

Validation of differentiable molecular barcodes as reference spike-in oligonucleotides for microbiome studies

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Introduction

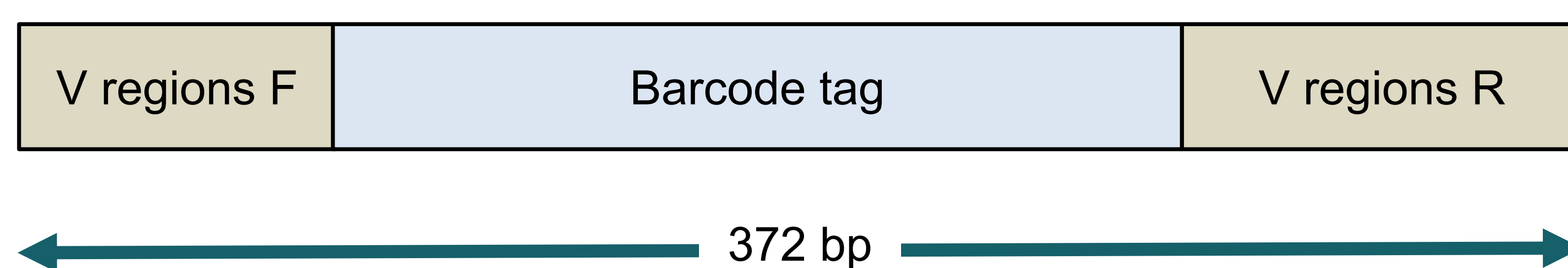
A shortcoming in microbiome research is the lack of reference standards to control biases introduced by differential DNA extractability, interference with amplification, library preparation, next-generation sequencing platforms, and data analysis.

Aim

To develop and test novel molecular barcodes for use as spike-in reference standards in the form of oligonucleotides or recombinants in microbiome studies.

Methods

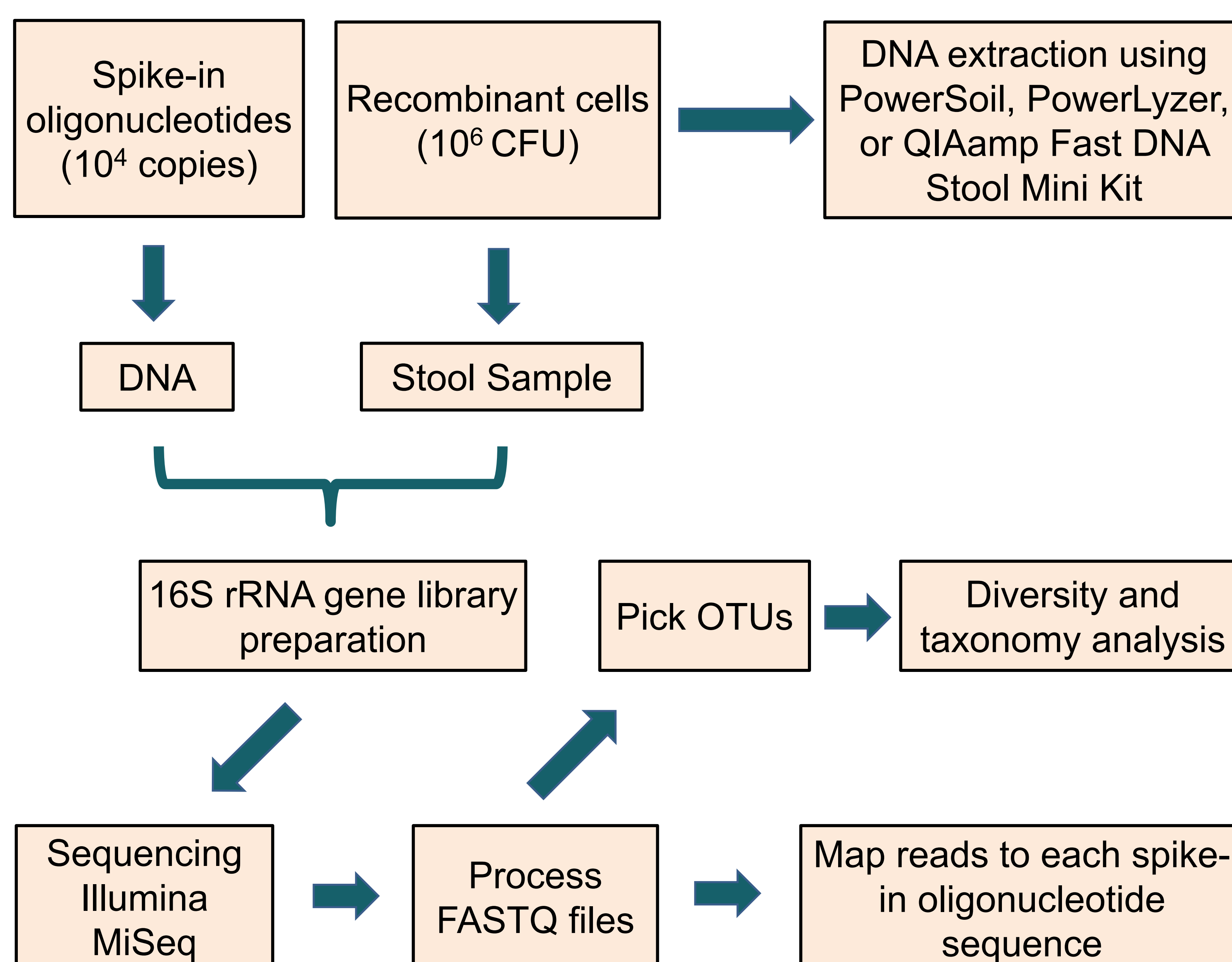
Structure of Reference Spike-in



There are three elements to the spike-in oligonucleotides:

1. 16S variable region forward primer sites
2. Identifier barcode (unique to each spike-in oligo)
3. 16S variable region reverse primer sites

General Methodology



Results

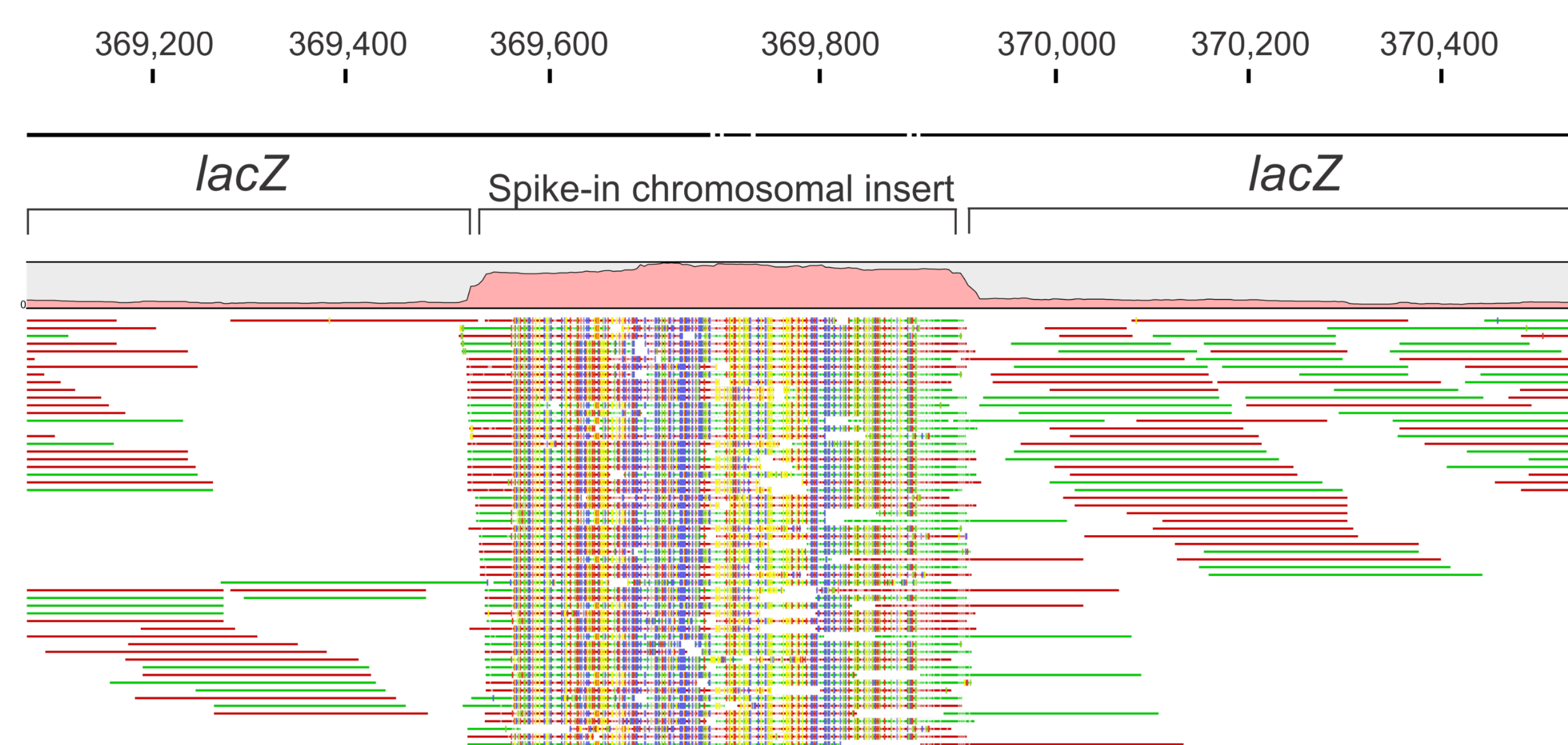


Figure 1. Example of chromosomal insert of a Gram-positive recombinant used as a spike-in

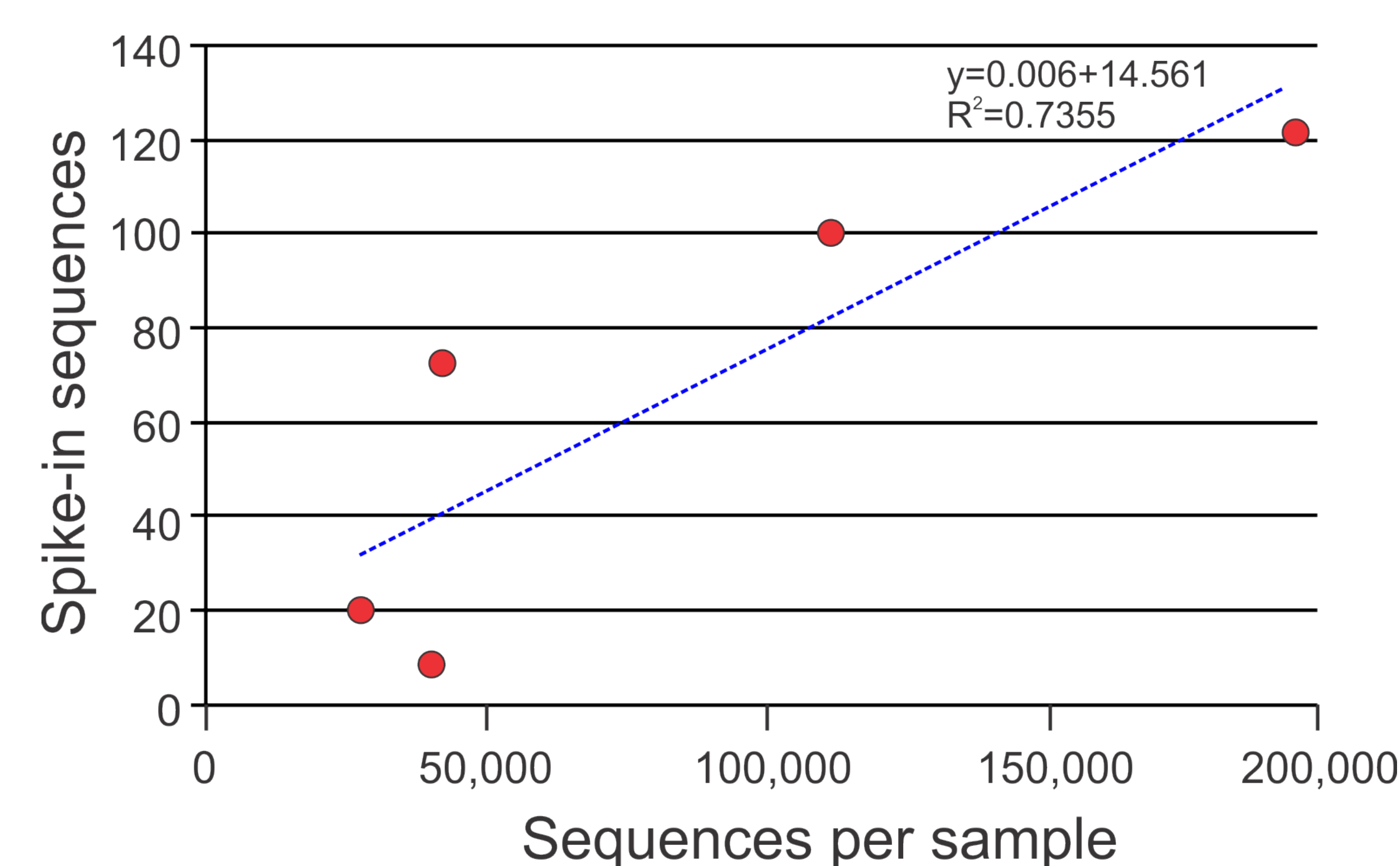


Figure 2. Number of reads mapping to spike-in oligonucleotides vs. sequences per samples

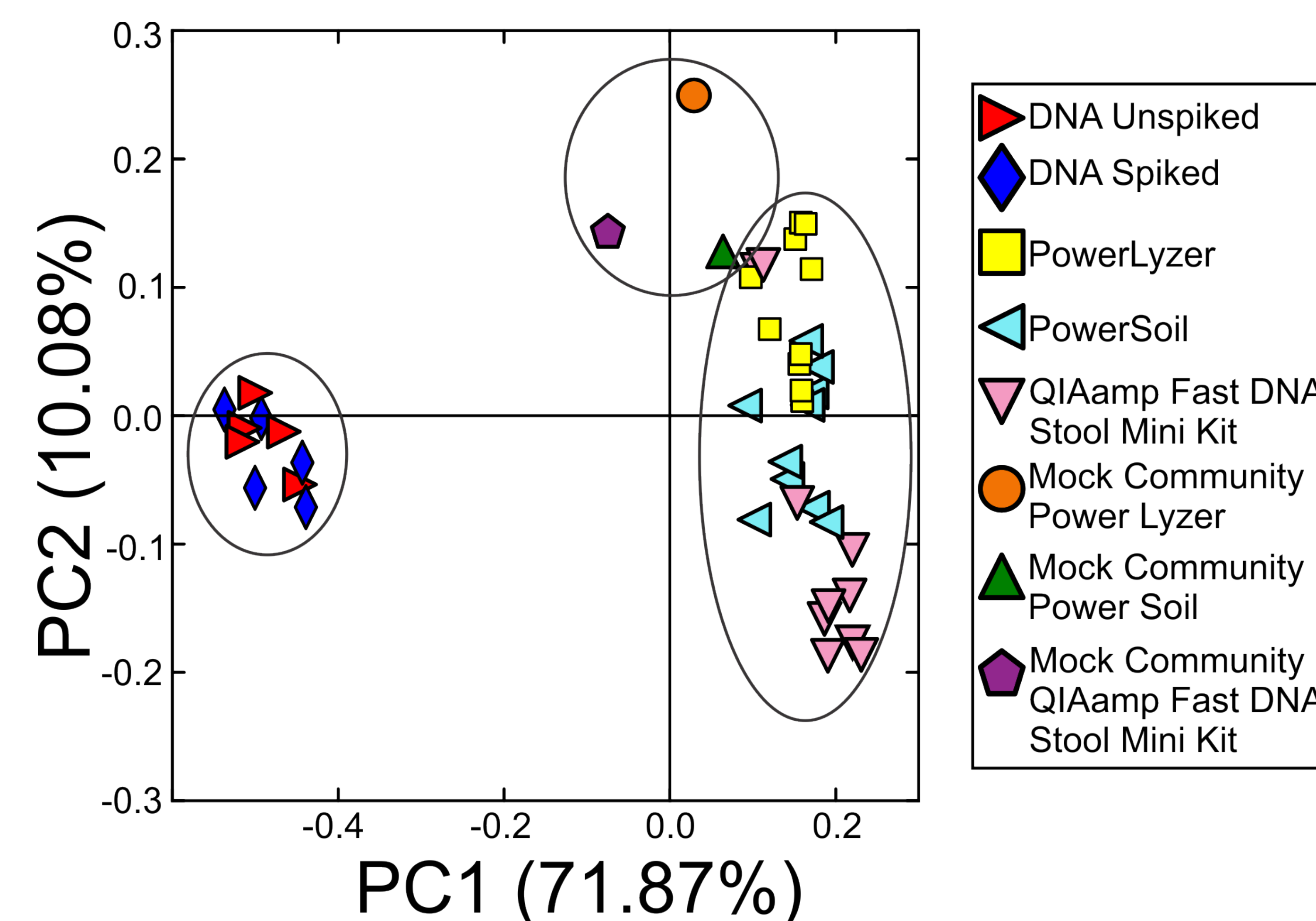


Figure 3. Beta-diversity

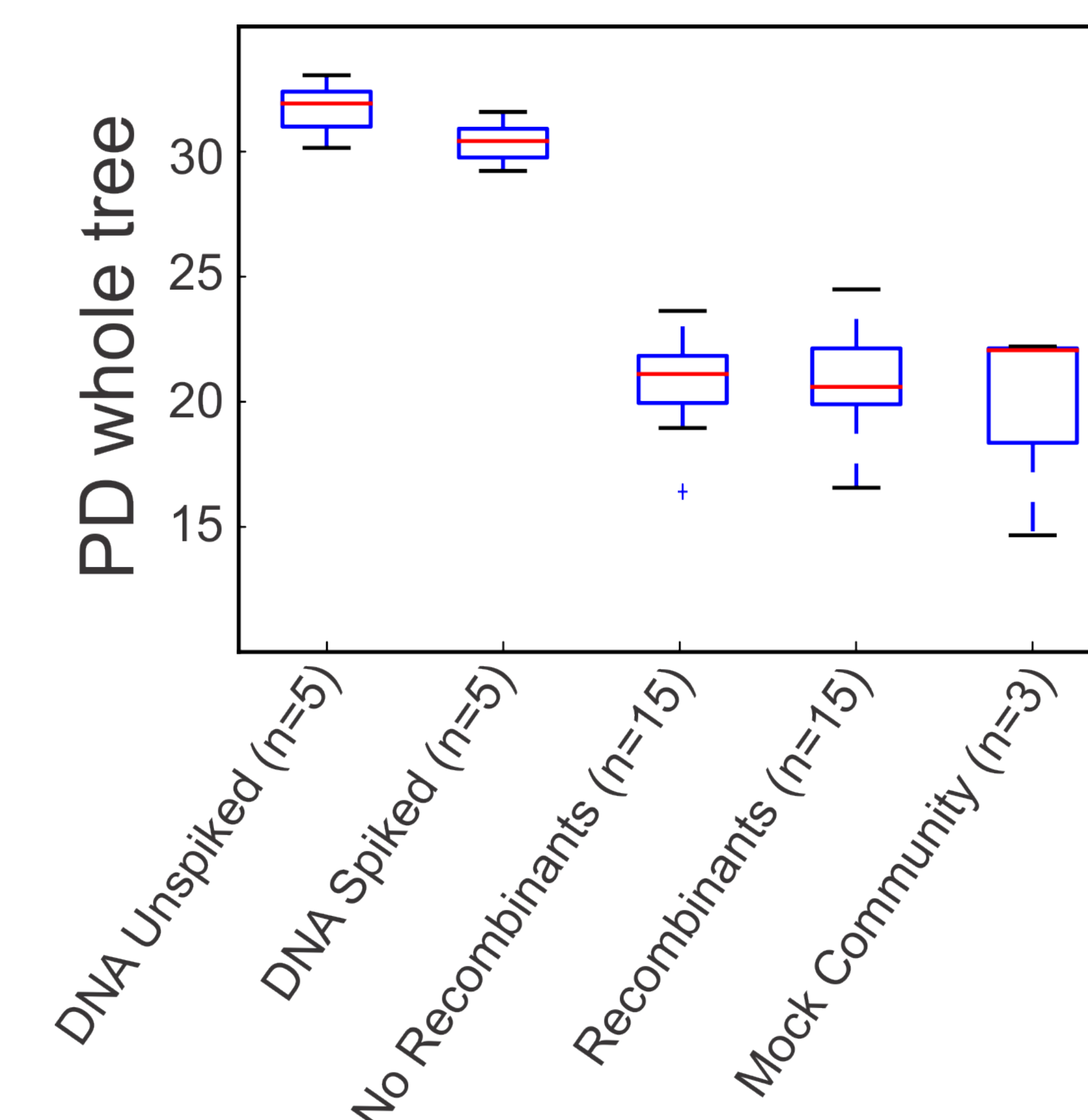


Figure 4. Alpha-diversity

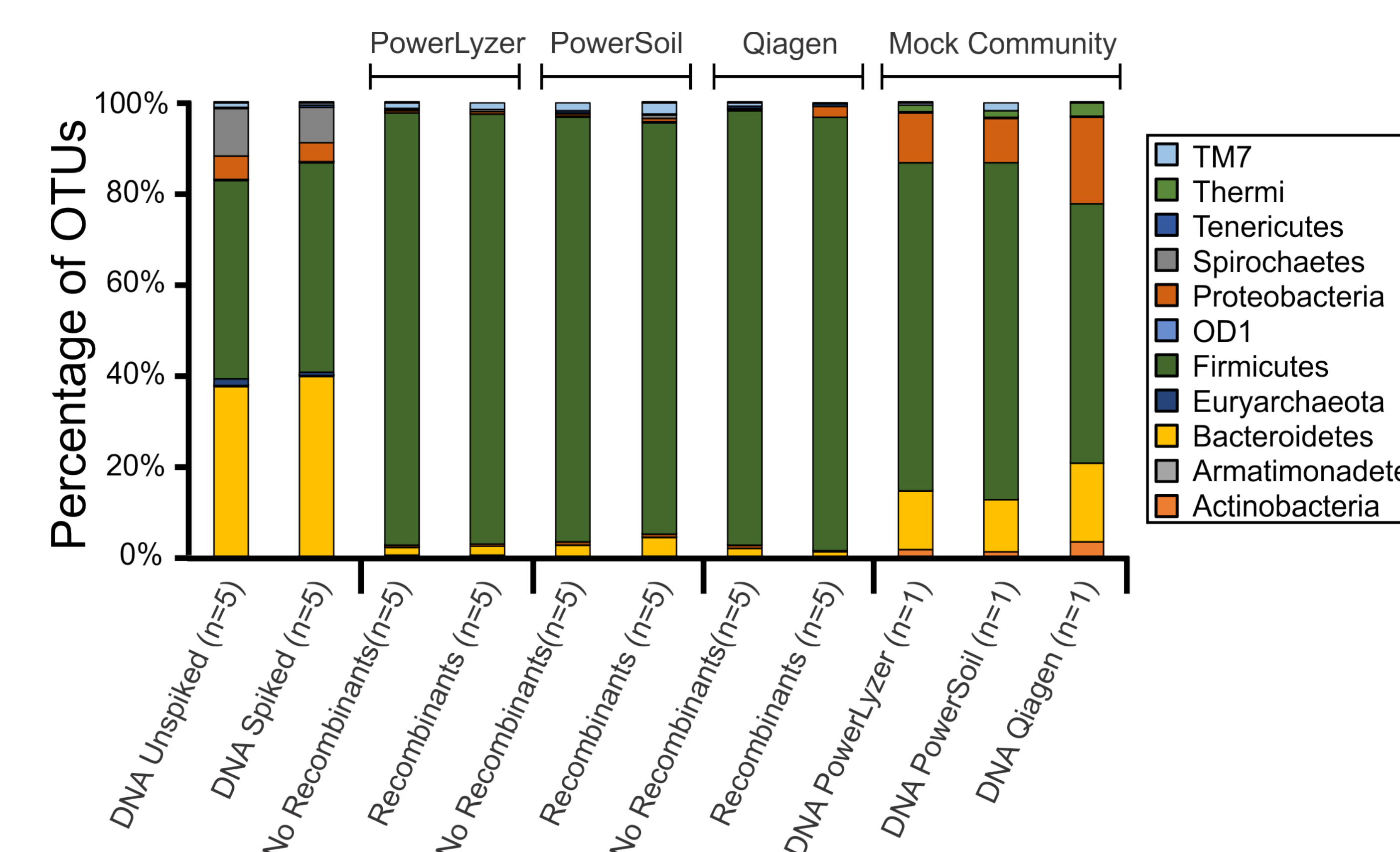


Figure 5. Taxonomy

Conclusions

- Results support the value of including reference standards in microbiome analyses to strengthen the validity of findings, correlations, and possible causations.
- Further validation studies are needed to assess the recoverability of the differentiable molecular barcodes and recombinants as in other sample types.
- DNA extraction methods, OTU picking strategy, and sequencing depth have a significant impact on the results
- The applications of spike-in oligonucleotides can be extended to human and veterinary medicine, personalized medicine, and animal models to study the association of the microbiome in health and disease.

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