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Optimizing Extraction Kits and Sequencing Techniques for Efficient Analysis of Cow Fecal Microbiome Diversity

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Cattle play a vital role in human livelihoods, providing essential livestock products and serving as agricultural labor. However, the cattle industry faces significant challenges due to diseases such as calf diarrhea, a major cause of calf mortality. Understanding the gut microbiome in diarrheal cattle is critical for developing effective preventive measures. To address this, we aimed to establish the cost-effective and high-resolution sequencing approach for analyzing the cattle gut microbiome, particularly in relation to diarrheal disease.

In this study, we evaluated two DNA extraction kits (feces and soil) and compared single-end versus paired-end sequencing techniques to determine the most effective method for metagenomic analysis of cattle fecal samples. Fecal samples from three cows (samples 2, 3, and 11) were used for preliminary testing to optimize the extraction and sequencing methods.

Our results showed that paired-end sequencing consistently produced higher total read counts and better bacterial and viral genome recovery compared to single-end sequencing. Notably, paired-end sequencing with the soil extraction kit yielded the highest bacterial abundance, while the feces extraction kit produced higher viral counts. Alpha diversity analysis revealed greater species richness in samples processed with paired-end sequencing, while beta diversity analysis showed distinct clustering patterns, indicating significant differences in microbial community composition between sequencing approaches.

While no statistically significant difference was observed between the feces and soil extraction methods, paired-end sequencing with the soil extraction kit is recommended for future metagenomic studies due to its superior overall read counts and microbial recovery. This optimized approach will be applied to sequence 110 cattle fecal samples, including both healthy and unhealthy individuals, to provide a comprehensive understanding of the gut microbiome across different regions, genders, and health conditions.