Sample prep

Microbiome characterization using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit and sequencing

Summary

The MagMAX Wastewater Ultra Nucleic Acid Isolation Kit isolates high-quality nucleic acid suitable for whole-genome and 16S rRNA gene sequencing.

Introduction

Wastewater testing is an important tool for public health and industrial monitoring programs [1]. However, wastewater samples consist of a complex matrix with high concentrations of organic compounds that are inhibitory to nucleic acid extraction and downstream processing. Hence, it is important to eliminate these contaminants during the extraction process to make samples amenable to downstream applications such as sequencing and quantitative PCR (qPCR). We have demonstrated that the Applied Biosystems™ MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit efficiently isolates nucleic acid, offering multiple workflow options for 200 µL to 500 mL of wastewater and 200 mg of sludge samples [2]. Advances in sequencing technologies allow for comprehensive identification of microbial populations in wastewater. We utilized whole-genome sequencing (WGS) to detect abundant bacteria, archaea, viruses, and eukaryotes, as well as 16S rRNA gene sequencing to identify the most prevalent microorganisms present in wastewater samples.

Methods

Sample extraction

Raw wastewater samples that have not undergone any treatment were collected from two wastewater reclamation facilities in northern Georgia, USA. Upon receipt, the wastewater samples were heat-inactivated and stored at 4°C prior to processing. For processing of 10 mL samples, wastewater specimens (starting volume of 15 mL) were vortexed at high speed and centrifuged for 10–15 min at 10,000 x *g*; then 10 mL of debris-free supernatant was divided equally between two 24 deep-well plates. The samples could then be processed on a Thermo Scientific™ KingFisher™ Flex, Apex, or Duo Prime Purification System (Figure 1). The scripts for 10 mL wastewater samples specify combining the divided samples from the two plates and eluting them into a single elution plate with the lysis buffer from the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, 500 µL per well. Proteinase K, binding buffer, and magnetic beads were added to the collected eluate, and total nucleic acid was purified using the MagMAX Wastewater Ultra kit on one of the KingFisher instruments (Figure 1). For 200 µL and 1 mL of wastewater, the samples were processed directly using 200 µL and 1 mL, respectively, of the lysis buffer from the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit. However, the workflows recommend preconcentrating these samples

by an ultracentrifugation or precipitation method of the user's choice. The Qiagen RNeasy™ PowerMicrobiome™ Kit was used to compare the performance with the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit. The Qiagen kit, a spin column–based isolation kit, was used to process 200 µL wastewater samples according to the kit instructions.

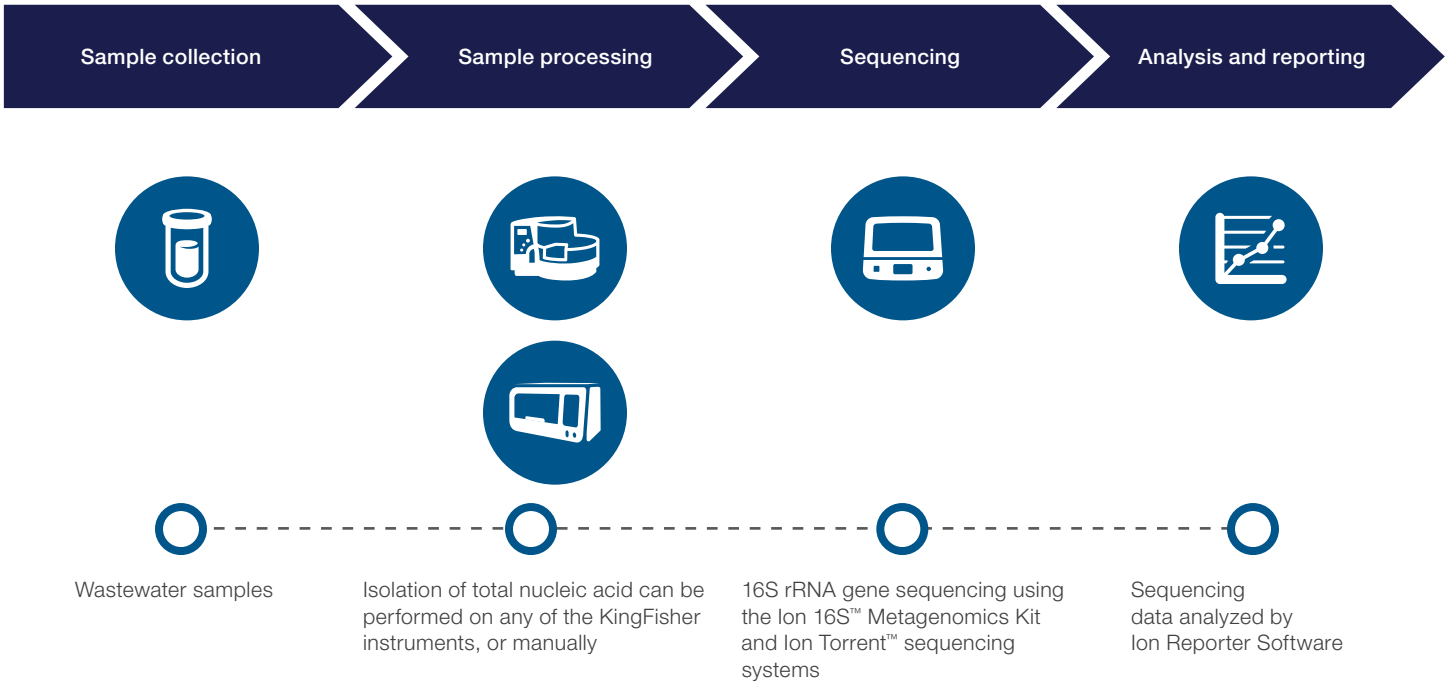

Figure 1. Simplified workflow for wastewater analysis.

Whole-genome sequencing (WGS) and 16S rRNA gene sequencing of wastewater

WGS was performed by an external vendor, and a taxon abundance heat map was generated with hierarchical clustering for 16S rRNA gene sequencing. Library creation, sequencing, and analysis were performed following nucleic acid extraction, to identify the microbial population (Figure 2). The Ion Plus™ Fragment Library Kit and the Ion 16S Metagenomics Kit were used to synthesize 16S rRNA gene libraries on the Ion Chef™ Instrument. The barcoded libraries were pooled and templated on the Ion Chef Instrument, then sequenced on the Ion GeneStudio™ S5 System. Automated analysis, annotation, and taxonomic assignment were performed using Ion Reporter™ Software. Heat maps were generated using RStudio™ software.

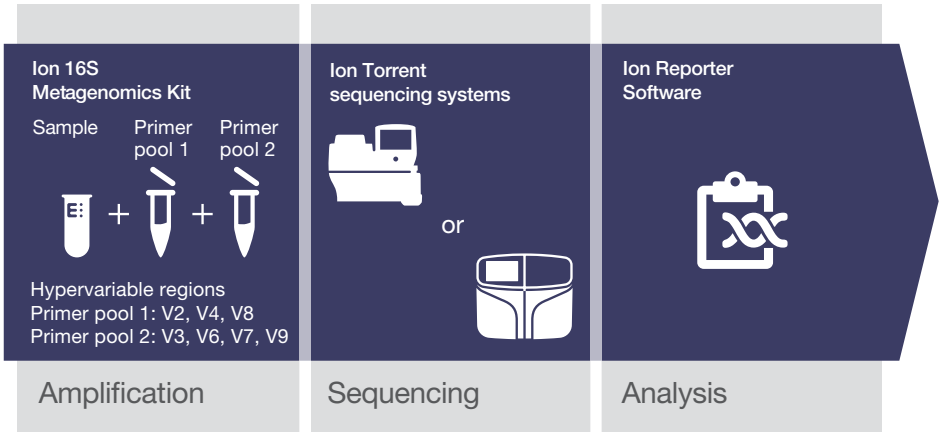

Figure 2. Simplified workflow of 16S rRNA gene sequencing of organisms in wastewater samples: library creation, sequencing, and analysis.

Results

The relative abundance of dominant taxonomic groups detected by WGS of wastewater samples for the 1 mL workflow is shown in a heat map (Figure 3). The most abundant phylum detected with WGS was Proteobacteria, which is consistent with previous studies [3,4]. Two independent samples showed similar results, indicating the consistency of the preparations using the MagMAX Wastewater Ultra kit.

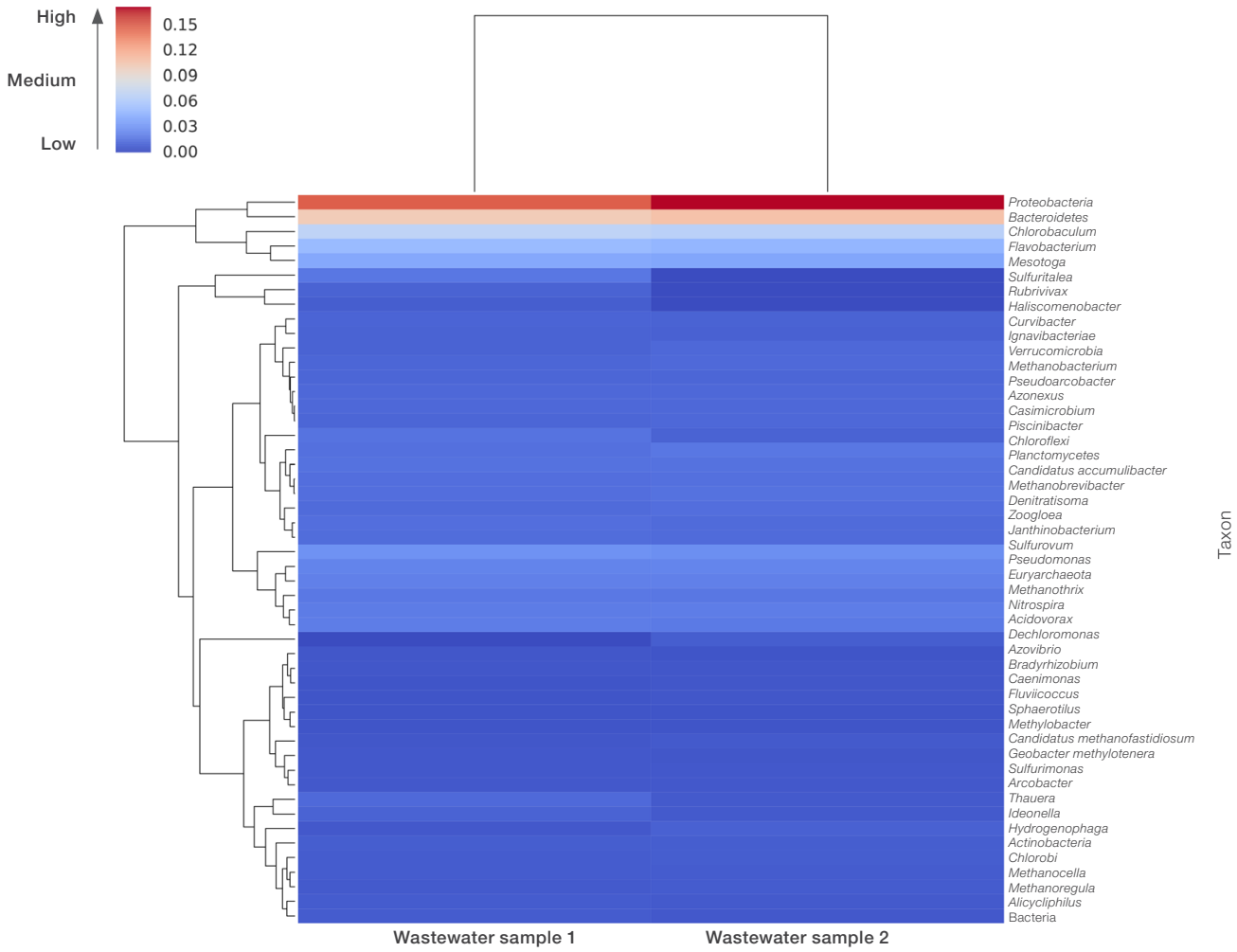

Figure 3. WGS of wastewater samples showing taxonomic abundance of microbial distribution at the phylum and genus levels. A color scale, shown at top left, indicates the abundance levels of targets.

16S rRNA amplicon sequencing shows the relative abundance of dominant families consistently detected across all the workflow options using different volumes of wastewater samples (Figure 4). The 10 mL workflow shows slightly better recovery of the microbial population than the 200 µL and 1 mL direct workflows, as a higher volume of wastewater was processed with 10 mL. We also compared the 200 µL workflow of the MagMAX Wastewater Ultra kit to the spin column–based extraction workflow of the Qiagen RNeasy PowerMicrobiome Kit. The 16S rRNA amplicon sequencing on eluates of both the RNeasy and MagMAX kits shows similar recovery of an abundant microbial population in wastewater across both methods (Figure 5). Predominant bacterial families detected by 16S rRNA gene sequencing include Campylobacteraceae, Comamonadaceae, Flavobacteriaceae, Moraxellaceae, Rhodocyclaceae, and Streptococcaceae. In general, WGS offers insight into a total microbial community, including viruses and fungi, while 16S rRNA gene sequencing identifies the bacteria present in the microbial community.

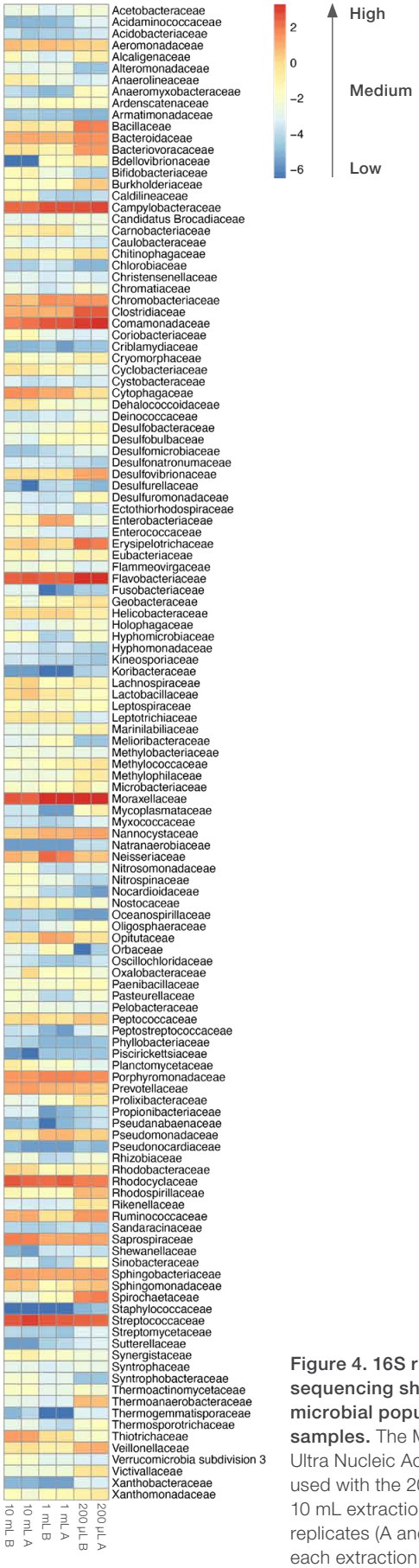

Figure 4. 16S rRNA gene sequencing showing an abundant microbial population in wastewater samples. The MagMAX Wastewater Ultra Nucleic Acid Isolation Kit was used with the 200 µL, 1 mL, and 10 mL extraction workflows. Two replicates (A and B) were used for each extraction workflow. The color scale (log values) indicates the relative abundance of targets.

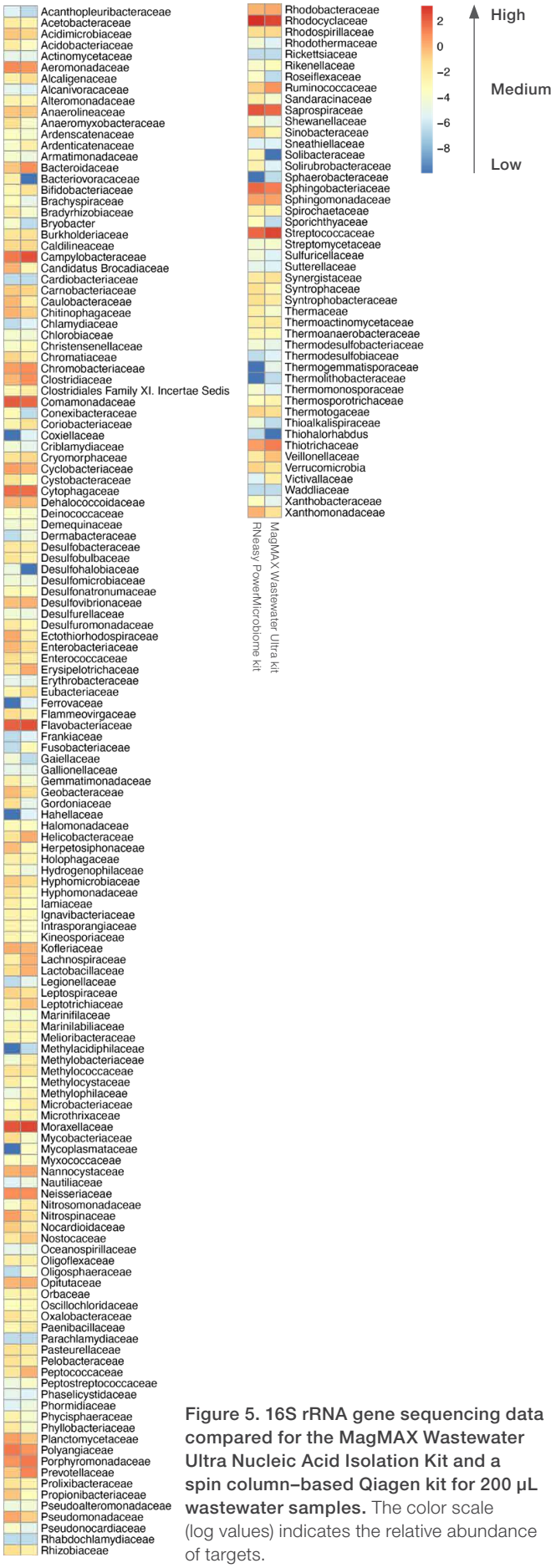

Figure 5. 16S rRNA gene sequencing data compared for the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit and a spin column–based Qiagen kit for 200 µL wastewater samples. The color scale (log values) indicates the relative abundance of targets.

Conclusion

We have shown previously that the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit is able to efficiently isolate total nucleic acid from wastewater samples [2]. Furthermore, the isolated nucleic acid is of sufficient quality to be used for sequencing applications. Here, we have shown successful sequencing (both WGS and 16S rRNA gene sequencing) of total nucleic acid isolated from wastewater. In addition, the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit shows results for 16S rRNA sequencing comparable to those of the Qiagen kit, which includes bead beating in the workflow to isolate gram-positive bacterial targets. The MagMAX Wastewater Ultra Nucleic Acid Isolation Kit eliminates the bead-beating step during sample processing and reduces hands-on time.

References

1. Sims N, Kasprzyk-Hordern B (2020) Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level. *Environment International* 139. doi:10.1016/j.envint.2020.105689.
2. Thermo Fisher Scientific (2021) Multiple workflow options for detection of SARS-CoV-2 in wastewater samples. (Application note COL34431)
3. Zhang T et al. (2012) 454 pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. *ISME J* 6:1137-1147. doi:10.1038/ismej.2011.188.
4. Xue J et al. (2019) Assessing the spatial and temporal variability of bacterial communities in two Bardenpho wastewater treatment systems via Illumina MiSeq sequencing. *Sci Total Environ* 657:1543-1552. doi:10.1016/j.scitotenv.2018.12.141.

Ordering information

Description	Quantity	Cat. No.
MagMAX Wastewater Ultra Nucleic Acid Isolation Kit	100 preps	A52606
MagMAX Wastewater Ultra Nucleic Acid Isolation Kit with Virus Enrichment	100 preps	A52610
KingFisher Flex Purification System KingFisher Apex Purification System KingFisher Duo Prime Purification System	1 instrument	Go to thermofisher.com/kingfisher
Ion Plus Fragment Library Kit	10 reactions	4471252
Ion 16S Metagenomics Kit	100 reactions	A26216
Ion Chef Instrument	1 instrument	4484177
Ion GeneStudio S5 System	1 instrument	A38194

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