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Protocol

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High-throughput identification and quantification of bacterial cells in the microbiota based on 16S rRNA sequencing with single-base accuracy using BarBIQ

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Supplementary Methods

Supplementary Method 1 | Preparation of a mock community

We prepared a mock microbial community that consisted of ten bacterial strains (ATCC29098, ATCC700926, DSM14469, JCM1297, JCM5824, JCM5827, JCM9498, JCM10188, JCM14656, and JCM17463) which were reported as representative bacteria in human gut[1] (details in the Supplementary Table 1 of our previously published study[2]). Briefly, we cultured the bacteria of each strain and stored each of them in their own culture medium with 10% glycerol or in PBS (phosphate-buffered saline, Thermo Fisher Scientific or FUJIFILM Wako) at −80 °C until the experiments (the medium and storing condition for each strain is listed in the Supplementary Table 1 of our previously published study[2]). After culturing and before the stocks were made, we washed the JCM14656 and DSM14469 cells once with PBS using centrifugation. We cultured JCM10188 on GAM agar (Nissui), collected the bacteria from colonies, and suspended the bacteria in PBS by vortexing at 3,200 rpm for 1 min (Vortex Genie 2, Scientific Industries).

We diluted the ten strains using PBS and then made the mock community by mixing the ten strains according to the designed concentrations (Supplementary Table 1 in our previously published study[2]) in a class II biosafety cabinet. After each step of dilution or mixing, the bacterial solution was homogenized by vortexing at 3,200 rpm for 1 min. We stored the mock community at −80 °C until the experiments.

Supplementary Method 2 | Preparation of cecal samples

All procedures for mouse experiment were performed in accordance with the ethical guidelines of the institute under the protocols approved by the Animal Experimentation Committee of the Institute for Frontier Life and Medical Sciences, Kyoto University or the Institutional Animal Care and Use Committee of RIKEN. We purchased three 6-week-old C57BL/6J male mice from CLEA Japan and maintained them in the RIKEN facility (temperature: 23 °C ± 2 °C, humidity: ≲70%, and light: 24-hour light/dark cycle (lights on at 7:00 and off at 19:00)) by being fed a CE-2 diet (CLEA Japan) for 3 days in the same cage before sampling; we named these mice as CE2 nutrient group. For the VA group (Fig. 3a in our previously published study[2]), we purchased 12 8-week-old C57BL/6J male mice from Japan SLC, Inc. and maintained them at the Experimental Research Center for Infectious Diseases in the Institute

for Frontier Life and Medical Sciences, Kyoto University under specific pathogen-free (SPF) conditions (temperature: 24 °C ± 2 °C, humidity: 50% ± 10%, and light: 24-h light/dark cycle (lights on at 8:00 and off at 20:00)). We randomly allocated the 12 mice into two cages (six per cage) and maintained them for 2 weeks by being fed a sterile nutrition-balanced diet (VA-sufficient diet; designed based on AIN-93G Diet; A18041301, Research Diets, Inc.) (Supplementary Table 2 in our previously published study[2]); in the middle of the 2 weeks, we randomly selected three mice from each cage and put them into the other cage. For subsequent vitamin A-dependent experiments, we randomly allocated all the 12 mice into four groups (three per cage). Then, we continuously maintained two of the four cages for 3 weeks by being fed the VA-sufficient diet, while we maintained the other two cages for 3 weeks by being fed a sterile VA-deficient diet (A21022401, Research Diets, Inc.; only vitamin A was not included compared to the VA-sufficient diet) (Supplementary Table 2 in our previously published study[2]).

For BarBIQ measurements, we randomly selected two of the three mice in each cage. We exteriorized the murine ceca by surgery within 10 min after cervical dislocation under isoflurane (FUJIFILM Wako) or sevoflurane (FUJIFILM Wako) anesthesia. Then, we sampled the cecal contents at two locations (Fig. 1b in our previously published study[2]) within 10 min after the surgery by slicing using sterile scissors in a class II biosafety cabinet. We collected the samples of each location in each mouse in a DNA LoBind tube (Eppendorf) (see Step 3). For controls, we subjected two empty tubes to the whole process of cecal content sampling as contamination controls (see Step 3). We measured the weight of each sample immediately after collection into the DNA LoBind tubes (see Step 3); the weights of the samples in the CE2-nutrient group were ranged from 8.57 to 19.82 mg and the weights of the samples in the VA group were ranged from 1.7 to 5.6 mg.

References

1. Faith, J. J., McNulty, N. P., Rey, F. E. & Gordon, J. I. Predicting a human gut microbiota's response to diet in gnotobiotic mice. *Science* **333**, 101–104 (2011).
2. Jin, J. *et al.* High-throughput identification and quantification of single bacterial cells in the microbiota. *Nat. Commun.* **13**, 1–7 (2022).