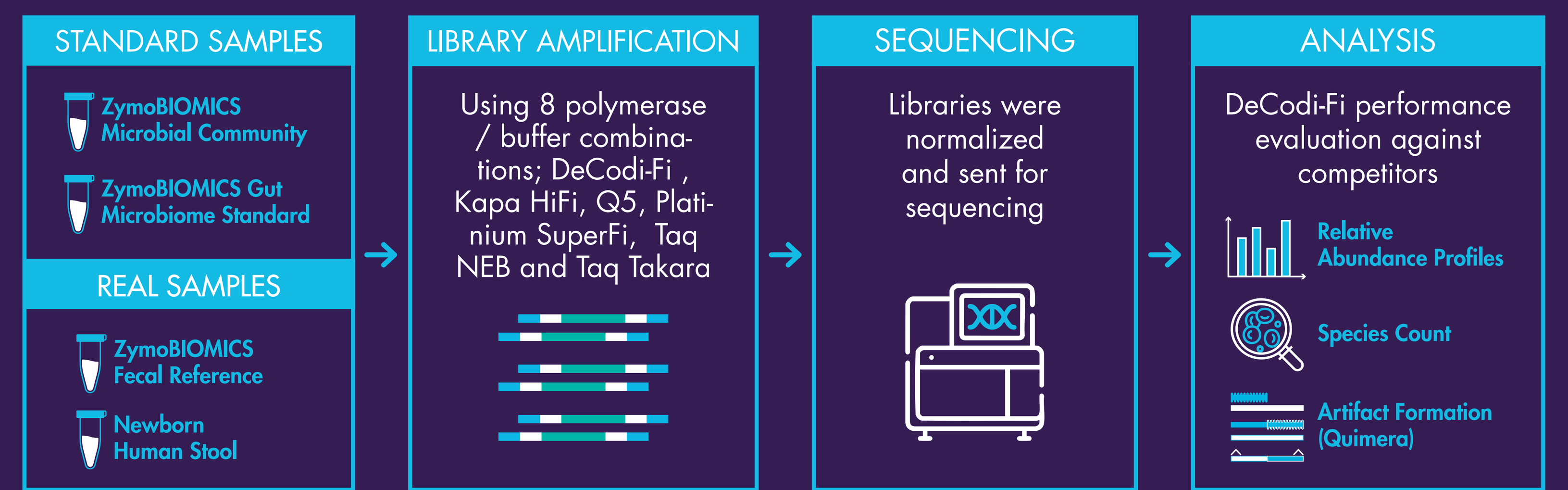
Unveiling Microbial Diversity: Cost-Effective 16S rRNA Amplicon Sequencing using DeCodi-Fi High Fidelity Polymerase

J.Gimpel, J. Hsieh, J. Sáez, J. Cáceres, I. Arriagada, S. Kamkar, I. Marx, M. Rozas
Kura Biotech Inc, Atlanta, GA, USA

CONTEXT

16S rRNA amplicon sequencing is a powerful tool for exploring microbial diversity, enabling the characterization of complex microbial communities across numerous samples. However, the choice of DNA polymerase for PCR amplification can significantly influence how accurately the composition of these communities are represented. This study compares the performance of eight polymerase/buffer combinations, including the cost-effective DeCodi-Fi High Fidelity Polymerase, across two mock communities and two real feces samples. DeCodi-Fi demonstrated to be accurate and reliable in 16S sequencing compared to yet more expensive HiFi options: KAPA HIFi, Platinium SuperFi, and Q5, evidencing it is a reliable alternative for 16S sequencing. Also, these findings highlight the importance of polymerase selection in microbiome studies, emphasizing DeCodi-Fi's cost-effectiveness and high-performance balance.

METHODS



HIGHER ACCURACY

DeCodi-Fi Ensures Accurate Profiling

Our results suggest that all DNA polymerases/buffer treatments, except Taq NEB, resulted in overall comparable 16S rRNA amplicon sequencing data from the 4 tested bacterial communities. These enzyme represented well the expected relative abundance of the mock communities, and demonstrated a consistent representation of the two real samples (Fecal Reference and Newborn Stool).

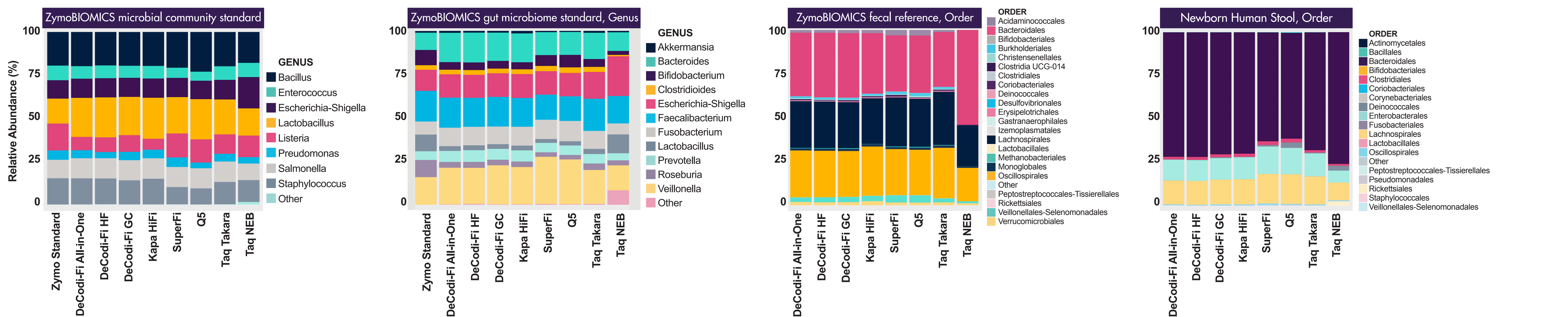


Figure 1: Relative abundance across four samples using eight different polymerase/buffer combinations to amplify the V4 region of the 16S rRNA gene.

LOWER BIAS

DeCodi-Fi Enables Reliable Species Count and Abundance in Real Samples

DeCodiFi, as well as other high-fidelity polymerases, except Taq NEB, yielded similar results regarding the number of identified species. The healthy adult Fecal Reference mix contained 10 times more species than the Newborn Human Stool, possibly due to the newborn being exclusively breastfed. Analysis of the top 15 species in both real samples revealed a consistent pattern in relative abundance for all polymerases in the Newborn Stool sample. The bias of Taq NEB is evident in showing reduced sensitivity for low-abundance species. Additionally, SuperFi and Q5 seem to differ in the representation of two minor species compared to the rest of the group (excluding Taq NEB), but otherwise, there is overall agreement.

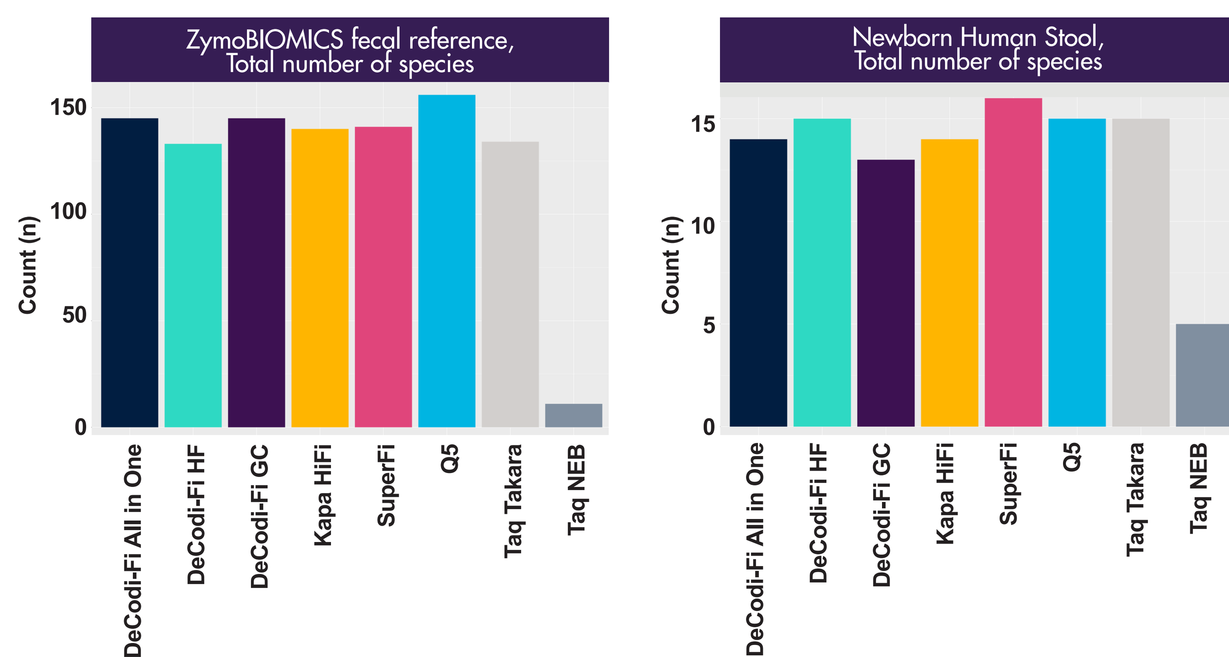


Figure 2: Species count across 2 real samples using eight different polymerase/buffer combinations to amplify the V4 region of the 16S rRNA gene.

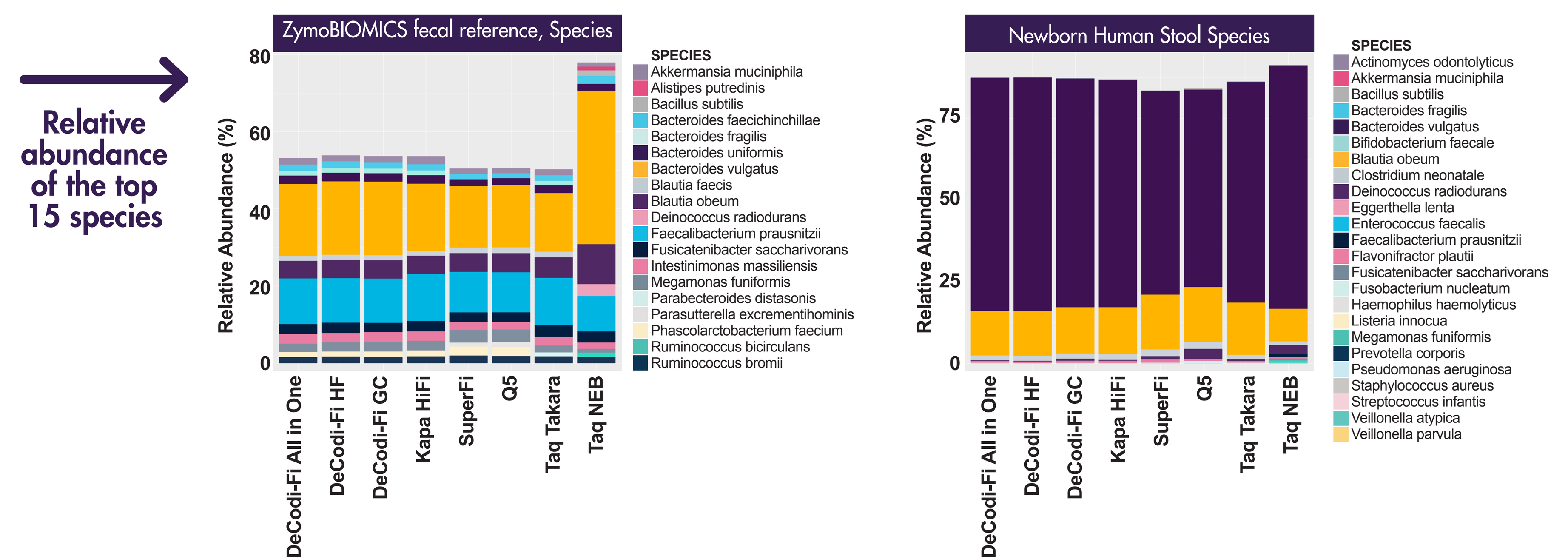


Figure 3: Relative abundance of the top 15 species from each real sample using eight different polymerase/buffer combinations to amplify the V4 region of the 16S rRNA gene.

LOWER ARTIFACTS

DeCodi-Fi Minimizes Artifacts for Accurate 16S rRNA Sequencing

Chimera formation (Artifact) was reduced with DeCodi-Fi and Kapa HiFi compared to Platinium SuperFI and Q5. Taq polymerases tend to present even lower chimera rates.

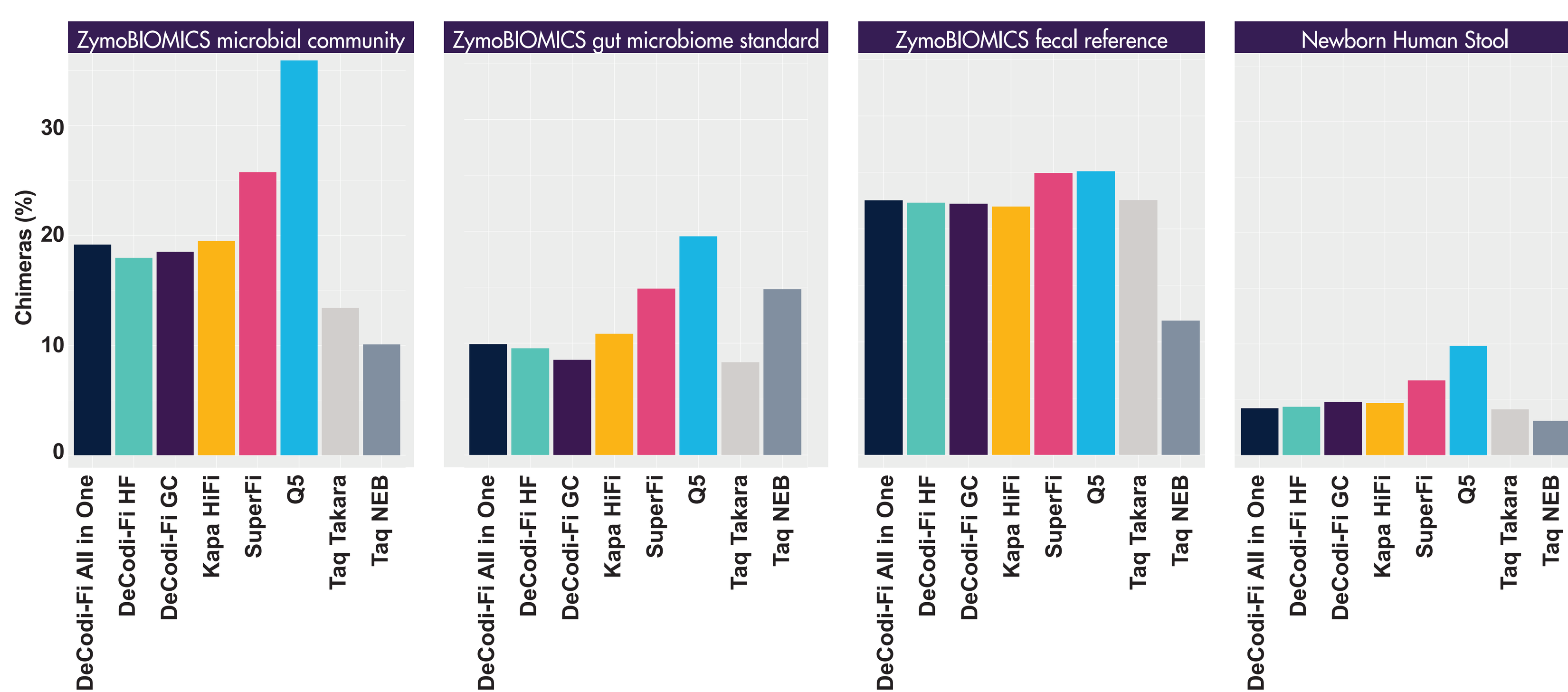


Figure 4: Percentages of Quimera reads across each sample using eight different polymerase/buffer combinations to amplify the V4 region of the 16S rRNA gene.

STUDY ROBUSTNESS

Ensuring Experimental Robustness

To validate our conclusions, we conducted a comparative analysis of the V4 and V3-V4 amplicon regions. The choice of 16S amplicon had no significant impact on the relative abundance structure across the four samples, demonstrating the robustness of the enzymes, laboratory methods, and bioinformatic pipelines. These results provide strong support for the consistency and reliability of our findings.

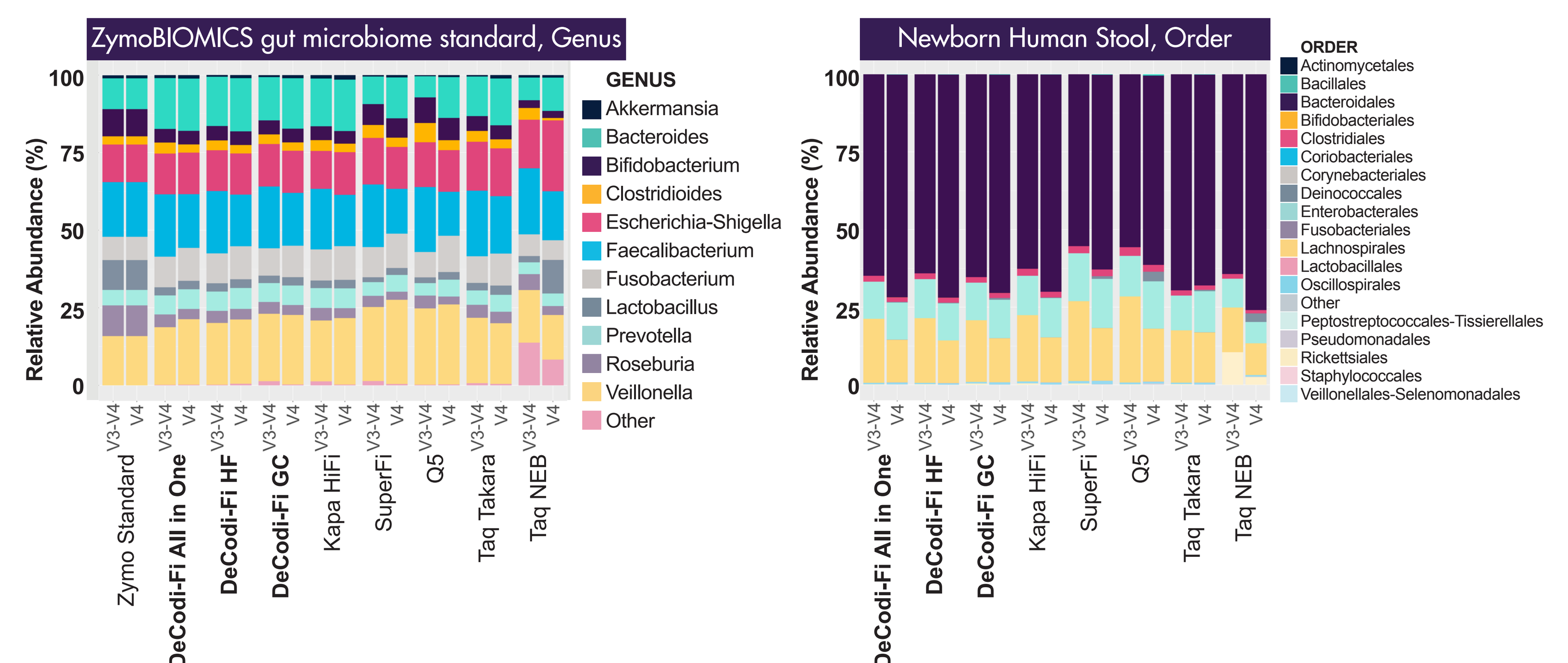
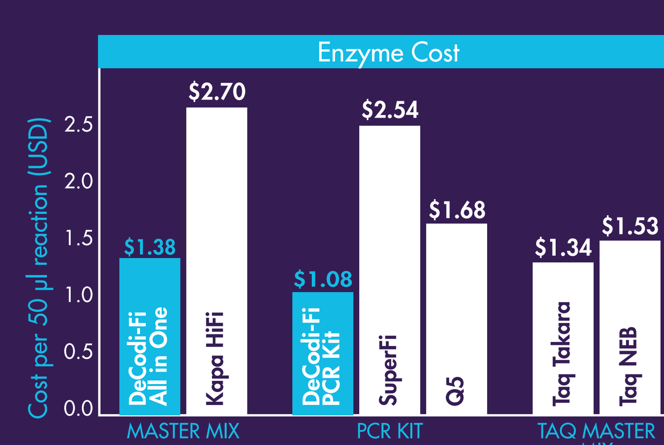


Figure 5: Relative abundance in a standard and real sample using eight different polymerase/buffer combinations to amplify the V4 and V3-V4 region of the 16S rRNA gene.

CONCLUSION

DeCodi-Fi High Fidelity polymerase offers a cost-effective alternative for 16S sequencing, matching the performance of pricier options like Kapa, Platinium Superfi, and Q5. At nearly half the cost of competing products, DeCodi-Fi provides reliable and robust results, making it an economic choice for high-fidelity applications in microbiome studies.



Download Poster:

Visit our website: