

Comparison of seven methods for extraction of bacterial DNA from fecal and cecal samples of mice.

Feces

CASE STUDY

Ferrand, J.; Patron, K.; Legrand-Frossi, C.; Fripiat, J.; Merlin, C.; Alauzet, C.; Lozniewski, A. *Journal of Microbiological Methods*. 2014, 105.

Overview

- Keywords:** DNA extraction, mice feces, mice cecal content, 16S rDNA, qPCR
- Aim of the study:** Selection of an optimal DNA extraction method for molecular assays
- Application:** Quantitative PCR
- Sample type:** Mice feces and intestinal contents
- Material:** FastDNA™ SPIN Kit for Soil, FastDNA™ SPIN Kit for Feces, QIAamp™ DNA Stool Mini Kit, MasterPure™ Gram Positive DNA Purification Kit, NucliSENS™ easyMAG, ZR Fecal DNA MiniPrep™, FastPrep-24™ instrument
- Buffer:** Buffers provided with each DNA extraction kit

Protocol and Parameters

1. Feces were pooled and frozen at -20°C immediately after collection.
2. Cecal samples were obtained shortly after dissection and immediately frozen in liquid nitrogen and stored at -80°C before use.
3. With each extraction method tested, DNA was extracted from 50 mg of starting material (wet weight) in five duplicates.
4. For three bead beating methods (FastDNA™ SPIN Kit for Soil, FastDNA™ SPIN Kit for Feces and ZR Fecal DNA MiniPrep™), DNA extraction was performed with the FastPrep-24™ homogenizer at speed 6 m/s for 40s.

Conclusion

Among seven DNA extraction methods, the FastDNA™ SPIN Kit for Soil was shown to be the most efficient extraction method for both feces and intestinal contents, providing the highest DNA yields and 16S rDNA. DNA fragments recovered were larger than 1.6 kb and suitable for PCR-analysis of microbiomes. This study reveals how FastPrep® technology (FastPrep® homogenizer and FastDNA™ SPIN Kit for Soil) can be adapted for detecting genes of various Gram-positive bacteria present in fecal and cecal matrices.

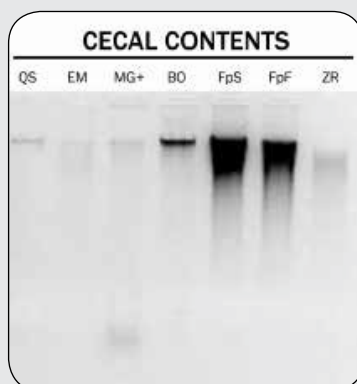


Figure 1

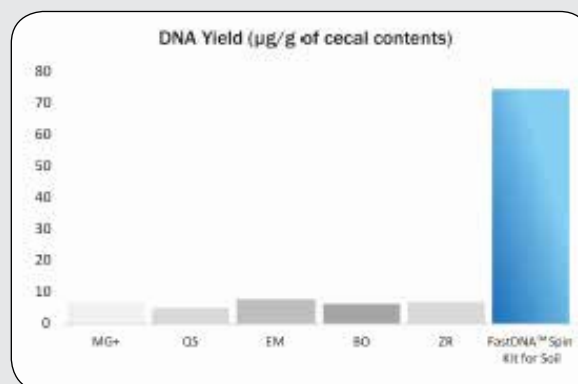


Figure 2

Fig. 1: Electrophoresis profiles of DNA extracted from cecal contents using the seven methods tested. MG+: MasterPure™ Gram Positive; QS: QIAamp™ DNA Stool; EM: NucliSENS™ easyMAG; BO: method from Bonot et al (2010); ZR: ZR Fecal DNA MiniPrep™; FpF: FastDNA™ SPIN Kit for Feces; FpS: FastDNA™ SPIN Kit for Soil.

Fig. 2: DNA yields from a 50 mg sample. MG+: MasterPure™ Gram Positive; QS: QIAamp™ DNA Stool; EM: NucliSENS™ easyMAG; BO: method from Bonot et al (2010); ZR: ZR Fecal DNA MiniPrep™; FpS: FastDNA™ SPIN Kit for Soil.