***DNA extraction, PCR amplification, and sequencing.***

All the samples were subjected to the same procedures for DNA extraction and PCR amplification by the same laboratory staff. The sample was suspended in 790μl of sterile lysis buffer (4M guanidine thiocyanate; 10% N-lauroyl sarcosine; 5% N-lauroyl sarcosine-0.1 M phosphate buffer [pH 8.0]) in 2 ml screw-cap tube containing 1g glass beads (0.1mm BioSpec Products, Inc., USA). This mixture was vortexed vigorously then incubate at 70℃ for 1h. After incubation by bead beating for 10min at maximum speed. DNA was extracted by following the manufacturer’s instructions for bacterial DNA extraction using The E.Z.N.A.Stool DNA Kit (Omega Bio-tek, Inc., GA), which excepted lysis steps and stored at -20°C for further analysis. The extracted DNA from each sample was used as the template to amplify the V3~V4 region of 16S rRNA genes.

The primers F1 and R2 (5’- CCTACGGGNGGCWGCAG -3’ and 5’-GACTACHVGGGTATCTAATCC-3’) correspond to positions 341 to 805 in the *Escherichia coli* 16S rRNA gene were used to amplify the V3~V4 region of each sample by PCR. PCR reactions were run in a EasyCycler 96 PCR system (Analytik Jena Corp., AG) using the following program: 3 min of denaturation at 95 ℃ followed by 21 cycles of 0.5 min at 94 ℃ (denaturation), 0.5 min for annealing at 58℃, and 0.5min at 72 ℃(elongation), with a final extension at 72 ℃ for 5min. The products from different samples were indexed and mixed at equal ratios for sequencing by Shanghai Mobio Biomedical Technology Co. Ltd. using the Miseq platform (Illumina Inc., USA) according to the manufacturer’s instructions.