



An Integrated Approach for Wastewater Surveillance

Pathogen Detection, AMR Monitoring, and Functional Analysis in Wastewater

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INTRODUCTION

Wastewater surveillance has emerged as a pivotal tool in public health epidemiology. Particularly catalyzed by the Covid-19 pandemic, modern culture-independent sequencing methods have become indispensable due to their ability to offer a comprehensive perspective. In this study, we present an innovative, integrated approach for simultaneous pathogen detection, antimicrobial resistance (AMR) monitoring, and functional analysis within wastewater systems. By harnessing advanced sample preparation technologies provided by Zymo Research and leveraging PacBio Onso™ short-read sequencing, our objective is to elevate our comprehension of microbial dynamics within wastewater environments.

METHODS

To evaluate the efficacy of Zymo Research's nucleic acid extraction technology, we conducted a study using real-world wastewater samples. Using Zymo Research's *Quick-DNA/RNA*™ Water kit, we processed these samples then analyzed the samples using the latest sequencing methodologies tailored for wastewater surveillance.

Sample Information

Wastewater samples pose significant challenges for

nucleic acid extraction, primarily due to their high turbidity and the abundance of PCR inhibitors. These samples have a diverse array of microbes, including viruses, bacteria, and fungi, all crucial targets for comprehensive wastewater surveillance. Notably, for effective pathogen detection, processing large volumes (>30 mL) is imperative, a task often daunting for conventional extraction kits. Here, 30 mL of wastewater (Samples 1 & 2) and up to 250 µl of primary sludge (Sample 3) were processed with the *Quick-DNA/RNA*™ Water Kit. (Table 1)

DNA/RNA Extraction

DNA/RNA extraction serves as the foundational step for all downstream molecular analysis applications. The quality of the extracted nucleic acid is critical to ensuring accurate analysis results. Zymo Research's *Quick-DNA/RNA*™ Water Kit offers a solution that delivers high-quality DNA/RNA eluates free of inhibitors, ready for next-generation sequencing (NGS) and polymerase chain reaction (PCR). Notably, the kit features an innovative concentration buffer and inhibitor removal technology that enables the concentration of microbes from large volume water samples and removes PCR inhibitors yielding ultra-pure DNA.

Sample ID	Sampling site	Description
Sample 1	Influent from wastewater treatment plant 1	The raw wastewater without treatment
Sample 2	Influent from wastewater treatment plant 2	The raw wastewater without treatment
Sample 3	Primary Sludge	The sediment from primary treatment process

Table 1. Sample Information

Next Generation Sequencing (NGS) Platforms:

Illumina NextSeq 2000™:

The Illumina NextSeq 2000™ platform is a widely utilized system for wastewater and environmental surveillance, particularly for pathogen detection and the identification of antimicrobial resistance (AMR) and mobile genetic elements (MGEs). Illumina sequencing employs a robust technology that utilizes reversible terminator chemistry, allowing for accurate and high-throughput sequencing of DNA fragments. Its versatility and scalability make it suitable for a wide range of applications, including metagenomic analysis and microbial community profiling.

PacBio Onso™ Short-Read Sequencing System:

The PacBio Onso™ short-read sequencing system utilizes sequencing by binding (SBB) technology. PacBio sequencing achieves high accuracy (Q40+) by measuring light signals emitted from fluorescently labeled nucleotides when they are bound, but not incorporated, by a polymerase on a DNA strand. This technology offers advantages in analyzing complex genomic regions, such as repetitive sequences and structural variations, making it valuable for comprehensive genomic characterization.

Bioinformatics Analysis

We downsampled Onso data from 130 M to 8 M to generate the same data size with NextSeq, then utilized the advanced bioinformatics pipeline provided by [Zymo Research Microbiome Sequencing Services](#) to generate a comprehensive wastewater report. These reports highlighted key functional bacterial groups vital for water monitoring plants. Specifically, our analysis focused on nitrogen removal bacteria, essential for effective wastewater treatment, and methyloprophs, pivotal in the anaerobic digestion of sludge.

RESULTS

All data was generated and provided by [Zymo Research Sequencing Services](#).

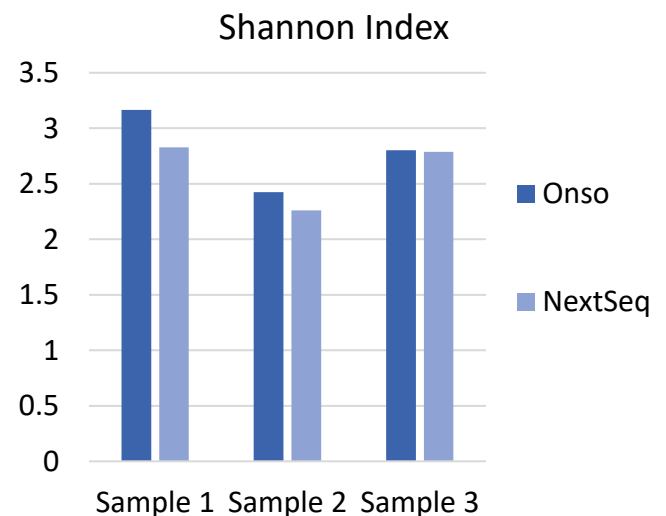


Figure 1. Shannon index for genus level microbial community analysis

A comparison of Shannon index values and read number of AMR class for the three samples are shown in Figure 1 and 2, respectively, for data generated using PacBio Onso™ and Illumina NextSeq 2000™.

Sample 1	Onso	NextSeq	Sample 2	Onso	NextSeq	Sample 3	Onso	NextSeq	Key
aminoglycoside	153	51	aminoglycoside	27	2	aminoglycoside	60	6	0
beta-lactamase	299	4	beta-lactamase	79	3	beta-lactamase	72	3	5
disinfecting agents	31	0	bicyclomycin-like	7	0	bicyclomycin-like	2	0	10
glycopeptide	9	0	disinfecting agents	27	0	disinfecting agents	1	0	100
lincosamide	54	6	fluoroquinolone	2	0	fluoroquinolone	8	0	1000
macrolide	512	13	lincosamide	1	1	glycopeptide	31	0	
multidrug	69	2	macrolide	93	0	lincosamide	25	2	
mupirocin-like	3	0	multidrug	26	0	macrolide	336	6	
nitroimidazole	1	0	peptide	1	0	multidrug	374	2	
peptide	14	0	phenicol	9	0	mupirocin	4	0	
phenicol	15	0	quinolone	1	0	nitroimidazole	1	0	
rifampin	5	0	rifampin	5	0	nucleoside	1	0	
streptogramin	48	0	streptogramin	4	0	phenicol	22	4	
sulfonamide	45	0	sulfonamide	103	0	rifampin	1	0	
tetracycline	194	6	tetracycline	53	1	streptogramin	38	0	
trimethoprim	2	0	trimethoprim	2	0	sulfonamide	8	0	
						tetracycline	224	2	
						trimethoprim	1	0	

Figure 2. Read number assigned to AMR class in 2 sequencing systems.

[Zymo Research Microbiome Sequencing Services](#) also provided a customized data report for the read number assigned to specific bacterial groups that are significant for wastewater monitoring. (Figure 3 & 4) Read number for data generated using PacBio Onso™ and Illumina NextSeq 2000™ sequencing methods are compared.

	Methylotrophs					
	Sample 1		Sample 2		Sample 3	
	Onso	NextSeq	Onso	NextSeq	Onso	NextSeq
Paracoccus	0	247568	18896	0	95976	0
Piscinibacter	40024	0	0	107488	0	0
Hyphomicrobium	81800	19776	0	0	0	68424
Methyloversatilis	111016	0	0	0	0	580904
Methylotenera	0	0	0	0	0	0
Methanosarcina	47064	0	0	0	339968	677248

Figure 3. Read number assigned to methylotrophs in 2 sequencing systems.

Nitrogen Removal Bacteria

	Sample 1		Sample 2		Sample 3	
	Onso	NextSeq	Onso	NextSeq	Onso	NextSeq
Zoogloea	317360	15296	38512	0	96696	0
Terrimonas	0	0	0	0	0	0
Thauera	31296	0	785192	0	186672	99552
Acidovorax	0	0	158784	241464	183232	17688
Hydrogenophaga	44104	0	0	0	10968	75064
Mesorhizobium	0	247568	0	0	0	0
Paracoccus	0	0	18896	0	95976	0
Novosphingobium	0	0	0	0	0	0
Pseudomonas	0	0	476832	0	372392	0
Hyphomicrobium	81800	19776	0	0	0	68424
Azoarcus	0	0	0	0	0	0

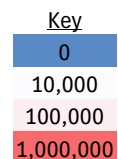


Figure 4. Read number assigned to nitrogen removal bacteria in 2 sequencing systems.

Conclusion

This study underscores the effectiveness of the *Quick-DNA/RNA™* Water Kit in conjunction with next-generation sequencing (NGS) for wastewater surveillance, offering crucial insights into microbial dynamics and antibiotic resistance patterns. By employing two sequencing technologies and analyzing microbial taxa, functional groups, and antibiotic resistance genes (ARGs) in real-world wastewater samples, notable insights have emerged.

The PacBio Onso™ system emerges as a standout technology boasting high-throughput short-read sequencing capabilities that provide detailed and precise microbial community structures, antibiotics resistance profiles, and functional bacteria detection. This precision equips researchers with the

tools necessary for predicting disease outbreaks and optimizing both public health management strategies and water treatment processes, thereby bolstering environmental and public health outcomes.

Despite lower accuracy and read counts compared to the PacBio Onso™ system, the Illumina NextSeq 2000™ system offers unparalleled speed, enabling real-time transmission tracking—a vital asset in scenarios where rapid response is paramount.

In essence, the integration of the *Quick-DNA/RNA™* Water Kit with NGS technologies holds immense promise for advancing wastewater surveillance practices.

Evaluate the *Quick-DNA/RNA™* Water Kit:

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