

Case study - Feces

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

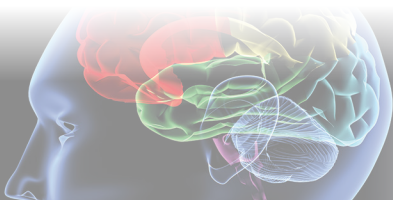
Janina Ferrand, Kevin Patron, Christine Legrand-Frossi, Jean-Pol Fripiat, Christophe Merlin, Corentine Alauzet, Alain Lozniewski.
Journal of microbiological methods. 2014. Vol 105.

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 name:** Mice feces and intestinal contents
- **Material:** FastDNA™ Spin Kit for Soil, FastDNA™ Spin Kit for Feces, QIAamp™ DNA stool minikit, 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, DNAs were 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.

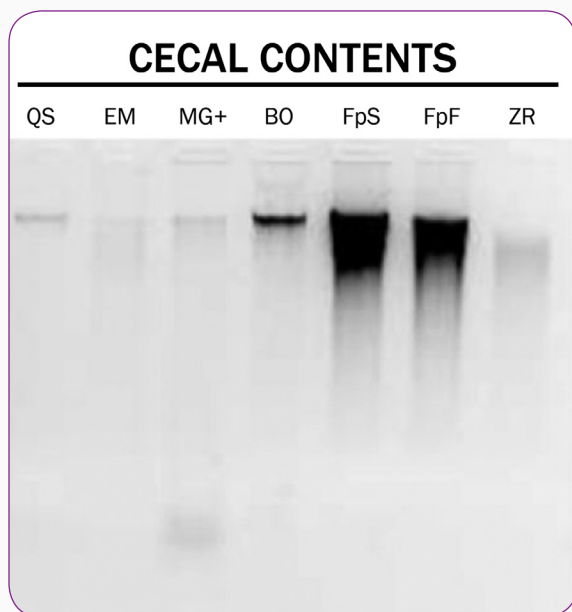


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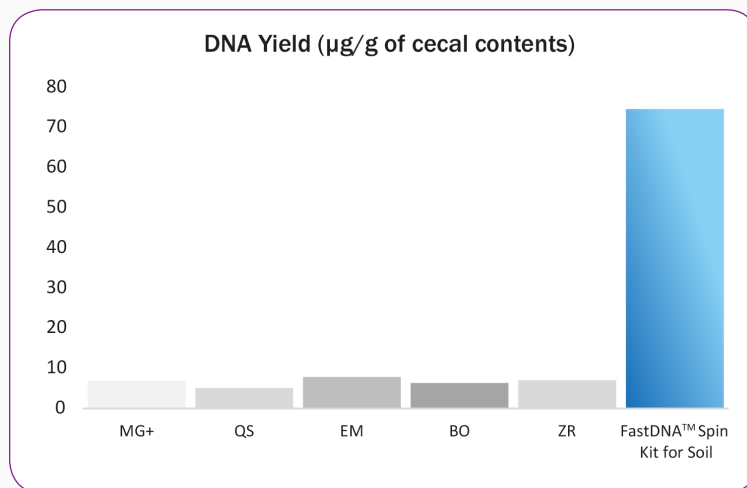
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Results

Effective DNA extraction method from mice cecal contents



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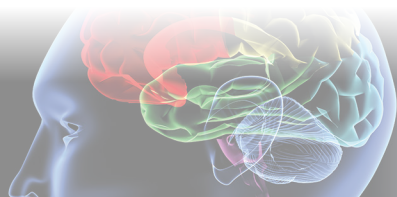
Electrophoresis profiles of DNA extracted from cecal contents using the seven methods tested. MG+: MasterPure™ Gram Positive; QS: QIAamp™ DNA Stool; EM: NucliSENS™ easyMAG; BO: method from Bonot et al (2010); ZR: ZR Fecal DNA MiniPrep™; FpF: FastDNA™ Spin Kit for Feces; FpS: FastDNA™ Spin Kit for Soil.

DNA yield 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™; FpF: FastDNA™ Spin Kit for Feces; FpS: FastDNA™ Spin Kit for Soil.

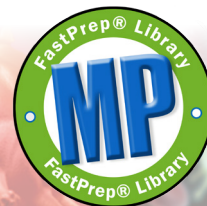
Conclusion

- Among seven DNA extraction methods, The FastDNA™ Spin Kit for Soil showed to be the most efficient extraction method for both feces and intestinal contents providing the highest DNA yield and 16S rDNA.
- DNA fragments recovered were larger than 1.6 kb making them suitable for PCR-analysis of microbiomes.
- This study shows that the FastPrep® technology (FastPrep® homogenizer and FastDNA™ Spin Kit for Soil) is adapted for detecting genes of various Gram-positive bacteria present in fecal and cecal matrices.

Successful sample preparation using the MP Biomedicals FastPrep® product line has been highlighted in thousands of scientific articles. To access articles and other materials, visit www.mpbio.com/FastPrepLibrary.



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