**SUPPLEMENTARY FILE**

**Mock community**

A mock community was created by combining anaerobic cultures from 4 bacterial species belonging to either one of the phyla Gammaproteobacteria, Firmicutes, Actinobactera and Bacteroidetes. Bacterial pellets were dissolved in PBS and adjusted to an optical density (OD) of 1. OD values are an estimation of the amount of bacterial cells in a suspension, however, it does not take into account the different sizes of each species, so an estimated ratio from 40: 30: 20: 10 was created of *Bacteroides vulgates* 3775 SL(B)10, *Escherichia coli* NC101 [1], *Streptococcus gallolyticus* subsp. *gallolyticus* UCN34 [2] and *Colinsella intestinalis* DSM 13280 respectively. The mock community was used to calculate PCR efficiencies of all the individual primer pairs.

**Optimization of stool DNA isolation protocol**

DNA quality and quantity also depends substantially on the DNA extraction method used [3–5]. The Mobio DNA isolation protocol was used previously in the Human Microbiome Project [6], however, it was shown before that the MP Bio Fast DNA spin kit for soil results in higher DNA yields compared to the Mobio DNA isolation protocol [7]. Furthermore, the introduction of bead-beating can significantly increase the yield of DNA of firmicutes and actinobacteria in the Mobio kit [7,8]. However, too extensive bead-beating can also result in loss of DNA via DNA–shearing from easier to lyse bacteria like bacteroidetes and gammaproteobacteria. To find an optimal balance we tested two different types of beads with the Mobio protocol (garnet beads and 0.1mm glass beads), at 3 different bead-beating speeds (4400, 5400 and 6400 relevations per minute (rpm)) and two different bead-beating durations (2x 30 seconds or 4x 30 seconds with intervals of 30 seconds on ice) on a MagNaLyser instrument (Roche). In addition, enzymatic lysis with mutanolysin for 60 minutes at 37°C was shown to help in breaking down gram-positive cells [9], therefore we also introduced an enzymatic lysis step before the bead-beating with glass beads. The Mobio protocols were compared with the MP Bio protocol (used according to the manufacturer’s instructions) in terms of DNA yield, DNA quality and 16S rDNA amplification.

DNA quantity was obtained with the Qubit platform high sensitivity protocol. Generally, a 2-fold higher DNA-yield was achieved with the MP Bio kit compared to the Mobio kit at all bead-beating speeds and times (Supplementary table 1). However, the DNA on 1% agarose gel resulted in DNA-shearing for the MP Bio kit compared to the Mobio kit. The Mobio kit showed DNA of high molecular weight. Higher bead-beating speeds and longer durations showed some decrease in DNA quality, however, the average DNA size was still above 4000 bp at 6400 rpm (Supplementary Figure 1A). Furthermore, with qPCR relatively more 16S rDNA amplification per ng extracted DNA was seen with the Mobio kit compared to the MP Bio kit for Gammaproteobacteria and Bacteroidetes, however, total amounts of 16S rDNA amplification were similar (Supplementary figure 1B). Based on these results we selected the Mobio kit at the highest bead-beating speed. Next we tested different beads, different bead-beating times and speeds in the Mobio protocol. The glass beads were combined with mutanolysin treatment before bead-beating. First of all, for both glass beads and garnet beads, increasing bead-beating speeds yielded more Firmicutes without significant loss of Bacteroidetes or Gammaproteobacteria, but bead-beating for 2x 30 seconds was found to be better in retaining Gammaproteobacterial DNA compared to 4x30 seconds, especially with glass beads (Supplementary figure 1C). The use of glass beads with mutanolysin showed similar DNA yield and quality compared to garnet beads (data not shown), however, there was an increase in detection of Gammaproteobacteria, Firmicutes and Actinobacteria phyla / ng input DNA compared to garnet beads (Supplementary figure 1D). This was observed in DNA isolated from both stool and the mock community. The fact that with the mutanolysin and glass beads a more effective extraction was achieved for Firmicutes and Actinobacteria is in accordance with what was found in the literature [9], confirming that enzymatic lysis and smaller beads are important additions to the DNA isolation protocol that allow a better detection of Gram-positive bacteria. Therefore we selected 0.1mm glass beads combined with mutanolysin pretreatment and bead-beating at 6400 rpm for 2x30 seconds combined with the MoBio protocol. In our hands this method had the highest yields (absolute Ct values/ ng DNA).

**Final DNA extraction protocol**

The OC-sensor liquid was transferred to eppendorf tubes and centrifuged at 14000 g for 1 minute. The pellet was used for the DNA-extraction protocol. For Hemmocult cards, the cards were cut into pieces from roughly around 0.5 by 0.5 cm. The OC-sensor pellet and Hemmocult card pieces were dissolved in Mo BIO bead solution with 0,2 KU of mutanolysin. After dissolving, the card pieces were removed and the samples were incubated at 37°C for 60 minutes. Next MoBio Bead solution was added (up to 750 μl) together with the glass-beads and the MoBio Powerlyser Powersoil DNA isolation protocol was followed with the inclusion of bead-beating with the MagNA lyser at 6400 rpm (2 x 30 seconds with 30 seconds on ice in between to prevent heating). Samples were eluted in 50 μl final volume. Isolated DNA quantity was measured with the Qubit High-Sensitivity protocol (Thermo-Fisher Scientific) and quality was evaluated on 1% agarose gel in TBE-buffer with ethidium bromide. As reference the 1kb Plus DNA ladder was used (Thermo-Fisher Scientific).

**qPCR protocol and primers**

Real-time PCRs were performed in a 7500 Fast Real-Time PCR system (Applied Biosystems®). Primers were selected based on of Yang et al. [10] and De Gregoris et al. [11] and primer pair specificity was evaluated using the RDPII database ([12]; Supplementary table 2 and 3). The program was run using the following protocol: 50°C for 2 min, 95°C for 10 min, 30 cycles of 95°C for 15s and 60°C for 1 min and 95°C for 30 seconds. Melting curves were run to evaluate the specificity of the real-time PCR signal. Samples were prepared with 400 nM forward and reverse primer, 2x Power SYBR Green PCR master mix, 100 to 0.1ng of template DNA and distilled water.

**Calculation of qPCR efficiencies**

We used the formula $E= 10^{( - \frac{1}{slope})}$ to calculate the primer efficiencies [13], in which the mentioned slope is from the curve created by the Ct values of a dilution series of DNA with each sample from the mock community group. The DNA input for each reaction to create standard curves was 100, 10, 1 and 0.1 ng DNA. We found that the Ct values from the reaction with 100 ng input DNA led to inhibition of the PCR reaction, so this value was excluded from the standard curve. The standard curves for the 5 bacterial primer pairs are shown in Supplementary figure 1D with qPCR efficiencies.

**Calculation of relative abundances**

For the calculation of relative abundances (ratio) the method of Pfaffl et al. [13] was used using the following formula: $ratio= 〖E target〗\^(ΔCt (control-sample) )⁄〖E reference〗\^(ΔCt (control-sample) )$. The ratio of each bacterial phylum (target gene) of a given sample was calculated to an internal control sample (control). As reference gene, universal 16S rDNA gene amplification was used for an estimation of the total input 16S rDNA in the DNA sample. First ΔCt values were calculated by subtracting the Ct value of the OC-sensor or Hemoccult II from the Ct value of the internal control for each target gene and the reference gene. Next, the calculated qPCR efficiencies of each target phylum (*E target*) and the reference gene (*E reference*) were used to obtain ratios of the OC-sensor and Hemoccult II relative to the reference sample.

**REFERENCES**

1 Kim SC, Tonkonogy SL, Albright CA, *et al.* Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. *Gastroenterology* 2005;**128**:891–906.

2 Boleij A, Muytjens CMJ, Bukhari SI, *et al.* Novel clues on the specific association of Streptococcus gallolyticus subsp gallolyticus with colorectal cancer. *J Infect Dis* 2011;**203**:1101–9.

3 Nechvatal JM, Ram JL, Basson MD, *et al.* Fecal collection, ambient preservation, and DNA extraction for PCR amplification of bacterial and human markers from human feces. *J Microbiol Methods* 2008;**72**:124–32. doi:10.1016/j.mimet.2007.11.007

4 Kennedy NA, Walker AW, Berry SH, *et al.* The impact of different DNA extraction kits and laboratories upon the assessment of human gut microbiota composition by 16S rRNA gene sequencing. *PLoS One* 2014;**9**:e88982. doi:10.1371/journal.pone.0088982

5 Tyler AD, Smith MI, Silverberg MS. Analyzing the human microbiome: a ‘how to’ guide for physicians. *Am J Gastroenterol* 2014;**109**:983–93. doi:10.1038/ajg.2014.73

6 A framework for human microbiome research. *Nature* 2012;**486**:215–21. doi:10.1038/nature11209

7 Ariefdjohan MW, Savaiano DA, Nakatsu CH. Comparison of DNA extraction kits for PCR-DGGE analysis of human intestinal microbial communities from fecal specimens. *Nutr J* 2010;**9**:23. doi:10.1186/1475-2891-9-23

8 Walker AW, Martin JC, Scott P, *et al.* 16S rRNA gene-based profiling of the human infant gut microbiota is strongly influenced by sample processing and PCR primer choice. *Microbiome* 2015;**3**:26. doi:10.1186/s40168-015-0087-4

9 Yuan S, Cohen DB, Ravel J, *et al.* Evaluation of methods for the extraction and purification of DNA from the human microbiome. *PLoS One* 2012;**7**:e33865. doi:10.1371/journal.pone.0033865

10 Yang Y-W, Chen M-K, Yang B-Y, *et al.* Use of 16S rRNA Gene-Targeted Group-Specific Primers for Real-Time PCR Analysis of Predominant Bacteria in Mouse Feces. *Appl Environ Microbiol* 2015;**81**:6749–56.

11 Bacchetti De Gregoris T, Aldred N, Clare AS, *et al.* Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa. *J Microbiol Methods* 2011;**86**:351–6. doi:10.1016/j.mimet.2011.06.010

12 Cole JR, Wang Q, Fish JA, *et al.* Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res* 2014;**42**:D633–42. doi:10.1093/nar/gkt1244

13 Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;**29**:e45.

**Supplementary Table 1: DNA yields with Mobio and MP Bio protocols with different bead-beating speeds and durations**

|  |  |
| --- | --- |
| Conditions | DNA concentrations (ng/µl) and (yield (μg)) |
| Speed | **Duration** | **Mobio kit** | **MP Bio kit** |
| 6400 rpm | 60s | 215 (21,5) | 470 (47,0) |
| 120s | 216 (21,6) | 526 (52,6) |
| 5400 rpm | 60s | 202 (20,2) | 462 (46,2) |
| 120s | 209 (20,9) | 434 (43,4) |
| 4400 rpm | 60s | 204 (20,4) | 456 (45,6) |
| 120s | 213 (21,3) | 410 (41,0) |

**Supplementary table 2: 16S rDNA qPCR primer pairs**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Primer pair**  | **Phylum** | **Forward** | **Sequence** | **Reverse** | **sequence** | **References** |
| 1 | universal | 926F | AAACTCAAAKGAATTGACGG | 1062R | CTCACRRCACGAGCTGAC | Yang and De Gregoris |
| 2 | Gammaproteobacteria | 1080γF | TCGTCAGCTCGTGTYGTGA | γ1202R | CGTAAGGGGCCATGATG | De Gregoris  |
| 3 | Firmicutes | 928F-Firm | TGAAACTYAAAGGAATTGACG | 1040FirmR | ACCATGCACCACCTGTC | De Gregoris  |
| 4 | Bacteroides | Bac960F | GTTTAATTCGATGATACGCGAG | Bac1100R | TTAASCCGACACCTCACGG | Yang  |
| 5 | Actinobacteria | Act664F | TGTAGCGGTGGAATGCGC | Act941R | AATTAAGCCACATGCTCCGCT | Yang​ |

**Supplementary Table 3: specificity of qPCR primer pairs based on the RDP II database**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Primer specificity RDP II (Probe Match)** |  |  |  |
|  |  | **primer set** |
|  |  | 1‡ | 2 | 3 | 4 | 5\* |
| **phylum/class** | Actinobacteria | 89,60% | 1,30% | **30,90%** | <0,01% | **8,40%** |
| Bacteroidetes | 92,90% | 3,80% | 0,16% | **48,00%** | 0,16% |
| Firmicutes | 93,30% | 1,30% | **65,90%** | <0,01% | 0,83% |
| Gammaproteobacteria | 54% | **54,80%** | 0,05% | <0,01% | 0,05% |
| Verrucomicrobia | 20,60% | 0,07% | 0,11% | <0,01% | <0,01% |

\* detects mainly *Streptomycinae* and *Coriobacteridae* (excludes *Bifidobacteriaceae*)

‡ Universal primers detect only 0,1% of the order *Enterobacteriales*

**Supplementary Figure 1**

**Supplementary Figure 2**

