**Supplementary Materials**

***Microbiome analysis***

**1. Human stool sample collection and sorting.**

Stool samples were collected from a healthy population who come to our hospital for physical examinations, combined with answering questionnaires and providing their blood lipid results and ultrasound results. The stools of newly diagnosed NAFLD patients and normal human subjects were collected after obtaining informed consent from each. The stools were collected for a period of time generally no longer than 3 days from the physical examination. Generally, on Wednesdays, we screened the human subjects who completed the physical examination on Mondays and Tuesdays and then we communicated with those who would be eligible for this study. Finally, we asked them to come back to our hospital for stool collection on Thursdays or Fridays and stored the samples in a freezer at −40ºC.

**2. Bacterial 16S rDNA sequencing.**

The bacterial 16S rDNA sequence consists of nine variable regions and ten constant regions. The variable regions vary among bacteria, and the degree of variation was closely related to the phylogeny of bacteria. By determining the variation and abundance of 16S rDNA sequences, we were able to describe the diversity of the microbial community in environmental samples.

**3. Steps for extraction of 16S rDNA samples.**

3.1 Prepared five 96-well deep plates, added 600 μL buffer with magnetic beads + 20 μL proteinase K + 5 μL RNase A + wash 1(700µl Buffer AW1+ 30µl MagPure Particles) + wash 2(700µl 70~75% ethanol) +wash 3(700µl 70~75% ethanol) +100 μL elution buffer.

3.2 Transfered 100–200 mg sample to the centrifuge tube with grinding beads. Added 1 mL ATL/PVP-10 buffer, ground the sample with the grinding machine, and incubated at 65℃ for 20 m.

3.3 Centrifuged at 14000 *g* for 5 m. Transferred the supernatant to a new tube. Added 0.6 mL buffer PCI and mixed thoroughly by vortex for 15 s.

3.4 Centrifuged at 12000 *g* for 10 m. Transferred the supernatant to deep-well plates with magnetic bead binding solution.

3.5 Transferred the deep-well plate to the proper place in Kingfisher Flex (Thermo Fisher). Started the corresponding program in the machine.

3.6 Transferred the DNA to 1.5 mL centrifuge tubes.

**4. Sample quality check.**

Two methods were used to test the whole genomics DNA samples: fluorescence quantitative and agarose gel electrophoresis. Concentration was tested with a fluorescence or microplate reader.

**5. PCR amplification and product recycle.**

Variable regions V3–V4 of bacterial 16S rRNA gene was amplified with degenerate PCR primers, 341F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR enrichment was performed in a 50 μL reaction containing 30 ng template, fusion PCR primer and PCR master mix. PCR cycling conditions were as follows: 94℃ for 3 m, 30 cycles of 94℃ for 30 s, 56℃ for 45 s, 72℃ for 45 s, and final extension at 72℃ for 10 m.

**6. Library quality check and sequencing.**

Quality inspection of the library was performed using an Agilent 2100 Bioanalyzer. Upon confirmation of library quality, sequencing was carried out on an Illumina HiSeq 2500 platform with a paired-end 300 (PE300) configuration.

**7. Sequencing and bioinformatics analysis.**

Raw reads were filtered to remove adaptors and low-quality and ambiguous bases, and then paired-end reads were added to tags by the Fast Length Adjustment of Short reads program (FLASH, v1.2.11)1 to obtain the tags. The tags were clustered into OTUs with a cutoff value of 97% using UPARSE software (v7.0.1090)2 and chimera sequences were compared with the Gold database using UCHIME (v4.2.40)3 to detect. Then, OTU representative sequences were taxonomically classified using Ribosomal Database Project Classifier v.2.2 with a minimum confidence threshold of 0.6, and trained on the Greengenes database v201305 by QIIME v1.8.0. 4 The USEARCH\_global5 was used to compare all tags back to the OTU to generate the OTU abundance statistics table for each sample.

**References**

1 Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 2011;27(21):2957-2963. doi: 10.1093/bioinformatics/btr507. PubMed PMID: 21903629.

2 Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 2013;10(10):996-998. doi: 10.1038/nmeth.2604. PubMed PMID: 23955772.

3 Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 2011;27(16):2194-2200. doi: 10.1093/bioinformatics/btr381. PubMed PMID: 21700674.

4 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, *et al*. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7(5):335-336. doi: 10.1038/nmeth.f.303. Epub 2010 Apr 11. PubMed PMID: 20383131

5 Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010;26(19):2460-2461. doi: 10.1093/bioinformatics/btq461. PubMed PMID: 20709691.