Author's response to reviews

**Title:** Comparison of DNA Extraction Kits for PCR-DGGE Analysis of Human Intestinal Microbial Communities from Fecal Specimens

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**Author's response to reviews:** see over
Dear the Editor of Nutrition Journal,

Please kindly find below our responses to the comments of the third reviewer. We did not extensively revise this manuscript because the comments from reviewer #3 were similar to those we received in the first round of reviews, which we felt had been sufficiently addressed at the time. It is unfortunate that acceptance of our manuscript was delayed due to this oversight. Nevertheless, we appreciate your time and look forward to a prompt and favorable response.

Regards,

Merlin Ariefdjohan, PhD

(on behalf of Dennis Savaiano, PhD and Cindy Nakatsu, PhD)
Comments from the reviewer as forwarded by the Editor of Nutrition Journal, and authors’ responses to the respective comments

We received the next comments again from the third reviewer. Since other two reviewers recommended to accept your manuscript after minor changes, we can accept your manuscript if you can appropriately address the comments from this reviewer or respond with your reasonable rebuttals.

The editor would like to pass on the following comments:

“ The authors have addressed some of the original comments, but not others. It would have been helpful if the authors were required to address each reviewer comment in a letter. Then, perhaps I would know what they were thinking as they revised (or chose not revise) the manuscript.

→ As specified in the instructions for authors stated in the Nutrition Journal, authors are required to respond in point-by-point format (i.e., one comment from reviewer, followed by specific response from the authors), which we have already followed.

The major comments that have not been addressed are as follows:

→ DNA quality is referred to quite often throughout the paper and was criticized in my initial review. However, the authors still never explain how they expressed the quality of the DNA generated by each method. They mention that agarose gel electrophoresis was used to assess quality, but not what was actually measured.

→ We have already addressed this comment previously. We mentioned that the quality of DNA was assessed based on the extent of shearing by looking at the banding pattern on an agarose gel. A gel figure was added to illustrate the extent of shearing that was observed. DNA was quantified by fluorometry and not using a spectrophotometer, therefore we were unable to obtain specific OD measurements at different wavelengths (i.e., numerical data). Unfortunately, since this project has been concluded, we are not able to reanalyze the samples using other methods. However, this is a good suggestion and we will take note of this method for future work in this area.
- I asked that they describe characteristics of the marker that was used in DGGE fingerprinting gels, their PCR positive and negative controls, as well as the settings used to analyze the gels with software. This is still not done.

→ We have also addressed these comments previously. Our response is as follows:

With regards to the marker used for DGGE printing gels, we previously mentioned that markers were not shown in any of the gels because profile comparisons were not extensively performed for this manuscript. All analyses were performed on fingerprints that were run on the same gel in adjacent lanes. However, marker was loaded flanking the four samples that were used for similarity analysis for this manuscript. In addition, we would like to add that the marker consisted of 15 bands of differing G-C contents that are sufficiently distinct to one another and that they flanked the uppermost and lowermost bands in all fingerprints analyzed.

Further, we did not go into detailed specifics of the software other than to explain that we used BioNumerics from Applied Maths to analyze bands on the DGGE gels. We previously mentioned that typically lanes in a gel are “normalized” using the software. Although the normalization step in Bionumerics software was used during gel processing it was not necessary for these gel mentioned in the manuscript since bands in the four adjacent lanes were even across the gel. The dendrograms were constructed using the UPGMA function in BioNumerics software. Since we did not perform complex band analyses in this manuscript (which is also beyond the scope of this manuscript), we do not wish to go into too much details with regards to software settings. If the reviewer wishes to find out more about this process for his own objective, he can directly contact Cindy Nakatsu (cnakatsu@purdue.edu), who is one of the authors of this manuscript specializing in such data analysis.

With regards to PCR controls, we have previously mentioned that negative control consisted of an aliquot of the mastermix, with the addition of water (instead of DNA sample). This was used to check for contamination of the PCR mastermix. All samples act as a positive PCR control since in any fecal sample we expect Bacteria to be present. Any sample that did not produce high concentrations of PCR amplicons would be re-amplified but this was not necessary for any of our samples.

- P. 10, L. 14 - "could be seen stretching" should be "were visible"

→ manuscript amended

- Figs. 1, 3 and 4. According to the figures, the maximum DNA extraction yields are in the neighborhood of 1/3 of the total dry mass of the fecal material. This is too high. Perhaps the units on the Y axis should be microg/g, not mg/g.
We have already addressed this comment, and our previous response is as follows:

We double-checked the data and the unit is correct. Studies have estimated that 60% or more of fecal dry weight can be bacterial. Also in other studies that we have performed the quantities can be lower (about 10x less) and appear to be subject/diet dependent. Unfortunately we have insufficient subject data at this time to publish the potential trend between subject and DNA yield.

- The caption for figure four - "also" should not be there...implies that the reader had read the other captions.

→ manuscript amended