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Comparison of Nucleic Acid Extraction Kits for Detecting Pathogens in Spiked Human Serum

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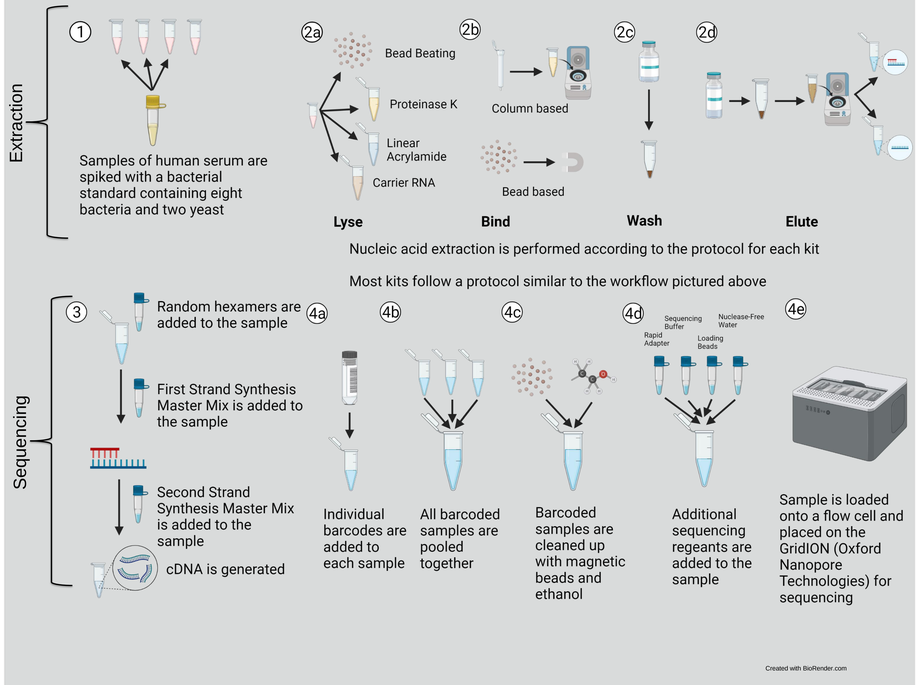
Background and Introduction

- Successful detection of pathogens depends first on the successful extraction of nucleic acids from the original sample[1]
- Many commercial extraction kits exist with varying costs, required materials, and processing costs
- High-throughput DNA sequencing has become a standard technique to characterize microbial communities[2]

Purpose

- Compare variations of commercial nucleic acid extraction kits to determine the most ideal protocol for future applications
- Consider which kits would be most effective for communities of varying resource availability

Methods



Mechanical and enzyme-based lysis techniques can increase the number of sequencing reads and increase the likelihood of detecting hard-to-lyse pathogens

Results

	Column-Based																	Bead-Based			Battery		Precipitation	
	Zymo DNA Mini		Zymo DNA/RNA Mini		Zymo Quick DNA/RNA Pathogen Mini		QIAamp Viral RNA Mini			QIAamp DNA Mini	QIAamp Cador Pathogen Mini							MagMax Viral/Pathogen	MagMax Microbiome		OmniLyse	PureLyse	Lucigen MasterPure	
	No Bead Beat	Bead Beat	No Bead Beat	Bead Beat	No Pro K	Pro K	Carrier	LA	No Carrier or LA	Gram Positive	Pretreatment and Carrier	Pretreatment and LA	Pretreatment	Carrier	LA	No Pretreatment, Carrier, or LA	Pro K	No Pro K	Pro K	No Pro K	No Pro K	No Pro K	Pro K
Gram Negative																								
Gram Positive																								
Yeast																								

Figure 1. Average number of sequencing reads for different categories of pathogens (per 100,000 total reads) detected by each kit

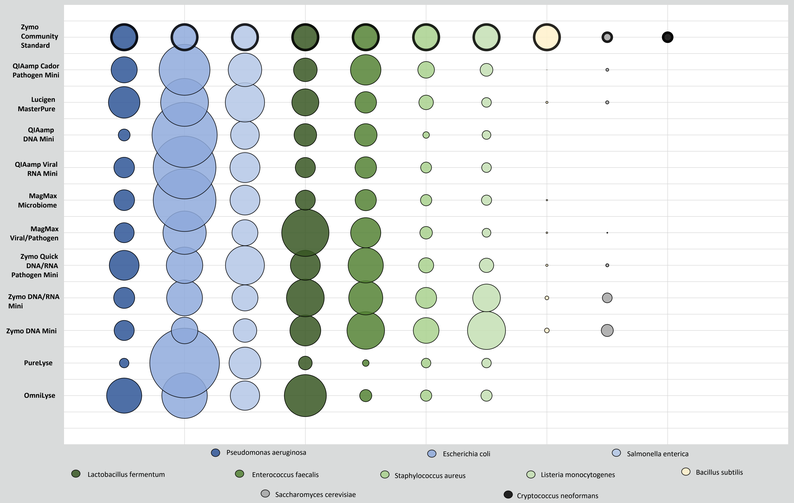


Figure 2. Proportions of pathogens present in the ZymoBIOMICS Microbial Community Standard as detected by each kit

Conclusions

- Protocols including bead beating, addition of Proteinase K, or addition of Carrier RNA typically resulted in a greater number of reads and more sensitive detection of yeast
- Kits from ZymoBIOMICS tend to result in proportions which are most like those of the ZymoBIOMICS Microbial Community Standard
- MagMax Viral/Pathogen kit could be an effective option for low-resource environments, as bead-based technology does not require a centrifuge
- OmniLyse kit could be an acceptable option for environments with very minimal resources, since it utilizes a battery pack rather than external power sources
- Would be beneficial in the future to also test the kits' abilities to detect viral pathogens

Acknowledgements

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References

1. Queipo-Ortuño, M. I., Tena, F., Colmenero, J. D., & Morata, P. (2008). Comparison of seven commercial DNA extraction kits for the recovery of Brucella DNA from spiked human serum samples using real-time PCR. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology, 27(2), 109–114. https://doi.org/10.1007/s10096-007-0409-y
2. Evans, J. S., López-Legentil, S., & Erwin, P. M. (2018). Comparing Two Common DNA Extraction Kits for the Characterization of Symbiotic Microbial Communities from Ascidian Tissue. Microbes and environments, 33(4), 435–439. https://doi.org/10.1264/jsme2.ME18031