MP Biomedicals

Case study - *Human Feces*

Health

Comparison of DNA extraction kits for PCR-DGGE analysis of human intestinal microbial communities from fecal specimens.

Merlin W Ariefdjohan, Dennis A Savaiano, and Cindy H Nakatsu.
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Overview

- **Keywords:** DNA extraction kits, PCR- DGGE analysis, fecal specimens.
- **Aim of the study:** Extraction of bacterial genomic DNA from human fecal specimens
- **Application:** PCR-DGGE
- **Sample Name:** Human fecal specimens
- **Material:** Mobio Ultra Clean Fecal DNA extraction kit (M), QIAamp DNA Stool Mini Kit (Q), FastDNA™ Spin Kit (FSp), FastDNA™ Spin Kit for Soil (FSo), FastPrep-24™ instrument, Vortex
- **Buffer:** Provided with each DNA extraction kit

Protocol and Parameters

1. Wet fecal specimen weight used for extraction: 10, 25, 50, 100 and 200 mg.
2. DNA extractions were made following each manufacturer instructions.
3. For FastDNA™ Spin Kit (Fsp) and FastDNA™ Spin Kit for Soil (FSo), samples were loaded in FastPrep-24™ homogenizer and processed 40 sec at speed setting of 6 m/s.
4. For M and Q Kits, samples were loaded in FastPrep-24™ homogenizer and processed 30 sec at speed setting of 5.5 m/s.

Results

Average DNA Yield using four different DNA extraction kits:

DNA extraction kit[1]	DNA yield (mg DNA/g dry wt feces)[3]
M	52.4 ± 14.5[b]
Q	57.0 ± 22.6[b]
FSp	151.3 ± 47.1[a]
FSo	187.2 ± 69.4[a]

1. *The following extractions accounted for various fecal specimen weights (10, 25, 50, 100, and 200 mg) and DNA yield was normalized by percent fecal dry matter (26%, 35%, and 41%) [2].*
2. *Values of DNA yield were based on n = 45/DNA extraction method and were normalized based on the dry weight of the respective fecal sample.*
3. *Treatment groups with different letters indicate significant differences between groups (P < 0.05). Values are means ± SE.*

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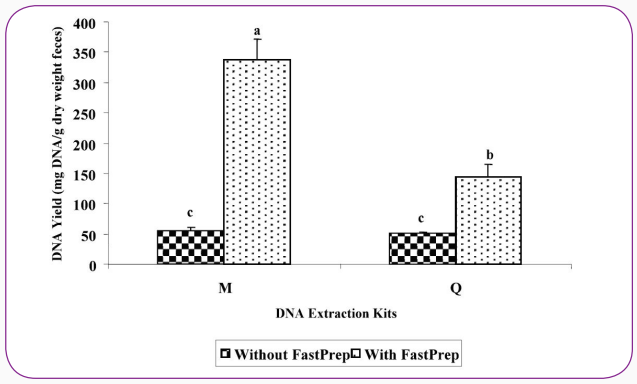
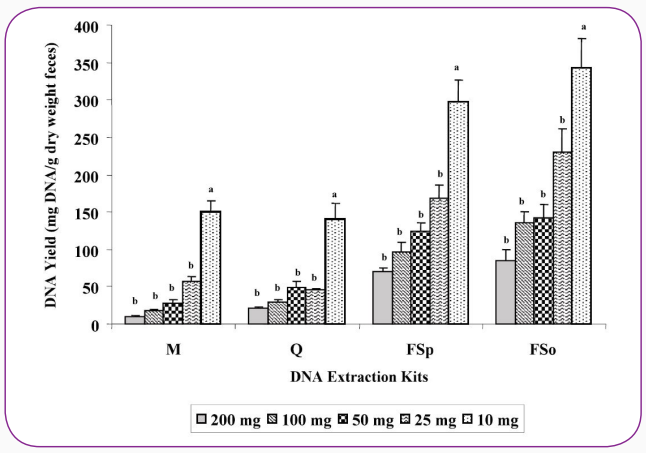
Results

Average DNA yield obtained using kits M and Q with and without FastPrep-24™ homogenizer:



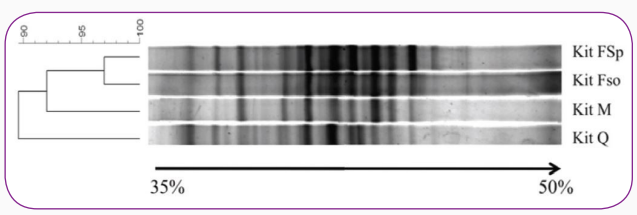
Comparison was made on the average DNA yield of these kits with and without the addition of vigorous mixing using the FastPrep-24™ Instrument (n = 3/kit). Values for DNA yield were normalized based on the dry weight of the respective fecal sample. Means with different letter designation are significantly different (P < 0.05).

Average DNA yield obtained as influenced by fecal specimen weights:

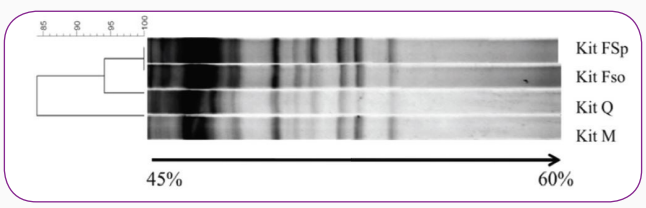


DNA was extracted from 200, 100, 50, 25, and 10 mg of human fecal specimens (n = 45/kit), Values for DNA yield were normalized based on the dry weight of the respective fecal specimen. Means with different letter designation are significantly different (comparisons within each extraction kit; P < 0.05).

Analysis of DGGE Fingerprint Result:



DGGE gel gradient 35~50%
The Dice similarity coefficient of bacterial community ranged from 0.88 ~ 0.97. FSo and FSp (similarity coefficient of 0.97) Q was the least similar to the others, where by its Dice similarity coefficient was 0.88 when compared to kit FSo.



DGGE gel gradient 45 ~60%
The Dice similarity coefficient of bacterial community ranged from 0.82 ~ 1.0. FSo and FSp were identical (similarity coefficient value of1) M was the least similar to the others with coefficient value of 0.82 when compared kits FSo and FSp.

Conclusion

Extraction kits that incorporated bead-containing lysing matrix and vigorous shaking produced high quality DNA from human fecal specimens (10 to 50 mg, wet wt) that can be resolved as bacterial community fingerprints using PCR-DGGE technique:

- DNA quantity was significantly improved when10 to 50 mg of fecal specimens (wet wt) were used.
- FastDNA™ Spin Kit and FastDNA™ Spin Kit for Soil extracted significantly larger amounts of DNA per g dry fecal specimens and produced more bands on their DGGE profiles than kits M and Q due to their use of bead-containing.
- DGGE of 16S rRNA gene PCR products was suitable for capturing the profiles of human intestinal microbial community and enabled rapid comparative assessment of inter- and intra-subject differences.

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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