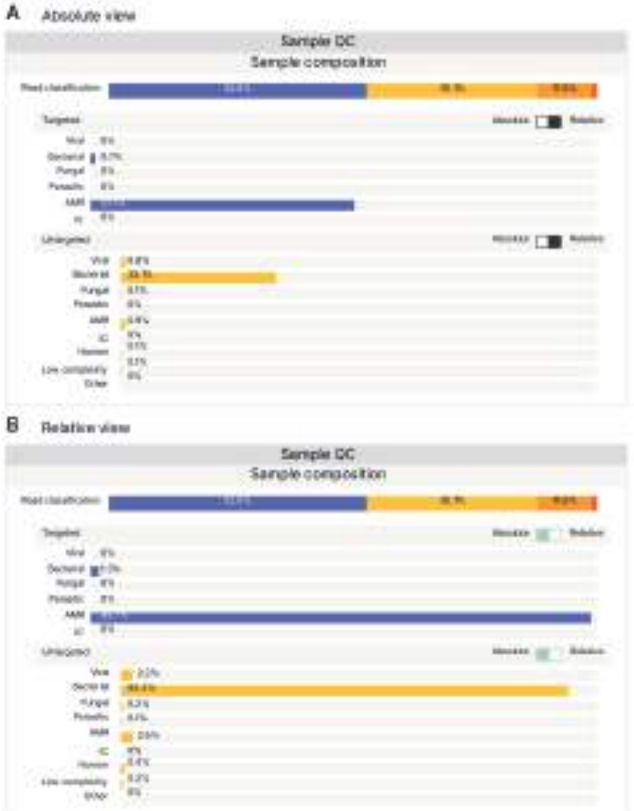

Figure 2: Composition of wastewater samples

Thread composition was breakdown into the following categories: targeted (blue), untargeted (yellow), ambiguous (orange), and unclassified (red). Sample composition displayed as (A) absolute shows the percentage of reads in each category relative to the total number of reads in the sample, and (B) relative shows the percentages relative to the proportion of reads within the targeted or untargeted categories themselves.

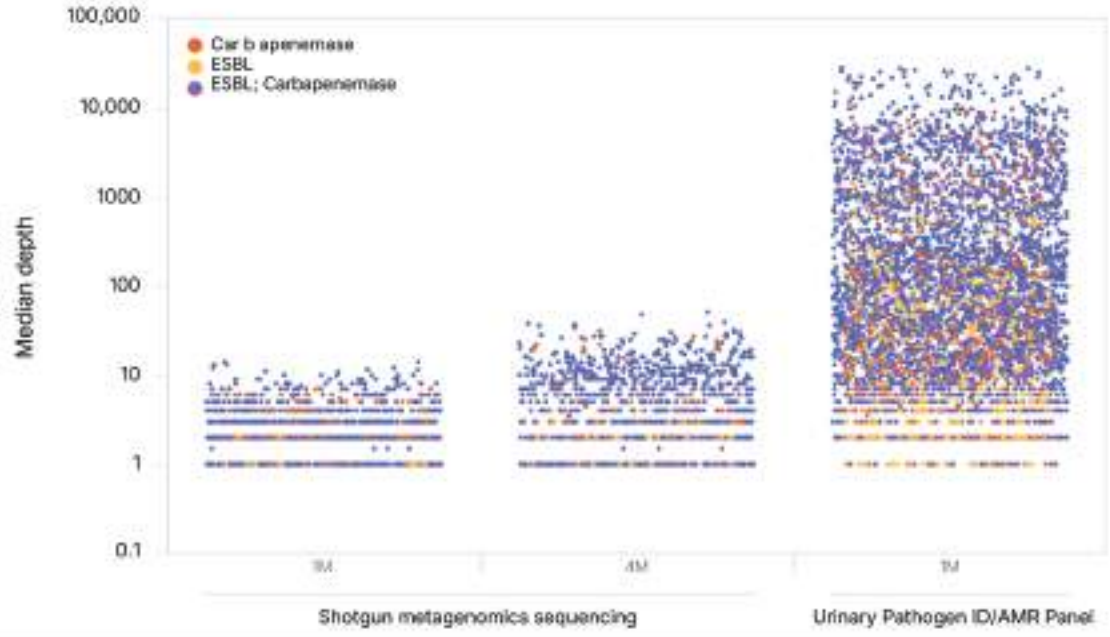

Figure 3: Comparison of AMR gene detection in Urinary Pathogen ID/AMR panel–enriched vs shotgun metagenomics libraries
After libraries were prepared and sequenced on the MiSeq i100 Plus System, the resulting FASTQ files were downsampled to 1M clusters or 2M PE reads (4M clusters or 8M PE reads for shotgun libraries) and analyzed with the DRAGEN Microbial Enrichment Plus app. A significant increase in the total number of ARGs was consistently detected in enriched libraries compared to shotgun libraries, both at comparable depth (1M clusters) and at 4× the sequencing depth for shotgun libraries. Genes associated with carbapenemases and extended-spectrum beta-lactamases (EBSLs) were identified using the analysis software.

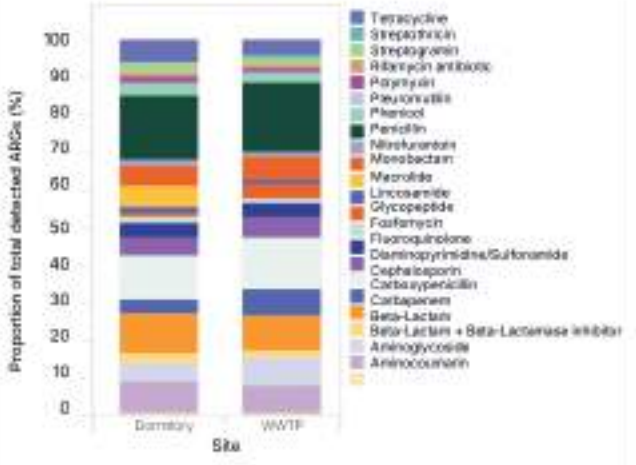

Figure 4: Resistomes of dormitory and WWTP samples
The distribution of AMR genes across 24 drug classes from the two collection sites (dormitory [n=33] and wastewater treatment plant [n=27]) shows the proportion of resistance within each drug class, allowing for a comparative assessment of the resistome between the two sites. DRAGEN Microbial Enrichment Plus reports putative associations between a given AMR marker detection and one or more drug classes based on public metadata.

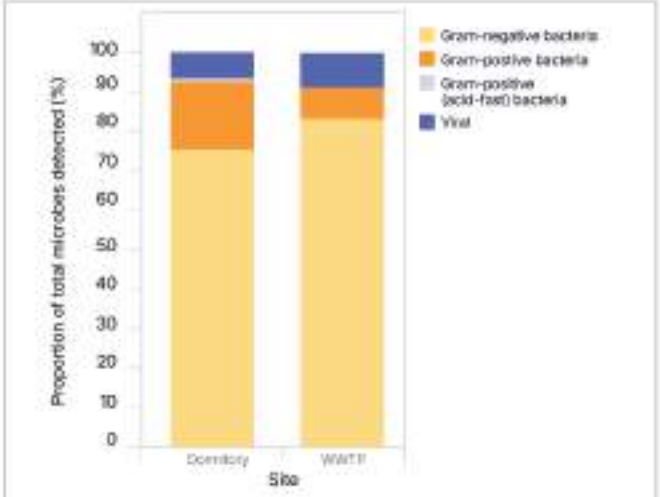

Figure 5: Microbe detection by collection site
The distribution of microbes detected from the two collection sites: dormitory (n=33) and wastewater treatment plant (n=27) highlights the differences in the prevalence of detected microbes at both sites. Samples from the dorms had a larger proportion of gram positive bacteria detected, including some acid fast bacteria. WWTP had more viral detections compared to dorms. Overall, the largest proportion of reads were associated with gram negative bacteria.

Samples from WWTPs exhibited greater viral diversity, likely reflecting the larger and more heterogeneous population from which the samples were collected from. Viral detections at both sites included JC polyomavirus and BK polyomavirus. Additionally, Human papillomavirus (HPV) was identified in the dormitory samples, and Adenovirus E was detected in the WWTP samples. These findings emphasize the focus of the Urinary Pathogen ID/AMR panel on detecting and quantifying bacteria and associated AMR genes relevant for human health, while also highlighting its capacity to capture broader microbial trends in complex sample types.

Summar y

The MiSeq i100 Series combined with the Urinary Pathogen ID/AMR Enrichment Kit provides a fast, comprehensive workflow that enables detection and characterization of ARGs and associated bacterial pathogens directly from samples to support wastewater surveillance as part of public health efforts.

Learn more

MiSeq i100 Series

Urinary Pathogen ID/AMR Enrichment Kit

References

1. Antimicrobial resistance: a silent pandemic. *Nat Commun.* 15, 6198 (2024). https://doi.org/10.1038/s41467-024-50457-z
2. Reyes R, Ahn R, Thurber K, Burke TF. Urbanization and Infectious Diseases: General Principles, Historical Perspectives, and Contemporary Challenges. *Challenges in Infectious Diseases.* 2012;123-146. Published 2012 May 19. doi:10.1007/978-1-4614-4496-1_4
3. Vassallo A, Kett S, Purchase D, Marvasi M. The Bacterial Urban Resistome: Recent Advances. *Antibiotics (Basel).* 2022;11(4):512. Published 2022 Apr 12. doi:10.3390/antibiotics11040512
4. Chau KK, Barker L, Budgell EP, et al. Systematic review of wastewater surveillance of antimicrobial resistance in human populations. *Environ Int.* 2022;162:107171. doi:10.1016/j.envint.2022.107171
5. Baba H, Kuroda M, Sekizuka T, Kanamori H. Highly sensitive detection of antimicrobial resistance genes in hospital wastewater using the multiplex hybrid capture target enrichment. *mSphere.* 2023;8(4):e0010023. doi:10.1128/msphere.00100-23
6. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Published 2017. Accessed April 22, 2025.
7. Berendonk TU, Manaia CM, Merlin C, et al. Tackling antibiotic resistance: the environmental framework. *Nat Rev Microbiol.* 2015;13(5):310-317. doi:10.1038/nrmicro3439
8. Kizny Gordon AE, Mathers AJ, Cheong EYL, et al. The Hospital Water Environment as a Reservoir for Carbapenem-Resistant Organisms Causing Hospital-Acquired Infections-A Systematic Review of the Literature [published correction appears in *Clin Infect Dis.* 2017 Oct 15;65(8):1431-1433. doi: 10.1093/cid/cix563.]. *Clin Infect Dis.* 2017;64(10):1435-1444. doi:10.1093/cid/cix132

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