SUPPLEMENTARY TEXT 2

METHODOLOGY OF DNA EXTRACTION AND GENERATION OF 16S rRNA AMPLICON READS

A 250 mg stool sample was incubated with 1 ml lysis buffer (500 mM NaCl, 50 mM tris-HCl, pH 8.0, 50 mM EDTA and 4% sodium dodecyl sulphate (SDS)) in a 2-ml screw cap tube with 0.5 g sterile 0.1 mm zirconia beads and four sterile 3.5 mm glass beads (BioSpec Products, Bartlesville, OK). This was homogenised three times for 60 s at maximum speed (Mini-Beadbeater™, BioSpec Products), with cooling on ice for 60 s between homogenisation cycles. Samples were incubated at 95 °C for 15 min to further lyse the cells. Samples were centrifuged (16,000g) at 4 °C for 5 min and the supernatant was collected. For increased yield, an additional 300 µl of RBB lysis buffer was added to the pellet and the RBB steps were repeated as before. The supernatants were pooled and incubated with 350 µl of 7.5 M ammonium acetate (Sigma Aldrich, …) for 10 min. The protein-free DNA was precipitated with isopropanol at 4 °C and centrifuged at 16,000g. The pellet was washed with 70% (v/v) ethanol, allowed to dry, re-suspended in TE buffer, and treated with 10 mg/ml RNAse A (Thermo Scientific, Ireland). Proteinase K treatment and remaining DNA isolation was performed on-column using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to manufacturers' instructions leading to 200 µl of DNA eluted in AE buffer. DNA was visualised on a 0.8% agarose gel for quality assessment and quantified using a NanoDrop 2000 system (Thermo Scientific). DNA was stored at −20 °C until use.

16S rRNA gene libraries for the Illumina MiSeq System were prepared manually following the manufacturer's protocol (15031942; Illumina, San Diego, CA, USA), with some modifications. V3 and V4 region of 16S rRNA genes were amplified using 15 ng of DNA template, Phusion HF Master Mix (Thermo Scientific) and 0.2 µM primers (98 °C 30 s; 25 cycles of 98 °C 10 s, 55 °C 15 s, 72 °C 20 s; 72 °C 5 min)(60). Amplicons were cleaned up using SPRIselect magnetic beads (Beckman Coulter, Indianapolis, IN) and checked for quality on a 1.2% agarose gel. Cleaned amplicons (5 µl) were used as template for Index PCR using Phusion HF Master Mix and Nextera XT Index Kit v2 Set A and D (Illumina) (98 °C 30 s; 8 cycles of 98 °C 30 s, 55 °C 30 s, 72 °C 30 s; 72 °C 5 min). Indexed amplicons were cleaned up using SPRIselect magnetic beads, run on a 1.2% agarose gel and quantified by Qubit dsDNA HS Assay (Thermo Scientific). The samples were pooled in equimolar amounts (40 ng DNA per sample) with up to 288 samples per library. Final library sizes were validated using Bioanalyzer DNA 1000 chips (Agilent Technologies, Santa Clara, CA). Libraries were denatured with 0.2 N NaOH

and diluted to 6 pmol/L with a 20% PhiX control before loading onto the MiSeq flow cell. Sequencing was performed on an Illumina MiSeq platform using a 2 × 250 bp paired end protocol, as per manufacturer's instructions (Illumina), on multiple sequencing runs.