

Across the “Sexome”: Understanding Genital Microbiomes

Julia Wang, MS¹; Grace Rutledge, MS¹; Rachel Houston, PhD¹; Ruby Dixon, BSc²; Brendan Chapman, BSc²; Sheree Hughes, PhD¹

¹Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

²Medical, Molecular and Forensic Science, Murdoch University, Perth, Australia



MU Murdoch University



MEMBER THE TEXAS STATE UNIVERSITY SYSTEM

ABSTRACT

Conventional methods for testing sexual assault evidence are based on preferential lysis during DNA extraction and rely on the presence of spermatozoa to identify perpetrators. Low concentrations of male DNA and mixtures of perpetrator and victim DNA complicate STR profile interpretation. However, in some cases no spermatozoa at all are present in evidence samples. In recent years, the “Sexome”, or genital microbiome, has gained particular attention in hopes of filling this gap left by spermatozoa-focused analysis methods^{1,2}.

Of the five methods tested in this study, the Invitrogen™ PureLink™ Microbiome DNA Purification kit was found to recover the most representative microbial communities. The microbiome genera of the penile swabs were found to be more diverse than the vaginal swabs. Unique genera were present in samples from most donors, highlighting the variability in microbiome composition and potential for use in forensic investigations.

INTRODUCTION

Forensic DNA laboratories routinely process sexual assault kit evidence that rely heavily on the presence of spermatozoa for screening and identifying potential male perpetrators. However, these methods are rendered ineffective in the absence of spermatozoa. With the growing accessibility of next generation sequencing technologies and a better understanding of the human microbiome, the analysis of genital microbiota may be an appealing alternative.

This study investigates alternative approaches to evaluate the applicability of bacterial 16S rRNA sequencing in sexual assault evidence analysis. The primary aim was to identify which commercially available kit for microbial DNA extraction and purification would obtain the most comprehensive and representative microbiota from representative skin and vaginal microbial communities.

The most effective DNA extraction kit was then used to recover microbial populations from authentic vaginal and penile swabs and report the variability observed in microbial populations and abundance.

ACKNOWLEDGEMENTS

Samples were collected from living donors using Sam Houston State University Institutional Review Board approved protocol 2020-166.

RESULTS & DISCUSSION

Average Relative Abundance of Genera Recovered from Mock Vaginal Microbial Communities

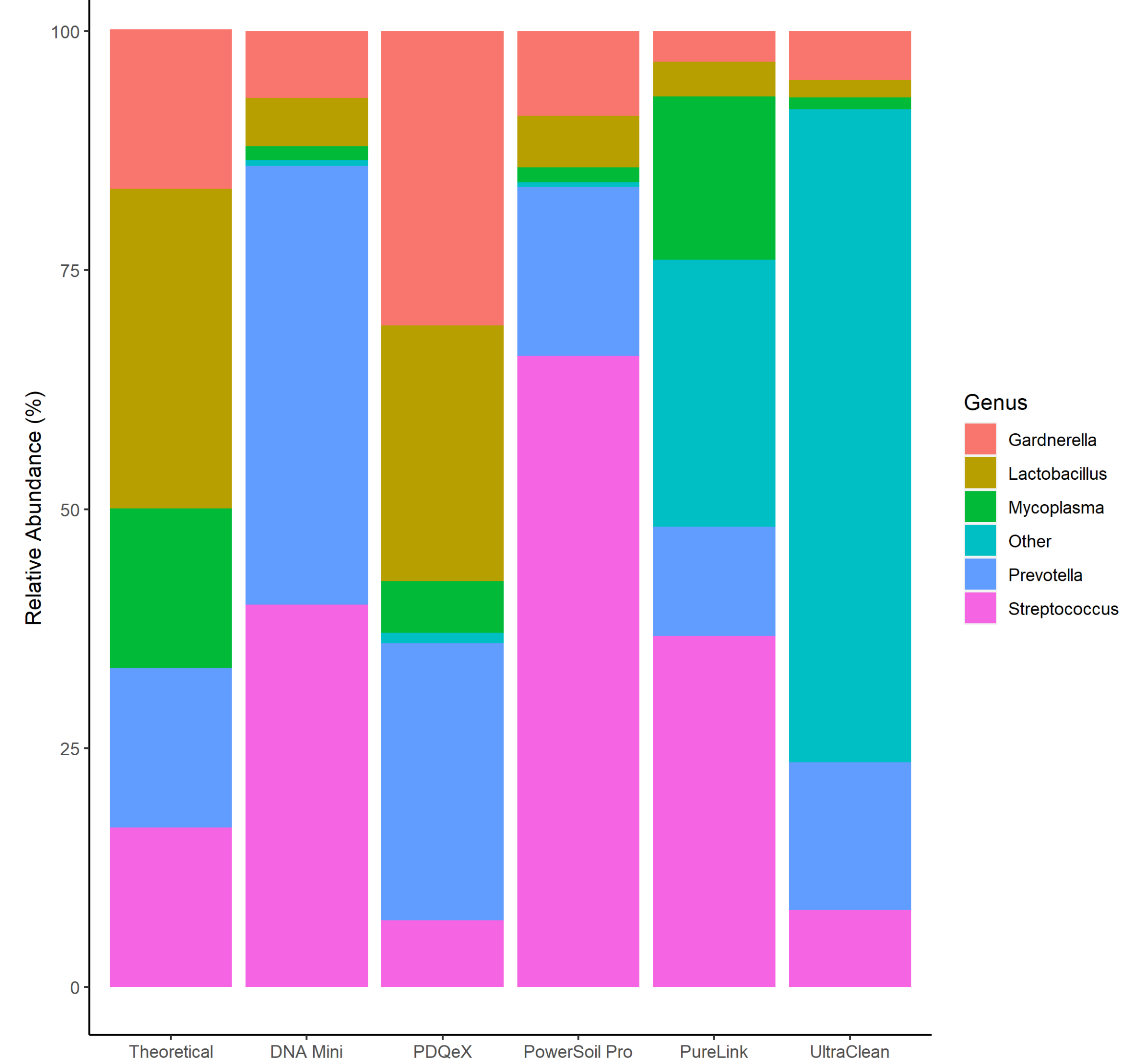


Figure 1. Relative microbial abundance recovered from mock vaginal microbial communities. All five expected mock vaginal genera were effectively extracted by all kits. The highest level of contamination was noted in samples extracted using the DNeasy® Ultraclean® kit. Samples subjected to extraction with the DNeasy® PowerSoil® Pro and QIAamp® DNA Mini kits displayed the most substantial disparities between the observed and expected abundance across the genera.

Average Relative Abundance of Genera Recovered from Mock Penile Microbial Communities

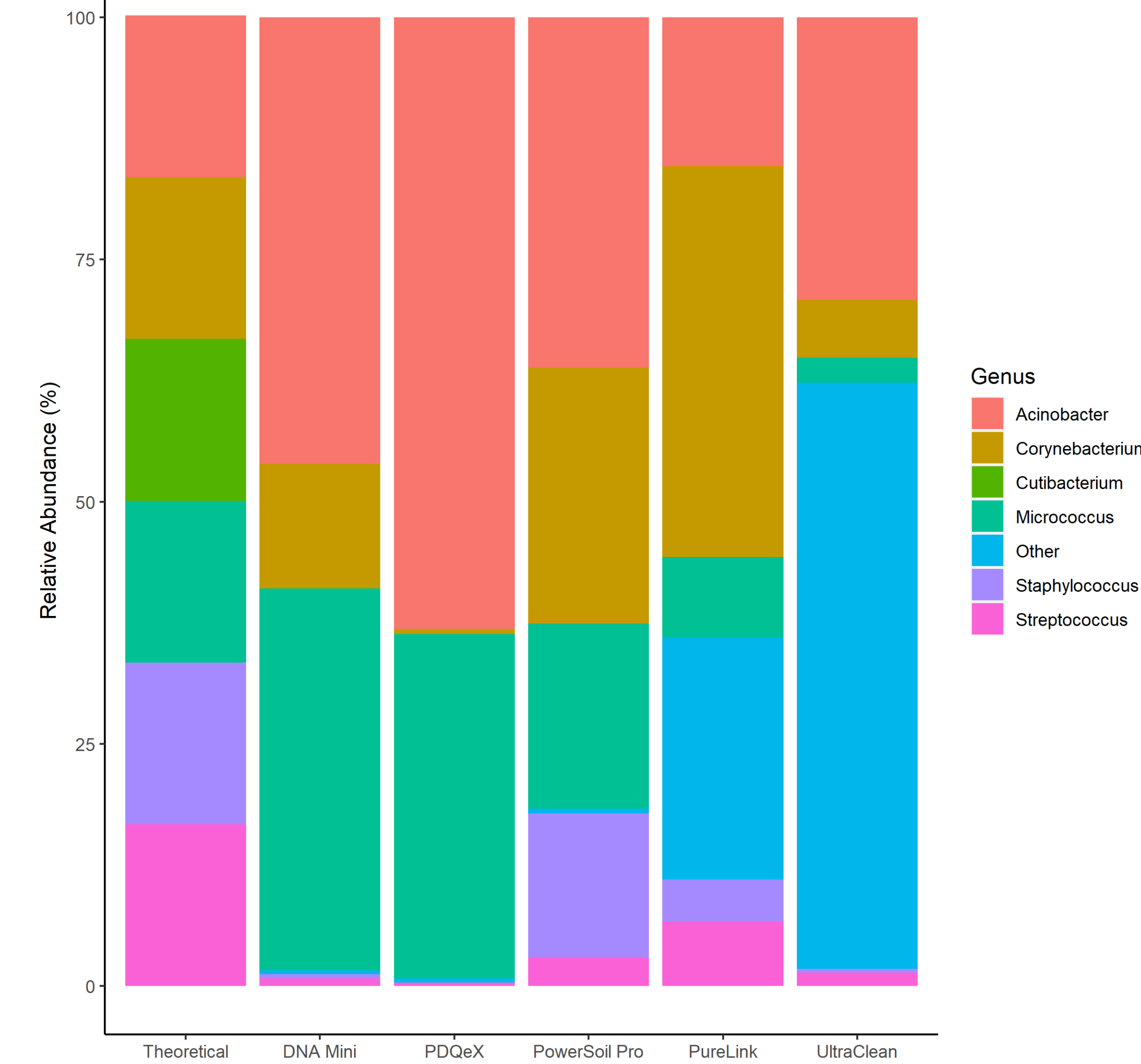


Figure 2. Relative microbial abundance recovered from mock penile microbial communities. The highest level of contamination was observed in DNeasy® Ultraclean® extracted samples. Samples extracted using the PDQeX prepGEM kit exhibited the greatest differences between observed and expected abundance across the genera.

Unique Taxa Observed in Donor Samples

Table 1. Unique genera and species reported in penile and vaginal swabs. All donors (except for one) displayed unique microbial profiles.

The degree of interpersonal variability was notably higher in penile swabs compared to vaginal swabs.

Unique Taxa Observed in Donor Samples						
Penile Swabs			Vaginal Swabs			
Donor	Genus	Species	Donor	Genus	Species	
#1	<i>Actinomyces</i>	<i>Corynebacterium glucuronolyticum</i>	#1	<i>Oscillospira</i>	<i>Lactobacillus coleohominis</i>	
	<i>Negativicoccus</i>	<i>Prevotella buccalis</i>		<i>Clostridium</i>	<i>Lactobacillus gigeriorum</i>	
	<i>Erwinia</i>	<i>Actinomyces radingae</i>		<i>Peptoniphilus</i>	<i>Lactobacillus kitasatonis</i>	
	<i>Escherichia</i>	<i>Actinomyces neuii</i>		<i>Anaerococcus</i>	<i>Peptoniphilus asaccharolyticus</i>	
#2	<i>Moryella</i>	<i>Erwinia dispersa</i>	#2	<i>Finegoldia</i>		
	<i>Lactobacillus</i>	<i>Peptoniphilus coxii</i>		<i>Enterococcus</i>		
	<i>Varibaculum</i>	<i>Corynebacterium tuberculostearicum</i>		<i>N/A</i>	<i>N/A</i>	
	<i>Campylobacter</i>	<i>Prevotella disiens</i>		<i>Megasphaera</i>	<i>Gardnerella vaginalis</i>	
#3		<i>Prevotella bivia</i>	#3	<i>Gardnerella</i>	<i>Lactobacillus johnsonii</i>	
		<i>Dialister microaerophilus</i>		#4	<i>Bifidobacterium</i>	<i>Lactobacillus intermedius</i>
		<i>Campylobacter ureolyticus</i>				<i>Pediococcus cellicola</i>
		<i>Corynebacterium atypicum</i>				<i>Megasphaera hominis</i>
#4	<i>Mycobacterium</i>	<i>Corynebacterium genitalium</i>				
	<i>Pseudoclavibacter</i>	<i>Corynebacterium jeikeium</i>				
	<i>Facklamia</i>	<i>Corynebacterium kutscheri</i>				
		<i>Anaerococcus octavius</i>				

REFERENCES

- Williams DW, Gibson G. Classification of individuals and the potential to detect sexual contact using the microbiome of the pubic region. *Forensic Science International: Genetics* 2019;41:177–87. <https://doi.org/10.1016/j.fsigen.2019.05.004>.
- Dixon R, Egan S, Hughes S, Chapman B. The Sexome - A proof of concept study into microbial transfer between heterosexual couples after sexual intercourse. *Forensic Science International* 2023;348:111711. <https://doi.org/10.1016/j.forsciint.2023.111711>.

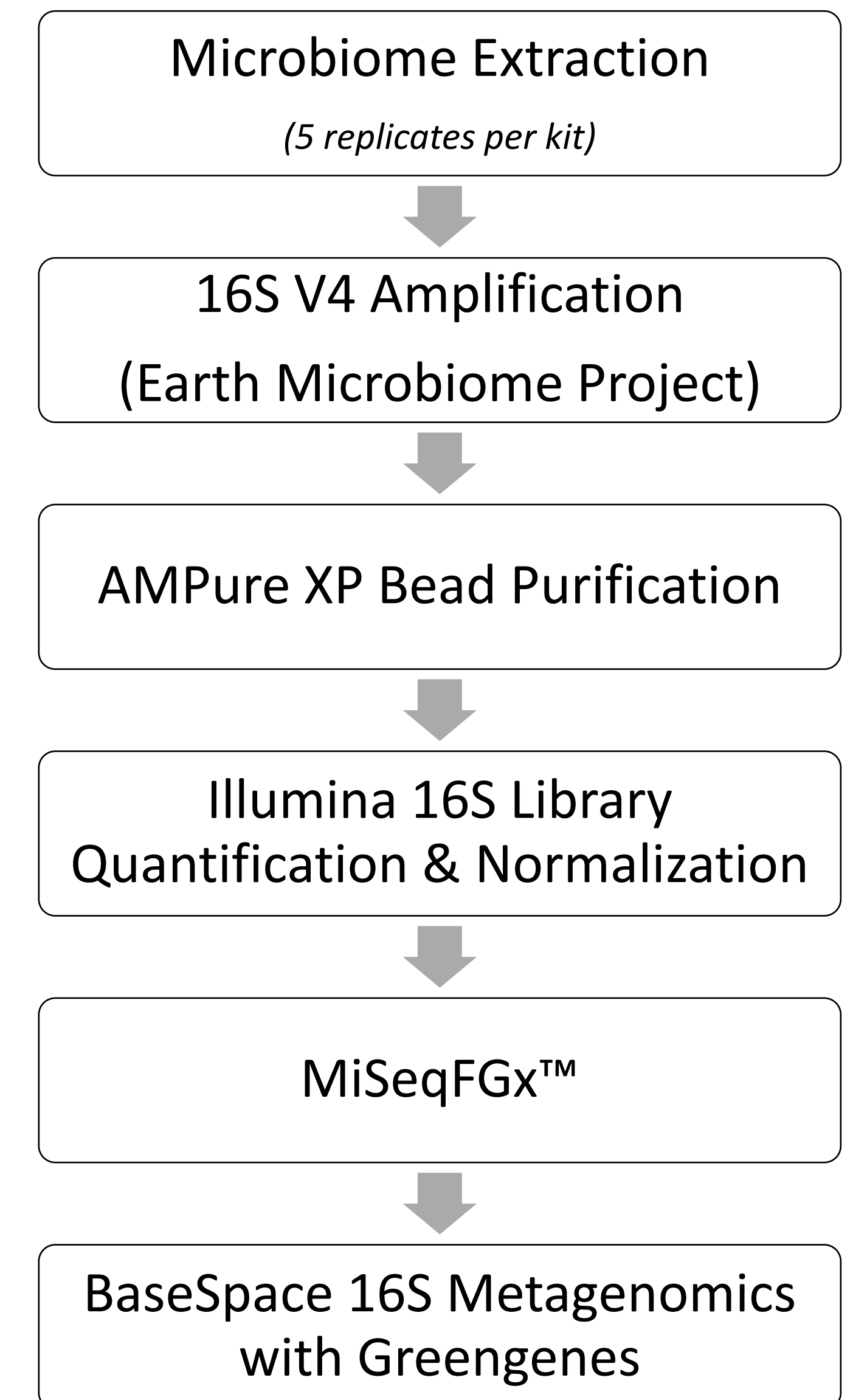
MATERIALS AND METHODS

Samples

- Mock microbial communities:
 - ATCC® Vaginal Microbiome Whole Cell Mix
 - ATCC® Skin (“Penile”) Microbiome Whole Cell Mix
- Authentic donor penile & vaginal swabs (N = 4 each)

DNA Extraction Kits

- PureLink™ Microbiome DNA Purification Kit
- PDQeX prepGEM Bacteria Kit
- QIAamp® DNA Mini Kit
- DNeasy® PowerSoil® Pro Kit
- DNeasy® UltraClean® Microbial Kit



CONCLUSIONS

- Invitrogen® PureLink™ Microbiome DNA Purification kit yielded the most representative bacterial profiles from both mock vaginal and skin (“penile”) microbial communities when compared to the other kits tested.
- Unique genera and species were present in vaginal and penile swabs with donors exhibiting distinct microbial profiles.
- Our results demonstrate the potential benefit in interrogating the “Sexome” for human identification and forensic investigative purposes when the presence of sperm is not detected in sexual assault evidence.