

1 **Optimization of DNA Extraction Kits for PCR-DGGE Analysis of Human Intestinal**
2 **Microbial Communities from Fecal Specimens**

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1 **ABSTRACT**

2 **Background:** The influence of diet on intestinal microflora has been investigated mainly
3 using conventional microbiological approaches. Although these studies have advanced
4 knowledge on human intestinal microflora, it is imperative that new methods are applied to
5 facilitate scientific progress. Culture-independent molecular fingerprinting method of
6 Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis (PCR-DGGE) has
7 been used to study microbial community structure in a variety of environmental samples.
8 However, these protocols must be optimized prior to their application in order to ensure PCR
9 efficiency and enhance the quality and accuracy of downstream analyses. In this study, the
10 relative efficacy of four commercial DNA extraction kits (Mobio Ultra Clean™ Fecal DNA
11 Kit, M; QIAamp® DNA Mini Stool Kit, Q; FastDNA® SPIN Kit, FSp; FastDNA® SPIN Kit
12 for Soil, FSo) were assessed and optimized for PCR-DGGE studies to test the efficacy of
13 these methods to monitor variations in human intestinal communities.

14 **Method:** Total DNA was extracted from varying weights of human feces, followed by PCR
15 amplification of bacterial 16S rRNA genes, and DGGE separation of the amplicons.

16 **Results:** Regardless of kit, maximum DNA yield was obtained using 10 to 50 mg (wet wt) of
17 feces and similar DGGE profiles were obtained. However, kits FSp and FSo extracted
18 significantly larger amounts of DNA per g feces and produced clearer DGGE profiles than
19 kits M and Q. DGGE of 16S rRNA gene PCR products was suitable for capturing the profiles
20 of human intestinal microbial community and enabled rapid comparative assessment of inter-
21 and intra-subject differences.

22 **Conclusion:** We conclude that with an optimized protocol, PCR-DGGE technique is highly
23 effective in monitoring variations in the human intestinal microflora community.

24

25

1 **BACKGROUND**

2 The microbial community colonizing the human gastrointestinal (GI) tract is known
3 to be diverse [1] and plays an important role in digestion, production of essential vitamins, as
4 well as protecting the GI tract from pathogen colonization [2, 3]. Dietary approaches such as
5 the ingestion of non-digestible oligosaccharides (prebiotics) and fermented food products
6 containing live culture (probiotics) have been speculated to confer health benefits by
7 enhancing the growth of beneficial intestinal bacteria [4]. The influence of diet on intestinal
8 microflora has been largely studied using conventional microbiological techniques. Many
9 limitations are associated with these techniques, but a significant drawback comes from their
10 reliance on the identification of appropriate growth nutrients and conditions. Estimates
11 indicate that only 20 - 40% [5] of the total intestinal microflora can be cultured using
12 standard laboratory protocols. This factor is further complicated by the need to collect
13 samples to maintain the viability of intestinal bacteria, many of which are anaerobic [6].
14 Thus, new analytical tools that can be applied in clinical studies are needed to overcome these
15 limitations.

16 In the past two decades, molecular techniques based on 16S rRNA gene and other
17 genetic markers have been developed to analyze bacterial communities in environmental
18 samples (e.g., lakes, soil) [7, 8]. These methods have an advantage over conventional
19 microbiological techniques because the presence of viable bacteria are not required [9].
20 Further, the use of genetic materials allows detection of species that cannot be cultured using
21 standard laboratory protocols. Thus, data derived from molecular techniques provide a more
22 complete analysis of the bacterial community. A molecular fingerprinting technique that
23 combines PCR-amplification of 16S rRNA gene and separation of amplicons using
24 Denaturing Gradient Gel Electrophoresis (PCR-DGGE) have produced successful results in

1 monitoring variations in microbial community in various environmental samples [7, 8].
2 However, its application in clinical studies have been limited [10-12].

3 The analytical success of molecular techniques, including PCR-DGGE, is greatly
4 affected by its reliance on cell lysis efficiency and the quality of DNA recovered from the
5 environmental samples. DNA isolation methods that contribute to insufficient cell lysis or
6 shearing of DNA may cause bias in PCR amplification [13, 14]. Inhibitors in feces, such as
7 bile salts and complex polysaccharides, will create similar problems [13, 15]. In addition, the
8 amount of fecal sample used in the extraction process affects extraction efficacy [14]. Hence,
9 it is important that upstream protocols (e.g., DNA extraction) are optimized in order to obtain
10 accurate results. Various commercial DNA extraction kits have been developed to simplify
11 and speed up the extraction process. However, the relative efficacy of these kits and the
12 optimum range of sample weight for extraction need evaluation.

13 The goal of this study is to compare the relative efficacy of four commercial DNA
14 extraction kits (Mobio Ultra CleanTM Fecal DNA Kit; QIAamp[®] DNA Mini Stool Kit;
15 FastDNA[®] SPIN Kit; FastDNA[®] SPIN Kit for Soil) in extracting bacterial genomic DNA
16 from human feces. These kits were selected due to their availability, cost, ease of use,
17 popularity, and differences in cell lysis methods. Although these kits have been tested
18 separately by different researchers on various biological samples [14, 16-18], our study
19 further extends the knowledge by direct comparison and application to PCR-DGGE.
20 Specifically, this study evaluates the influence of cell lysis techniques, fecal sample weight
21 used in extraction, and fecal dry matter content on DNA yield. By using optimized protocols,
22 this study intends to assess the feasibility of applying PCR-DGGE technique in a clinical
23 nutrition study that evaluates variations in human intestinal microbial community. Ultimately,
24 we aim to demonstrate that an optimized DNA extraction method is essential for applying
25 PCR-DGGE as an analytical tool for applications in nutritional studies.

1 **METHODS**

2

3 **Subjects**

4 Healthy volunteers aged 20 – 30 yr (n = 4) were recruited from a college community.
5 Volunteers were non-smokers, did not have any food allergies, had not used antibiotics for
6 the past 6 months, and did not have any history of GI diseases (stomach ulcers, colon cancer,
7 recent bouts of diarrhea, acid reflux disease, heartburn). Female participants were not
8 pregnant or lactating at the time of study. Protocols were approved by the Committee on the
9 Use of Human Research Subjects of Purdue University, West Lafayette, IN.

10

11 **Fecal Collection and Fecal Dry Weight Determination**

12 Feces were collected from each volunteer once a month for a total of 4 specimens per
13 volunteer, which were then stored at -20°C prior to being analyzed. To determine fecal
14 moisture content, frozen specimens were thawed at 4°C, and approximately 0.5 g (wet wt) of
15 feces was placed in a vacuum dryer for 3 d and re-weighed. Percent fecal dry weight was
16 calculated by using the following formula:

17
$$\text{Fecal dry wt (\%)} = (\text{Fecal dry wt} / \text{Fecal wet wt}) \times 100\%$$

18 To ensure sample homogeneity, remaining samples were diluted with sterile water
19 (1:2 wt/vol), and then kneaded in separate sterile plastic bags using a stomacher at high speed
20 for approximately 5 min. A sub-sample was aliquoted for DNA extraction and the remainder
21 was stored at -20°C.

22

23 **DNA Extraction and Quantification**

24 The following four commercial DNA extraction kits were evaluated:

25 **M** - Mobio Ultra Clean™ Fecal DNA Kit (MO BIO Laboratories, Inc., Carlsbad, CA)

1 **Q** - QIAamp[®] DNA Mini Stool Kit (Qiagen Inc., Valencia, CA)

2 **FSp** - FastDNA[®] SPIN Kit (MP Biomedicals, Irvine, CA)

3 **FSo** - FastDNA[®] SPIN Kit for Soil (MP Biomedicals, Irvine, CA)

4 Three variables were tested for kit extraction efficacy: ratio of water to dry matter content of
5 contributed feces, wet fecal weight used for extraction, and cell lysis method. Fecal
6 specimens submitted by the subjects typically vary in their percent dry matter content, which
7 may correlate with the concentration of microflora and subsequently the quantity of DNA
8 extracted from fecal samples. This would make it difficult to use a standardized method in
9 clinical studies. To investigate this, three fecal specimens with differing dry matter content
10 (26%, 35%, and 41%) from different individuals were selected for extraction.

11 Protocols supplied with the kits recommended different amounts of starting materials
12 for extraction (Table 1). Preliminary experiments showed that greater than 200 mg of fecal
13 specimen was not feasible. This amount overloaded the purification matrix and caused the
14 filter to rupture. Thus, five specimen weights (10, 25, 50, 100, and 200 mg (wet wt)) were
15 selected to evaluate the efficacy of the DNA extraction kits. Otherwise extraction of these
16 fecal specimens was according to the manufacturers' instructions. Additional experiments
17 with modifications to the standard protocol were conducted to determine whether the use of a
18 vigorous shaking steps, specifically using the FastPrep[®] Instrument, was the key determinant
19 in influencing DNA yield. The two kits, M and Q, that did not typically use a FastPrep[®]
20 Instrument, were tested by homogenizing 25 mg of a fecal specimen (26.3% dry matter) in
21 their respective lysing matrices using the FastPrep[®] Instrument for 30 seconds at speed
22 setting of 5.5. Note that lysis solution Q does not contain any beads typically required for
23 lysis with mechanical beating and none were added. Supernatant from each mixture was then
24 processed using subsequent steps in the protocols of kits M and Q.

1 DNA yield was quantified by fluorometric analysis (Picofluor, Turner BioSystems,
2 Sunnyvale, CA) using calf thymus DNA as a standard. Values for DNA yield were
3 normalized based on the dry weight of the respective fecal specimen. DNA quality was
4 evaluated using gel electrophoresis on 0.8% agarose stained with ethidium bromide and
5 visualized on a UV transilluminator (UVP BioImaging system, UVP LLC, Upland, CA).

6

7 **PCR-DGGE Analysis**

8 Once the DNA extraction method had been optimized, all fecal specimens were
9 evaluated for their microbial community profiles using the PCR-DGGE technique. Bacterial
10 16S rRNA gene V3 region was amplified by PCR using primers PRBA338F (5' **CGC CCG**
11 **CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG** GAC TCC TAC GGG AGG
12 CAG CAG 3'; GC-clamp is in boldface) [19] and PRUN518R (5' ATTA CCG CGG CTG
13 CTGG 3') [20]. The final PCR reaction mixture (50 µl total volume) contained 5 µl of 10x
14 PCR Buffer, 4 µl of 25 mM MgCl₂, 0.4 µl of 100 mM deoxynucleotide triphosphate mixture,
15 2.5 µl of 20 mg/ml bovine serum albumin, 0.75 µl of each primer (at 25 µM), 1 µl of 5U/µl
16 Taq polymerase, and 1 µl DNA template (approximately 1 – 10 ng/µl). The amplification
17 condition was 94°C for 5 min (initial denaturation), followed by 30 cycles of denaturation
18 92°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec. Final
19 extension step was carried out at 72°C for 15 min. Presence of PCR products were confirmed
20 by gel electrophoresis on 1.5% agarose on 1X TAE buffer, stained with ethidium bromide
21 and visualized on UV transilluminator (UVP BioImaging system, UVP LLC, Upland, CA).

22 PCR amplicons were separated using DGGE, which was conducted using the
23 DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA), with
24 slight modifications to the method previously described by Muyzer et al. [21]. Equal
25 quantities of PCR products were separated on 8% (wt/vol) polyacrylamide gels (40%

1 acrylamide/bis solution, 37.5:1; Bio-Rad Laboratories, Hercules, CA) in 1X TAE (40 mM
2 Tris, 20 mM Acetate, 1.0 mM Na₂-EDTA) using denaturing gradients of 35 to 50%, 45 to
3 60%, and 35 to 60%, where a 100% denaturant contained 7 M urea and 40% (vol/vol)
4 deionized formamide. Electrophoresis was performed at 50V for 10 min, then at 200 V for
5 5.5 hr. Electrophoresis buffer (1X TAE) was maintained throughout at 60°C. Gels were then
6 stained using SYBR Green I nucleic acid stain (Cambrex Bio Science, Rockland, ME) and
7 visualized on UV transilluminator (UVP BioImaging system, UVP LLC, Upland, CA).

8

9 **Analysis of Bacterial DGGE Banding Profiles and Sequencing**

10 Similarities between banding patterns on the DGGE profile were calculated based on
11 the presence and absence of bands and expressed as similarity coefficient. In this study, Dice
12 similarity coefficient was used to calculate pairwise comparisons of the DGGE fingerprint
13 profiles obtained from the four DNA extraction kits. This similarity coefficient is calculated
14 based on the following formula: $D_{sc} = [2j/(a+b)]$, where a = number of DGGE bands in lane
15 1, b = number of DGGE bands in lane 2, and j = number of common DGGE bands in lane 1
16 and lane 2, and $D_{sc} = 1$ indicates identical profile [22]. Dendograms showing clustering
17 according to the similarity of banding patterns of individual samples were constructed by the
18 unweighted pair group method of arithmetic averages (UPGMA) [23].

19

20 **Statistical Analysis**

21 All extractions were performed in triplicate to account for analytical variability.
22 Means of DNA yield were analyzed using SAS (version 9.1; SAS Institute, Cary, NC) by
23 one-way and two-way ANOVA. Differences between treatments were grouped by Tukey test.
24 Data are expressed as means \pm SE. Differences were considered as significant when $P < 0.05$.

25

1 **RESULTS**

2

3 **Relative Efficacy of DNA Extraction Methods**

4 Despite differences in extraction variables (weight of fecal specimen used in
5 extraction and fecal dry matter content) and protocols (Table 1), the four commercial kits
6 evaluated were found to be successful in extracting DNA from human feces (Table 2).
7 However, kits FSo and FSp produced approximately three times more DNA than kits M and
8 Q when quantities were normalized by fecal dry weight. DNA yield from kits Q and M did
9 not significantly differ from each other. A negative correlation was observed between the
10 amount of fecal weight used for extraction and DNA yield, whereby lower sample weight
11 produced higher DNA yield per gram of feces (Figure 1). Fecal specimen weight of 10 mg
12 produced significantly the highest DNA concentration, regardless of extraction kit. Fecal
13 sample weights of 100 and 200 mg, but not 10, 25 and 50 mg, produced large quantities of
14 sheared DNA. Hence, the optimum DNA yield (i.e., in terms of DNA quantity and quality)
15 was obtained by extracting 10 – 50 mg feces (data not shown).

16 The percentage of dry matter in the feces also influenced DNA yield (Figure 2). Kits
17 Q, FSp, and FSo produced significantly higher DNA yields from feces containing the lowest
18 percent of dry matter (26%), followed by 35% and 41%. In contrast, DNA yield obtained
19 from Kit M did not significantly differ regardless of the percent dry matter in the feces.

20 DNA yield from Kit M was significantly improved by incorporating a vigorous
21 shaking step using the FastPrep[®] Instrument instead of vortexing (as suggested by the
22 manufacturer's protocol) (Figure 3). Although Kit Q did not contain beads, which may have
23 contributed to its significant lower DNA yield than kit M, the additional step of vigorous
24 shaking using the FastPrep[®] Instrument contributed to a higher DNA yield in Kit Q than
25 extraction performed without this step (Figure 3).

1 **Comparative Analysis of DGGE Fingerprint Profiles**

2 A DGGE gel gradient of 35 – 50% showed a fingerprint profile of the bacterial
3 community having lower guanine–cytosine (G-C) content (Figure 4; Profile A) than those
4 captured on 45 – 60% DGGE gel (Figure 4; Profile B). However, the 45-60% gel focused on
5 the high G-C region and clearly illustrated the common bands between the profiles at the
6 lower end of the gel. It is imperative that PCR amplicons from the same set of samples be
7 separated using DGGE gels of multiple gradients in order to better distinguish common and
8 less common bands. Consequently, this practice will increase scoring accuracy. Based on a
9 DGGE gel gradient of 35-50%, the Dice similarity coefficients of community profiles from
10 DNA extracted using the four kits ranged from 0.86 to 0.97. Comparison of profiles based on
11 DNA extracted using Kit M to Kit Q had a similarity coefficient of 0.96, Kit M to Kit FSp
12 had a coefficient value of 0.86, and Kit M to Kit FSo had a coefficient value of 0.89. Profiles
13 from Kit FSo and Kit FSp had the highest similarity coefficient of 0.97 (Figure 4; Profile A).
14 Less variability in the DGGE banding profiles of DNA extracted using these kits were
15 observed for higher G-C content DNA. Banding profiles on DGGE gradient 45 – 60%
16 showed identical profiles from Kits Q, FSp, and FSo (i.e., similarity coefficient value of 1),
17 but relative band intensities varied. However, the profile from Kit M had the fewest bands
18 contributing to the lowest coefficient value of 0.89 when compared to profiles from the other
19 three kits (Figure 4; Profile B). This suggests that Kit M was not able to extract DNA from all
20 the bacteria present in the sample and hence, was the least efficient with regards to total cell
21 lysis than the other three kits. A broader gradient (35-60%) showed the entire range of the
22 intestinal bacterial community profile (Figure 5), which reflected intra- and inter-subject
23 variations.
24
25

1 **DISCUSSION**

2 Our data indicate that optimum DNA yield can be extracted from human fecal
3 samples using commercial extraction kits that consist of lysing matrix with beads, an
4 instrument that produce a vigorous shaking motion (bead-beating system), and chemical
5 reagents. From the four extraction kits that we evaluated, a combination of FastDNA[®] SPIN
6 Kit (FSp) or FastDNA[®] SPIN Kit for Soil (FSO) with FastPrep[®] Instrument appeared to
7 produce superior quality DNA that can be amplified using PCR and resolved clearly on a
8 DGGE gel. Further, the amounts of fecal specimen influenced extraction efficiency. Hence, it
9 is imperative that lysing and purification matrices are not overloaded with fecal specimen
10 during the extraction process. Too much fecal sample may cause the purification filter to
11 rupture. Consequently, this lowers the purity of DNA and the quality of downstream
12 analyses. Data from this study indicate that 10 – 50 mg of fecal specimen (wet wt) was
13 optimal for maximum DNA extraction per gram of feces and produced PCR products that
14 could be separated on DGGE gel of varying gradients. A broad gradient of 35 – 60%
15 appeared to include all the bands in the community with maximum resolution that facilitated
16 bacterial fingerprint profile comparisons. Our study provides evidence that an optimized
17 DNA extraction-PCR-DGGE protocol can be applied to nutritional studies that investigate
18 changes in the human intestinal microbial communities.

19 One of the objectives of this study was to compare the relative efficacy of four
20 commercial DNA extraction kits and to select one that would best reflect the human intestinal
21 microbial community as profiled using PCR-DGGE technique. Each of the four extraction
22 kits evaluated was supplied with specific proprietary reagents, but the overall extraction
23 concept is similar: i) breaking open cell walls to access the genetic materials contained
24 within; ii) a series of washing and filtration to eliminate inhibitors and contaminants; iii)
25 binding of DNA to a matrix; iv) purifying the bound DNA; and v) eluting DNA off the

1 matrix. Our data indicated that the quantity of DNA extracted from fecal samples is highly
2 influenced by how well an extraction kit performs in lysing the bacterial cell wall in the first
3 step of the extraction process. This step is crucial since recovering more DNA from a sample
4 increases the detection of rare species in the community [17]. The motion of a table-top
5 vortex, even when fitted with a specially designed vibrating tray (as the one used in Kit M),
6 was not sufficiently vigorous for extracting optimum DNA yield, unlike the FastPrep[®]
7 Instrument. As such, kits M and Q that used table-top vortex to induce cell lysis in bacteria
8 present in the fecal specimens produced significantly lower DNA yield than kits FSp and FSo
9 that used the latter equipment. Furthermore, DNA yield improved significantly when kits M
10 and Q were subjected to homogenization by the FastPrep[®] Instrument even though the rest of
11 the protocol remained unchanged (Figure 3). However, Kit Q, which did not have bead-
12 containing lysing matrix and relied solely on chemical lysis, produced lower DNA yield than
13 Kit M even after being shaken vigorously by the FastPrep[®] Instrument. Others have
14 demonstrated that the addition of bead beating to kit Q considerably improves DNA yield
15 [18]. On the other hand, kits FSp and FSo consistently produced significantly higher DNA
16 yield than kits M and Q. Thus, it appears that an effective and thorough lysis of the bacterial
17 cell wall in human feces can only be achieved by an extraction system that incorporates bead-
18 containing lysing matrix, a vigorous shaking motion, and chemical reagent. Subsequently,
19 this combination of lysing system will result in an optimum DNA yield. This observation has
20 also been verified with studies performed in soil [24, 25].

21 In contrast to our data, a recent study by Li et al. [18] showed that Kit Q produced
22 higher quantity of DNA than Kit FSo (respectively referred to as “stool kit” and “soil kit” in
23 their publication). Modifications in extraction protocol were performed by Li et al. [18],
24 which may have contributed to their results. Specifically, zirconia/silica beads (from another
25 supplier) were added into the lysing matrix of Kit Q and then the mixture was mixed (hence

1 inducing bacterial cell lysis) using FastPrep[®] Instrument. In addition, incubation (at 70°C and
2 95°C) was also performed to enhance cell lysis efficiency. Conversely, all DNA extractions
3 performed in our study closely followed the protocols supplied in the respective kits using
4 raw fecal samples. The above modifications were not included in the protocol for Kit Q. This
5 suggests that researchers should carefully study the manufacturer protocols and modify them
6 where necessary to obtain a better DNA yields. In retrospect, our data may indicate that Kit
7 FSo is sufficiently reliable for obtaining optimum DNA yield from human fecal sample, such
8 that modifications to its protocol is not necessary. In comparing DNA yield obtained from
9 kits FSp and M, our data is consistent with Scupham et al. [17], whereby kit FSp produce a
10 ten-fold higher DNA yield than Kit M.

11 DNA extraction efficiency is influenced by the amount of fecal specimen used for
12 extraction. Loading a smaller amount of specimen creates a greater mixing space within the
13 lysing tube. This allows for a more forceful mixing between beads, lysing buffer, and fecal
14 specimen during the bead-beating process, which improves cell lysis efficiency and DNA
15 yield. It has been suggested that DNA yield can be increased by prolonging bead-beating
16 time [26]. However, such treatment may produce smaller fragments DNA that create bias
17 during PCR amplification and affects the quality of the fingerprint profile [27].

18 Fecal samples obtained from our subjects showed variability in moisture content. On
19 average, fecal moisture content varied from 60% to 80% (i.e., reflecting dry matter content of
20 20% to 40%). More importantly, the percent fecal dry matter appeared to influence overall
21 DNA yield (Figure 2). Fecal specimens containing fibrous materials and a high percentage of
22 dry matter may have overloaded the purification system leading to lower DNA yield. It is
23 also possible that there are greater numbers of cells in the aqueous phase of feces and since
24 they do not contribute significantly to the dry biomass they are likely bacterial cells. Further,
25 kits M and Q, but not kits FSp and FSo, incorporated specific chemicals to adsorb PCR

1 inhibitors. However, bacterial 16S rRNA was successfully amplified using all extraction kits
2 and similar DGGE banding profiles were produced. Thus, either PCR inhibitors did not seem
3 to limit the quality of DNA extracted using kits FSp and FSo, or a proprietary reagent
4 contained in these kits were equally efficient in removing inhibitors. The quality of Taq
5 polymerase, the addition of bovine serum albumin in PCR, and thermal cycler setting have
6 also been shown to improve the quality of PCR products amplified from DNA extracted from
7 clinical samples [28, 29]. A combination of these factors may have contributed to our success
8 in getting high quality PCR products that produce clear DGGE fingerprinting profiles (Figure
9 4 and Figure 5).

10 The efficiency of DNA extraction varies depending on the kits and protocols being
11 used for the procedure. Subsequently, extraction efficiency highly influences the profile of
12 bacterial community when analyzed using PCR-DGGE technique. It has been observed that
13 higher extraction efficiency allows for better recovery of DNA from an environmental sample
14 that results in a more comprehensive and complete profile of the bacterial community within
15 the sample [26, 30-32]. Poor DNA extraction may mislead data analysis since a portion of the
16 bacterial community may not be clearly represented on a DGGE fingerprint profile (i.e.
17 presence of very light bands or total absence of bands). Further, having more DNA recovered
18 from a sample increases the chance of having rare species to be detected and represented in a
19 DGGE bacterial fingerprint [33, 34].

20 In general, a bacterial community comprising of 15 to 25 bands was observed within
21 an individual. There was a high similarity in banding profiles within an individual over time
22 but subject-to-subject variation was large. This observation is consistent with a previous
23 study indicating that intestinal microbial communities are host-specific [35]. Thus, within
24 subject assessment may be more meaningful than subject-to-subject comparisons when
25 evaluating a treatment effect.

1 **CONCLUSIONS**

2 Our data indicate that optimum DNA yield from human fecal specimen can only be
3 obtained by using extraction system that incorporates bead-containing lysing matrix, an
4 instrument that affords a vigorous shaking motion (bead-beating system), and chemical
5 reagents. The quantity and quality of DNA were also significantly improved by using 10 – 50
6 mg of fecal specimen (wt weight) in the extraction procedure since these amounts did not
7 overload the extraction matrices. Ultimately, we provided evidence that using optimized
8 conditions and appropriate analytical software, PCR-DGGE can be an important tool for
9 clinical studies, especially if the outcome measure is to evaluate changes in intestinal
10 microbial community responding to dietary treatments.

11

12 **LIST OF ABBREVIATIONS**

13 ANOVA, Analysis of Variance; DGGE, Denaturing Gradient Gel Electrophoresis; DNA,
14 Deoxyribonucleic Acid; FSo, FastDNA[®] SPIN Kit for Soil; FSp, FastDNA[®] SPIN Kit; M,
15 Mobio Ultra Clean[™] Fecal DNA Kit; PCR, Polymerase Chain Reaction; Q, QIAamp[®] DNA
16 Mini Stool Kit; SE, Standard Error; UPGMA, Unweighted Pair Group Method of Arithmetic
17 Averages

18

19 **COMPETING INTERESTS**

20 - None of the authors have any conflict of interest.

21

22 **AUTHORS' CONTRIBUTION**

23 - MWA was involved experimental design, recruited subjects, collected specimens,
24 performed experiments including data analyses, and wrote the first draft of the manuscript.

1 - DAS was involved in experimental design, data analyses and interpretation, and edited
2 drafts of the manuscript.
3 - CHN provided input into experimental design, data analyses and interpretation, other
4 important technical support associated with the experiments, as well as editing drafts of the
5 manuscript.

6

7 **ACKNOWLEDGEMENTS**

8 The authors wish to thank Solae LLC (St. Louis, MO) for funding for this study and Meyke
9 Ausman for technical assistance.

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21

1 **FIGURE LEGENDS**

2

3 **Figure 1. Average DNA yield obtained using the four commercial kits as influenced by**
4 **fecal specimen weights.** DNA extraction was performed on fecal specimen weights of 200,
5 100, 50, 25, and 10 mg (n = 45/ kit). Values for DNA yield were normalized based on the dry
6 weight of the respective fecal specimen. Asterisk (*) indicates the amount of fecal specimen
7 weight that produced the highest average DNA yield (significant at P < 0.05) by the
8 respective extraction kit.

9

10 **Figure 2. Average DNA yield obtained using the four commercial kits as influenced by**
11 **fecal dry matter.** The percent dry matter in the fecal specimens were 26%, 35%, and 41% (n
12 = 45/kit). Values for DNA yield were normalized based on the dry weight of the respective
13 fecal sample. Asterisk (*) indicates the amount of fecal sample weight that produced the
14 highest average DNA yield (significant at P < 0.05) by the respective extraction kit.

15

16 **Figure 3. Average DNA yield obtained using kits M and Q.** Comparison was also made on
17 the average DNA yield of these kits with and without the addition of vigorous mixing using
18 the FastPrep[®] Instrument (n = 3/kit; M, Mobio Ultra Clean[™] Fecal DNA Kit; Q, QIAamp[®]
19 DNA Mini Stool Kit). Values for DNA yield were normalized based on the dry weight of the
20 respective fecal sample. Means with different letter designation are significantly different (P
21 < 0.05).

22

23

24

1 **Figure 4. Dendograms generated from PCR-DGGE profiles obtained from DNA**
2 **extracted using the four commercial kits.** The dendograms were based on Unweighted Pair
3 Group Method with Arithmetic Averages (UPGMA). Dendogram A is based on 35 – 50%
4 DGGE gradient gel and Dendogram B is based on 45 – 60% DGGE gradient gel.

5

6 **Figure 5. A comparison of DGGE profiles of PCR amplified bacterial 16S rRNA gene.**

7 DNA was extracted using Kit FSo from 25 mg of fecal specimens collected from four human
8 subjects (Subject A, B, C, and D; n = 4 for each subject). Bacterial fingerprint profile is based
9 on 35 – 60% DGGE gel gradient. Lane 1 – 4 shows bacterial fingerprint profile of
10 consecutive fecal samples collected from each subject.

11

1 **TABLES**

2

3 Table 1 **Comparison of recommended DNA extraction protocols based on**
 4 **technical booklets included with respective extraction kits.**

5

| Extraction Kit/ Steps | M¹ | Q¹ | FSp¹ | FSo¹ |
|--|-------------------------------|--------------------------------------|---|---|
| Fecal wt (mg) | 250 - 1000 | 180 - 220 | 200 | 500 |
| Beads | Unknown beads | None | Ceramic + garnet | Ceramic + silica |
| Cell lysis and homogenization | Flat bed vortexer (10 min) | Centrifuge (14,000 rpm, 1 min) | Fast Prep [®] Instrument (speed 6.0, 40 s) | Fast Prep [®] Instrument (speed 5.5; 30 s) |
| Adsorption of inhibitors | IRS ² solution | InhibitEx tablet | None listed | None listed |

6

7 ¹ M, Ultra Clean[™] Fecal DNA Kit; Q, QIAamp[®] DNA Mini Stool Kit; FSp, FastDNA[®] SPIN

8 Kit; FSo, FastDNA[®] SPIN Kit for Soil

9 ² Abbreviations are not defined by manufacturers due to being proprietary in nature

10

1 Table 2 **Average DNA yield obtained using the four commercial DNA extraction**
2 **kits¹**. The following extractions accounted for various fecal specimen weights (10, 25, 50,
3 100, and 200 mg) and DNA yields were normalized by percent fecal dry matter (26%, 35%,
4 and 41%)².

5

| DNA extraction method¹ | 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 |

6

7 ¹ M, Mobio Ultra CleanTM Fecal DNA Kit; Q, QIAamp[®] DNA Mini Stool Kit; FSp,
8 FastDNA[®] SPIN Kit; FSo, FastDNA[®] SPIN Kit for Soil

9 ² Values of DNA yield were based on n = 45/DNA extraction method and were normalized
10 based on the dry weight of the respective fecal sample

11 ³ Treatment groups with different letters indicate significant differences between groups (P <
12 0.05). Values are means ± SE.

13

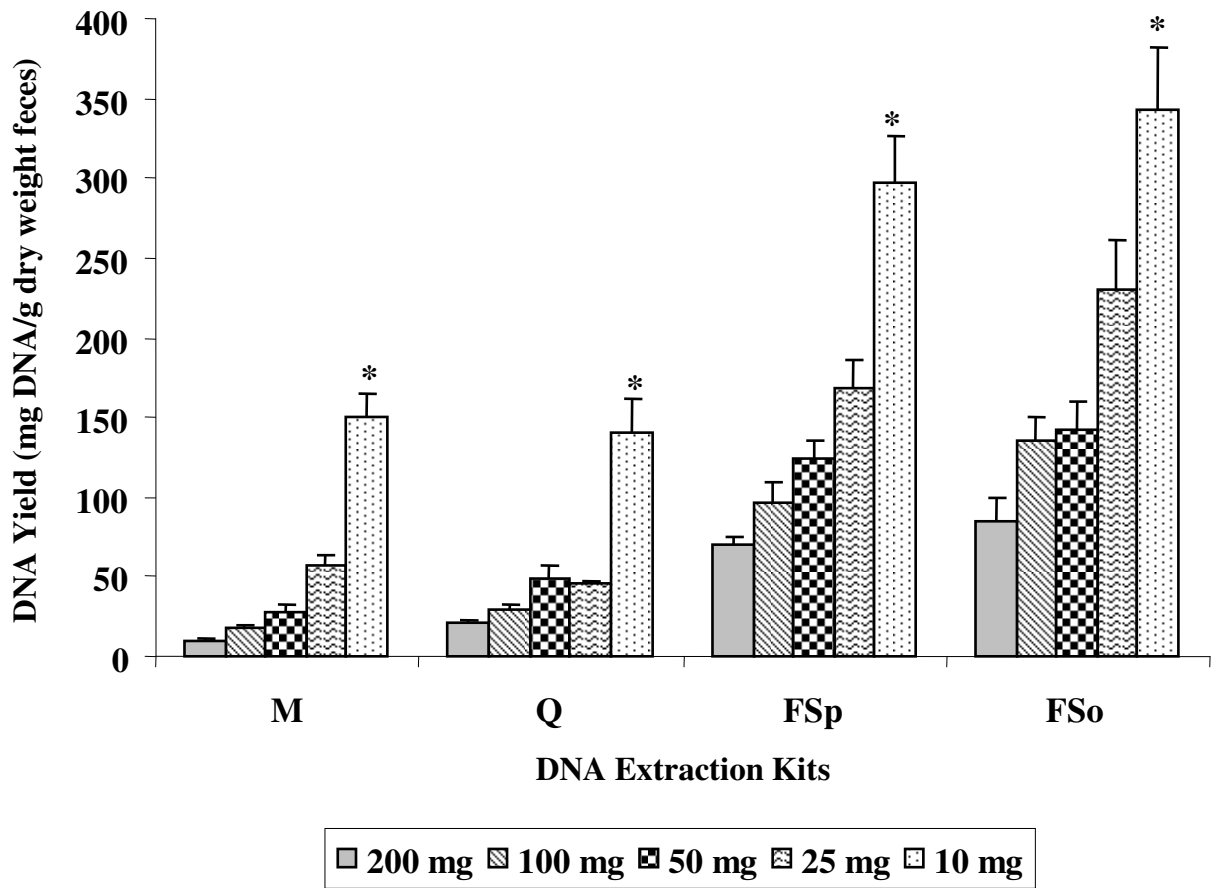


Figure 1

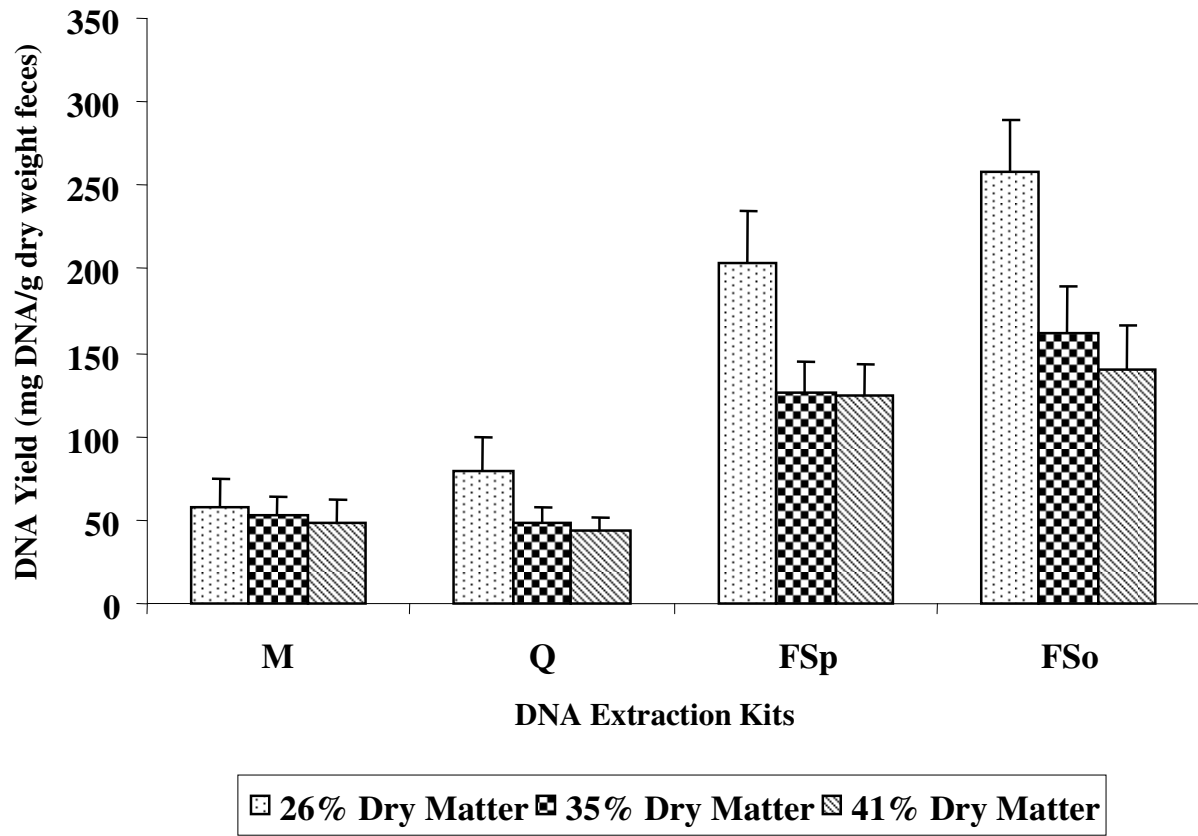


Figure 2

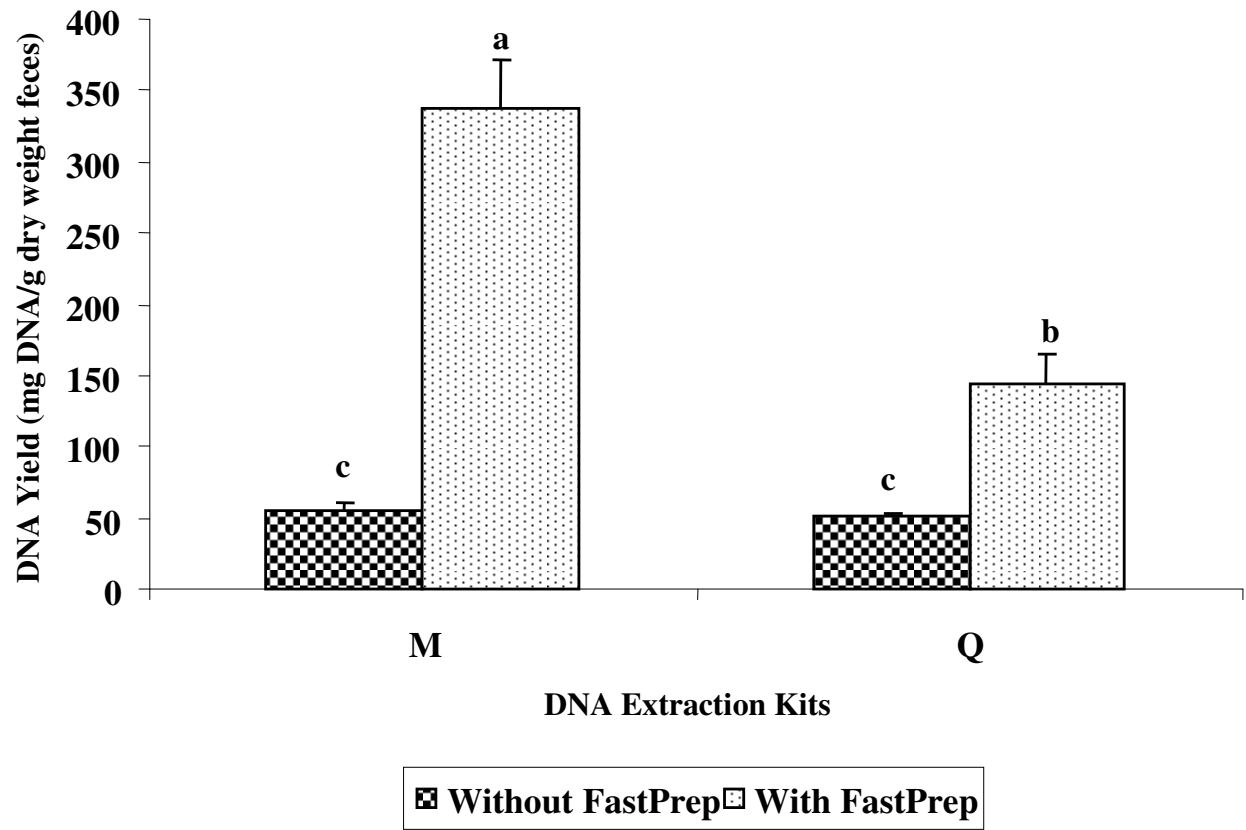


Figure 3

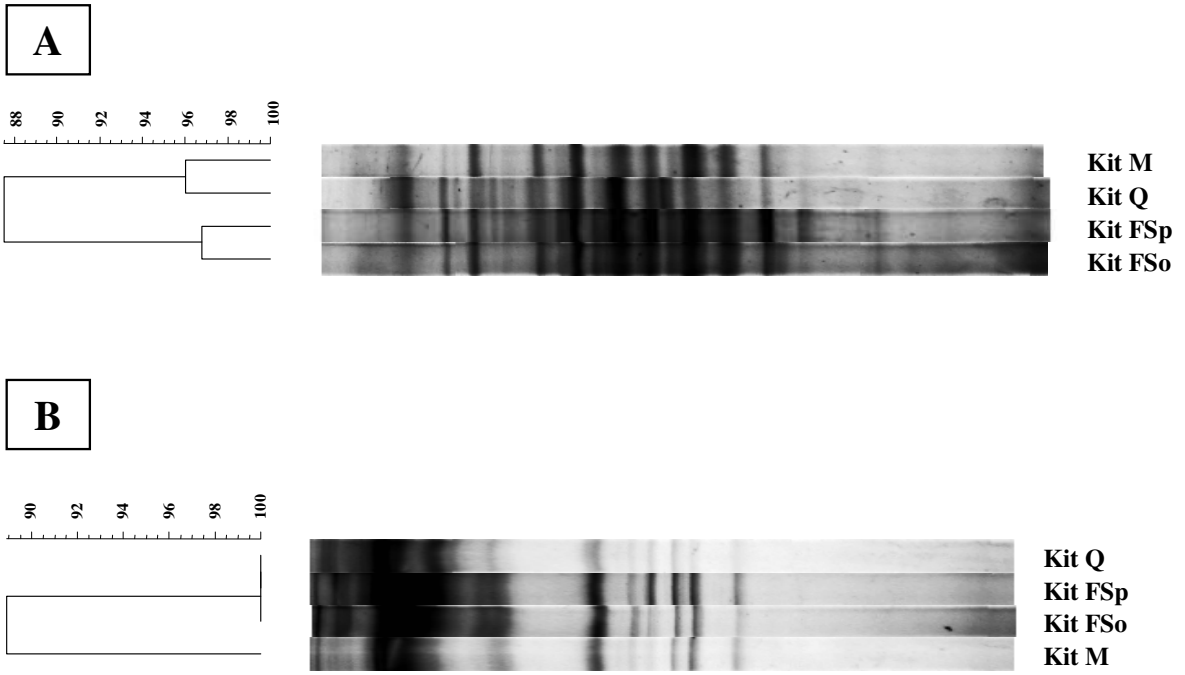


Figure 4

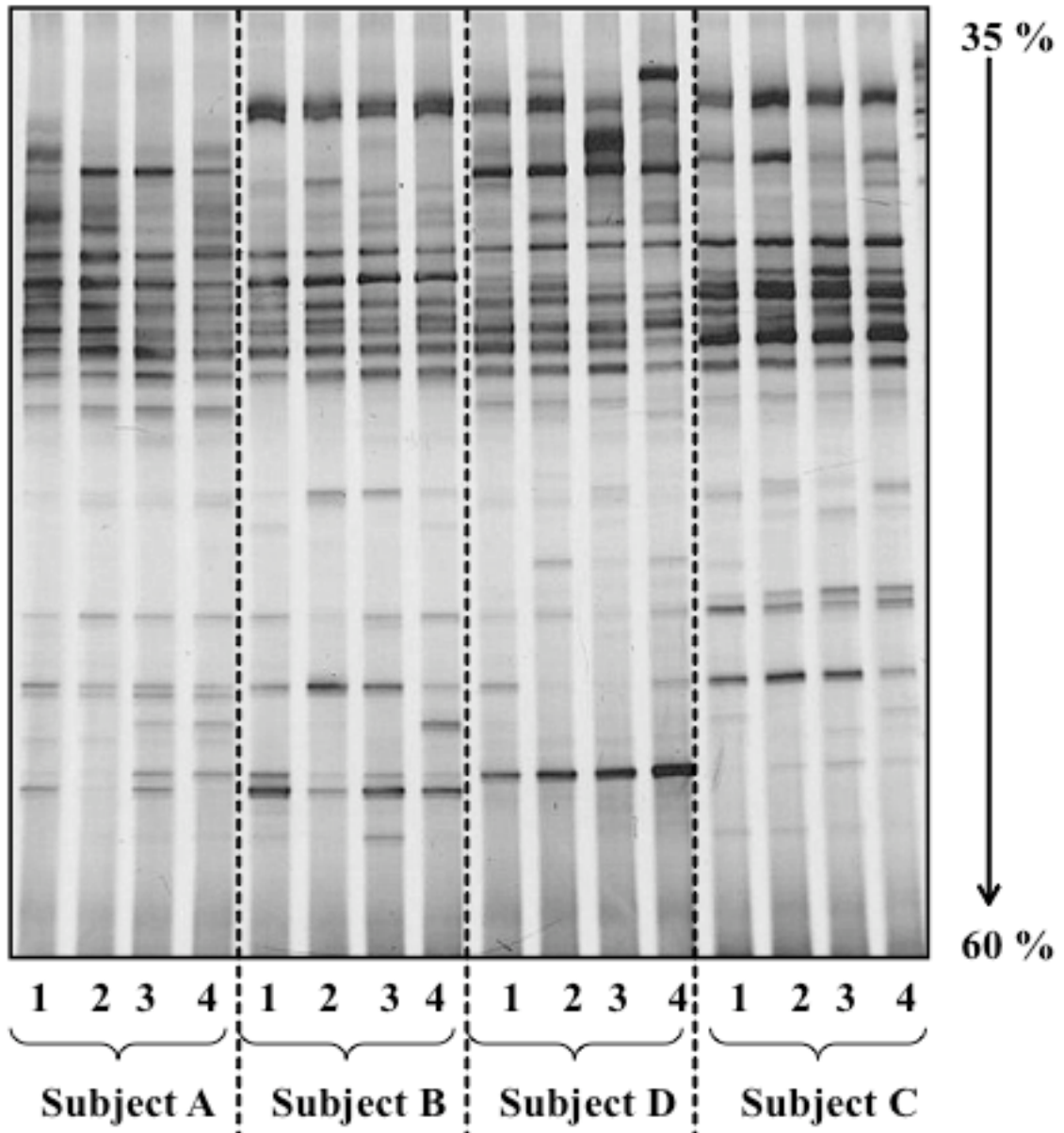


Figure 5