

Optimization of Collection, Storage, and Extraction of Samples for Large-Scale 16s Sequencing Project

EQUINE R E S E A R C H

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Introduction

Correlations between the microbiome of the horse and various physiological parameters continue to be determined¹. Most evaluations of the equine microbiome have been regionally focused with limited numbers or variability. In order to elucidate the role of the microbiome on equine health, a large-scale trial has been initiated with a primary objective of analyzing and characterizing the microbiome of the horse. A study of this scope relies on horse owners from around the country to collect samples and ship them to a centralized lab. Therefore, collection protocols and extraction techniques need to be optimized to maintain sample integrity². A trial was conducted to evaluate the storage conditions and extraction methodologies necessary to develop an optimized method of sample collection, preparation, and extraction.

Background

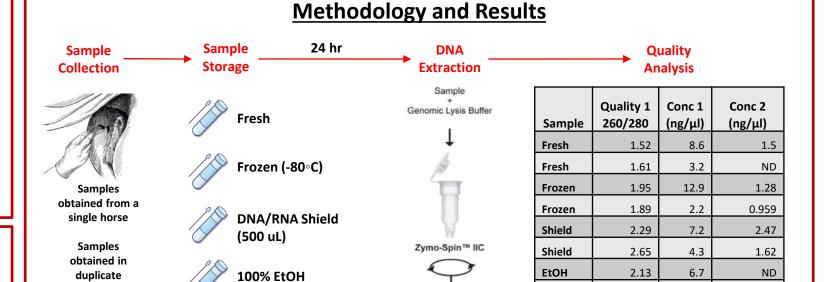
Reproducibility remains a challenge in microbiome research³. Sample collection, storage, processing, and data evaluation represent significant areas of variability between studies. Optimization of sample storage and DNA extraction reduce the potential for error, resulting in more consistent and repeatable data.





Objectives

Evaluate four storage methods on the quality and quantity of DNA extracted from fecal swabs obtained from horses to be utilized in downstream 16s sequencing.



Elute

Conclusions

Samples

obtained at the

same timepoint

(500 uL)

The necessity for swabs to be collected from around the country and shipped to a single location requires a buffering solution capable of preserving the DNA at varying temperatures prior to processing.

Utilization of a commercially available DNA/RNA preservation solution (DNA/RNA Shield; Zymo Research, Irvine, CA) resulted in the highest quantity and quality of DNA extract from fecal swabs.

References

2.20

4.6

Quality 1 and Conc 1 by Nanodrop

ND= Not detectable

Conc 2 by Qubit

EtOH

- 1- A microbiological map of the healthy equine gastrointestinal tract. Ericsson et al., 2016. PLOS One. November 11, 2016
- 2- Optimizing methods and dodging pitfalls in microbiome research. Kim et al., 2017. Microbiome 5:52
- 3- Identifying and overcoming threats to reproducibility, replicability, robustness, and generalizability in microbiome research. P.D. Schloss. 2018. mBio. Volume 9, Issue 3