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Microbial DNA extraction from intestinal biopsies is improved by avoiding mechanical cell disruption

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Abstract

Currently, standard protocols for microbial DNA extraction from intestinal tissues do not exist. We assessed the efficiency of a commercial kit with and without mechanical disruption. Better quality DNA was obtained without mechanical disruption. Thus, it appears that bead-beating is not required for efficient microbial DNA extraction from intestinal biopsies.

Keywords

Intestinal mucosa; DNA extraction; Bead-beating; Quantitative PCR

Obtaining high yield of high quality microbial DNA from intestinal samples often proves to be a challenge. It has been extensively shown that the DNA extraction protocol is the most critical step to obtain a representative and unbiased picture of microbial diversity in environmental samples (de Liphay et al., 2004). A positive effect of mechanical cell lysis has been demonstrated for stool samples, which are often used as a representation of intestinal tissue (Salonen et al., 2010). Consequently, mechanical disruption was also suggested for protocols of nucleic acid extraction from intestinal biopsies (Zoetendal et al., 2006). However, whereas the positive impact of mechanical disruption on stool samples is evident due to the often recalcitrant biostructure of stool (Swidsinski et al., 2008), mechanical disruption of mucosal biopsies might increase the concentration of eukaryotic DNA, which can bias PCR based methods by co-amplification of 16S rRNA gene sequences (Huys et al., 2008). Mechanical disruption also results in DNA shearing, and fragmented nucleic acids induce biases in subsequent PCR reactions (Liesack et al., 1991). Here, we

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compared DNA isolation from human ileal and colonic biopsies with a commercial extraction kit recommended but not yet tested on intestinal samples (Zoetendal, et al., 2006), with or without mechanical disruption by bead-beating.

A set of eight biopsies from two healthy individuals was used to assess the effect of bead-beating on DNA extraction efficiency. The manufacturer's protocol (Qiagen DNA stool kit) was followed and the solution obtained after chemical lysis were aliquoted into two equal volumes. One aliquot for each sample was transferred into Lysing Matrix E tubes (MP Biomedicals, Ohio) (containing 1.4 mm ceramic spheres, 0.1mm silica spheres and one 4 mm glass sphere). These aliquots were then subjected to three cycles of bead-beating (15 seconds at 30 Hz) using a TissueLyser (Qiagen,). Subsequently, the protocol was carried out according to manufacturer's instructions for both samples. DNA concentration were determined using the QubitR assay (Invitrogen) Real-time quantitative PCR (qPCR) was performed on all mucosal DNA extracts using the SYBR Green PCR Master Mix (Applied Biosystems) with a particular emphasis on low abundant hydrogenotrophic microbes. Primer pair FTHFSf/FTHFSr (Leaphart and Lovell, 2001) targeting functional formyltetrahydrofolate synthetase (*fhs*) gene of reductive acetogens, ME1/ME2 (Hales et al., 1996) targeting the methyl coenzyme-M reductase (*mcrA*) gene of methanogenic archaea (MA) and DSR1fdeg/DSR4rdeg (Leloup et al., 2007) targeting the dissimilatory sulfate reductase (*dsrA*) gene of sulphate-reducing bacteria (SRB) were used. Primer pairs targeting 16S rRNA genes of Bacteria (8f/1541r (Embley, 1991)) and the Gram-positive genus *Desulfotomaculum* (DFM140/DFM842 (Daly et al., 2000)) were also used. Samples were amplified on a 7900HT Fast Real-Time PCR System (Applied Biosystems) using a dissociation curve. Standard curves were determined simultaneously using plasmids containing *dsrA*, *mcrA* and *fhs* or diluted PCR products from reference strains for the 16S rRNA genes. Gene copy numbers per ng of DNA were inferred from the standard curves.

The addition of a bead-beating step clearly increased the DNA yield, which was generally two to four times higher (1.46-2.08 ng/μL for the DNA stool kit alone, 2.73-5.86 ng/μL with bead-beating) than with the kit protocol alone. The quality of the DNA in terms of detection of microbial target genes was assessed by calculation of the number of gene copies per ng of DNA. The quantity of Bacteria 16S rRNA gene copies was similar in DNA extracted with and without bead-beating (Figure 1). Detection of low abundant hydrogenotrophic microbiota in intestinal mucosa was of particular interest. Methanogenic archaea have also been reported to be refractory to chemical lysis (Jarrell et al., 1992, Kandler and Hippe, 1977). A greater relative abundance of *mcrA* and *dsrAB* was detected in extracts that were not subjected to mechanical cell disruption (Figure 2). Gram-positive bacteria are generally more recalcitrant to chemical and enzymatic lysis, and thus it is assumed that their effective lysis requires mechanical disruption (von Wintzingerode et al., 1997). Most reductive acetogens harbouring the *fhs* gene and all *Desulfotomaculum* spp. are Gram-positive (Drake et al., 2002, Junier et al., 2010) and their corresponding genes were also detected at greater relative abundance in extracts that were not subjected to mechanical cell disruption (Figure 3).

Thus, in the case of intestinal biopsies, the benefit of bead-beating is not supported and the present data indicate that better DNA quality is obtained without mechanical cell disruption. Overall, the bead-beating step increases the quantity of microbial DNA, but it also most likely increases the presence of eukaryotic DNA from epithelial cells. Eukaryotic DNA can be co-amplified by bacterial 16S primers (Huys, et al., 2008), which might explain the observation Bacteria 16S rRNA gene copy numbers were more similar in reactions using primers targeting this domain (Figure 1). Increased eukaryotic DNA can also have inhibitory effects on microbial targeted PCR by physical obstruction (Cogswell et al., 1996). Importantly, low abundant microbes such as MA and SRB were detected in higher relative

abundance in DNA extracted without mechanical disruption. Substantial MA and SRB diversity was described using this method on another set of colonic biopsies (Nava et al., 2011), and these observations motivated the present comparative study. Even more unexpected was the observation that Gram-positive bacteria were also detected in greater abundance. The mucosa-associated microbiota represents only a thin layer of cells in biopsy tissue and it is likely that gentler mechanical disruption (vortexing) coupled with chemical and enzymatic lyses is sufficient to dislodge and break microbial cells from intestinal tissues.

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Highlights

- Standard microbial DNA extraction protocols for intestinal biopsies are needed
- DNA quality is improved when cells are not mechanically disrupted
- A commercial DNA extraction kit is more efficient for such delicate samples

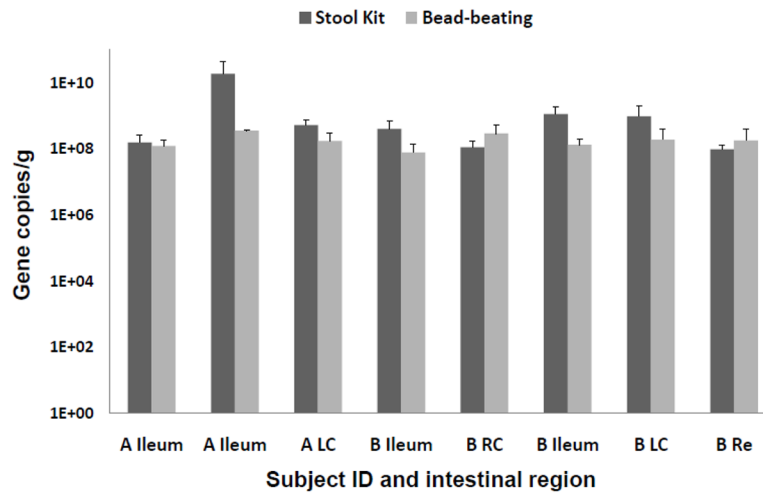


Figure 1. Bacteria 16S rRNA gene copy numbers per ng of DNA extract obtained from the DNA stool kit with and without bead-beating for two individuals (A and B). Error bars indicate the Standard Error to the Mean (SEM) based on three technical replicates. RC: Right Colon, LC: Left Colon, Re: Rectum.

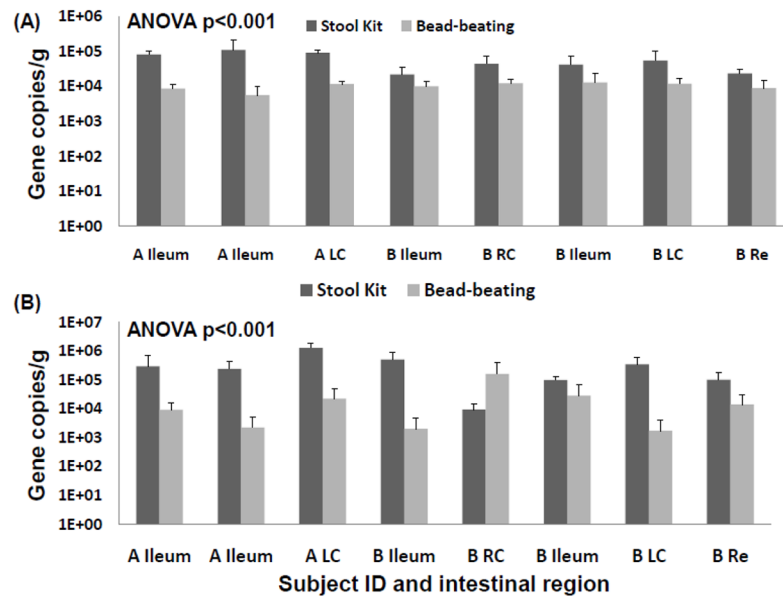


Figure 2. Bacteria 16S rRNA gene copy numbers per ng of DNA extract obtained from the DNA stool kit with and without bead-beating for two individuals (A and B). Error bars indicate the SEM based on three technical replicates. RC: Right Colon, LC: Left Colon, Re: Rectum.

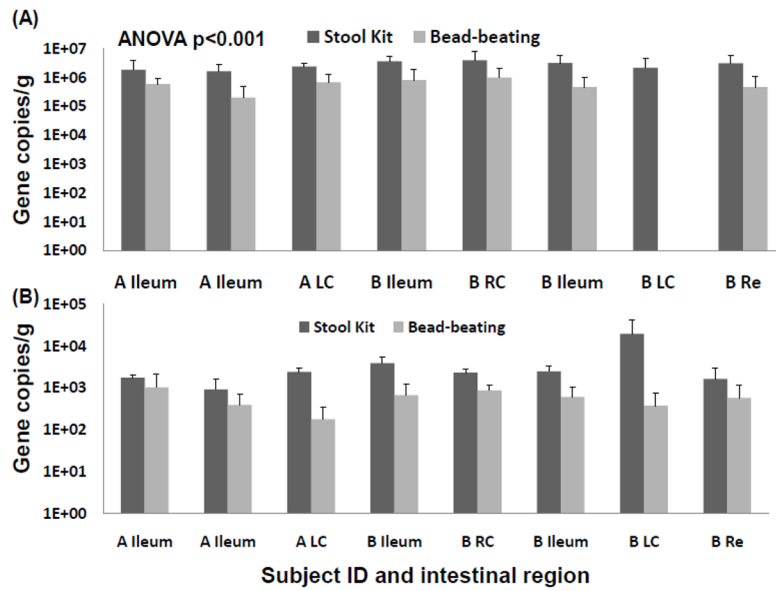


Figure 3. (A) *fhs* and (B) *Desulfotomaculum* 16S rRNA gene copy numbers per ng of DNA extract obtained from the DNA stool kit with and without bead-beating for two individuals (A and B). Error bars indicate the SEM based on three technical replicates. RC: Right Colon, LC: Left Colon, Re: Rectum.