

Reviewer's report

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

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Reviewer: Von Sigler

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Review of: Optimization of DNA Extraction Kits for PCR-DGGE Analysis of Human Intestinal Microbial Communities from Fecal Specimens

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The purpose of this research was to compare the efficacy of four commercial DNA extraction kits (Mobio Ultra Clean Fecal DNA Kit; QIAamp DNA Mini Stool Kit; FastDNA SPIN Kit; and FastDNA SPIN Kit for soil) in analyzing human intestinal communities of microflora. The authors are suggesting that PCR-DGGE techniques (polymerase chain reaction amplification with subsequent denaturing gradient gel electrophoresis) be applied in clinical studies using human intestinal communities to allow the broad detection bacteria. DNA was extracted from varying weights of human fecal material following the protocol for each kit. The 16S rRNA gene was amplified by PCR and the products were separated using DGGE. The two kits using the FastPrep Instrument (FastDNA SPIN Kit; FastDNA SPIN Kit for Soil) produced three times more DNA than the other kits. The authors also determined that the weight of the sample and percentage of dry matter both influenced the concentration and quality of DNA extracted. Overall the experiments appear to be correctly conducted and have provided results that will allow researchers to choose an extraction method appropriate for their needs. I would recommend this paper for publication after improvement through clarification, the specifics of which are listed below.

Major Compulsory Revisions

In general, the entire paper needs to be proofread thoroughly. Writing should reflect the past tense, but is expressed in the present tense in several places. Several instances of improper English also appear throughout the paper. Some are listed below, as examples, but many are not listed here.

P. 4, L. 2. "have" should be "has" OR "application" should be "applications"

P. 6, L. 2. "vary" should be "varied"

P. 6, L. 17. delete "a"

P. 8, L. 18. "...72o for 30 sec. A final..."

P. 8, L. 2. should be "denaturing gradient ranges"

P. 8, L. 11. "...expressed as a similarity coefficient...", "...In this study, the

Dice...”

P. 8, L. 16. “...indicates identical profiles...”

P. 9, L. 12. “...produced significantly the highest...” is awkward. Consider revising.

“DNA quality” is referred to quite often throughout the paper. However, the authors 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. They mention the impact of DNA shearing in the discussion, so maybe some measurement of “spread” of the DNA on the agarose gel could be used as an expression of shearing. In addition, quality of DNA could be quantified by measuring the A260:A280. While this would not provide an assessment of shearing, it would give the reader an idea of how clean the DNA was (and therefore a measure of its quality) following each extraction procedure.

Analysis of DGGE fingerprints– Was a marker used? What was its composition? With what frequency was it loaded? What software was used to create the dendrograms? What were the user-controlled settings in the software? Was normalization of the fingerprints to the marker or internal bands performed prior to dendrogram construction?

The authors imply that bands in the higher denaturing ranges of the gel contain DNA with high G-C content. Because other factors can impact band migration, specifically the position of G-C pairs in addition to the quantity, I don’t think that the authors can accurately make this statement. Furthermore, since no real elaboration of the importance of the G-C content is presented, its mention can probably be avoided. It would suffice to simply state that using multiple denaturing ranges can facilitate the detection of a broader profile of bacteria (a very good point), but not to bring G-C content into the discussion.

Minor Essential Revisions

P. 7, L. 8. How were the DNA extraction methods “optimized”? With the exception of the additional side experiment with the fast prep instrument, wasn’t the kit protocol followed exactly? Please clarify.

P. 7, L. 11. Not everyone who reads this paper will know what a GC-clamp is. Please provide a one sentence explanation of it for those readers. Same section – please describe your positive and negative PCR controls and if a DNA ladder was used to identify the correct PCR product size.

P. 7, L. 24. “Equal quantities of PCR product...” were these equal volumes or equal masses of DNA?

P. 8, L. 10. ...patterns in the DGGE profile...

P. 9, L. 10. “whereby lower...per gram of feces” can be deleted. This reasoning is already implied by the preceding clause.

P. 9, L. 20 – 25. This is an important observation, but this is also where the concept of DNA shearing and quality come into play. Yes, quantity might have

been higher with additional shaking, but if the DNA is sheared, the higher quantity might not be desired.

P. 10, L. 2. "...gradient of 35% - 50% showed..." "gradients" don't "show". Perhaps try "The fingerprint generated using a DGGE gradient of 35% - 50% revealed..."

P. 10, L. 6. "...lower end of the gel..." This is awkward terminology.

P. 10, L. 9. Why was the 35% - 50% gel picked for the dendrogram analyses, and not the 45% - 60% gel?

P. 10, L. 19. Fecal material was collected from four individuals, but the fingerprints from only one individual were used to compare the kits with regard to fingerprint similarity (Figure 4). The story would be greatly strengthened if the comparison was expanded to include at least one more individual, especially if the similarities were consistent among individuals one and two; OR justify why only information from one individual was used..

P. 13, L. 11-17. This discussion would be more appropriately incorporated into paragraph 1 on P. 11, where the masses of fecal material are discussed (or bring the appropriate material from P. 11 to here). Also, the material on P. 14, L. 10 – 19 could also be incorporated to make one cohesive section about appropriate sample mass# extraction efficiency#improved DGGE profiles. As it stands now, these three sections overlap, repeating a lot of information.

Figs. 1, 2 and 3. 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 #g/g, not mg/g.

Figure 1. The purpose of the asterisks is unclear, please clarify.

Figure 2. Asterisks are mentioned in description but not shown in figure. Please clarify.

Figure 4. Please add the denaturing range % to the figures.

Discretionary Revisions

Table 2. Can a column be added that expresses some measure of DNA quality (either an indication of shearing, since it is discussed in the text, or A260/A280)?

P. 11, L. 14. The authors mention in the results the importance of using multiple denaturing ranges to effectively detect the broad diversity of organisms present in the sample, but in the discussion mention that a single assay using a wide denaturant range of 35% - 60% appeared to include all of the bands in the community. I know this is picky, but the authors appear to contradict themselves with regard to their method philosophy. Please add a statement of justification for using 35% - 60% gels for the "final comparison".

P. 11, L. 16. The concluding statement of this paragraph mentions that this study has shown that DGGE can be applied to nutritional studies and to detect changes in intestinal microflora. However, I would not express the method's utility

on a nutritional study basis or on the basis of intestinal changes, as the fecal samples collected were not based on any type of nutritional treatments, nor were changes in the detected microflora discussed thoroughly. In actuality, this study effectively identified an optimized DNA isolation method for fecal material and that the DNA could be used to characterize the bacteria community in the sample. Perhaps in the conclusion section the authors can state that the method can be used in subsequent studies to improve on nutritional and intestinal change research, which would further justify their work.

P. 12, L. 1-3. How did the data indicate that DNA quantity was highly influenced by lysis efficiency? While this is a true statement overall, the authors should discuss how their data show this, since they make a direct link between the two here.

Table 1. Could two rows be added that include (i) time to completion and (ii) average cost per sample? This information might be valuable to those evaluating these protocols.

Perhaps refer to the material as “fecal material” instead of “feces”.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests