Supplemental Information

Primer Selection: Several optimization and quality control measures were taken to ensure appropriate standardization, efficiency, and reliability of the experiments and appropriate primer taxonomic coverage as described [1]. Using the Ribosomal Database Project (RDP) probe-match website [2], 16S rRNA gene (rDNA)-targeted oligonucleotide primers were selected for to analyze mammalian gut microbial taxa at the phylogenetic order-level as well as the broadest 'universal' or panbacterial (total bacterial) primer we could find.

The four bacterial orders quantified were based on associations to dietary changes in murine gut microbiota. The Bacteroidales order of the Bacteroides class from the phylum Bacteroidetes was chosen because they are one of the two most-prevalent gut orders [3,4];

The Bacteroidales order of the Bacteroides class from the phylum Bacteroidetes. This F/R primer has been referenced [1]: Tm was 61, amplicon size was 151 bp, 16S position was 1038-1189, covered was 56/59%, serially diluted standard curve genome was *B. fragilis* at 81500000 copies in 2 µL nuclease-free water. F Primer: GGTGTCGGCTTAAGTGCCAT; R Primer: CGGAYGTAAGGGCCGTGC.

The Clostridiales order of the Clostridia class from the low-C+C phylum Firmicutes. This F/R primer has been referenced [1]: Tm was 60, amplicon size was 429 bp, 16S position was 477-906, coverage was 34/33% with subgroup coverage of Lachnospiraceae at 76/65%, serially diluted standard curve genome was *R. productis* at 44000000 copies in 2 µL nuclease-free water. F Primer: CGGTACCTGACTAAGAAGC; R Primer: AGTTTYATTCTTGCGAACG

The Lactobacilliales order of the Bacilli class also from the phylum Firmicutes. This F/R primer has been referenced [1]: Tm was 59, amplicon size was 189 bp, 16S position was 158-347, coverage was 41/83% with subgroup coverage of *Lactobacillaceae* at 78/78%, serially diluted standard curve genome was *L. delbrueckii* at 20800000 copies in 2 µL nuclease-free water. F Primer: GGAAACRRWGCTAATACCG; R Primer: GAAGATTCCCTACTGCT

The Enterobacteriales order of the γ-Proteobacteria class from the phylum Proteobacteria. This F/R primer has been referenced [1]: Tm was 60.5, amplicon size was 177 bp, 16S position was 1475-1652, covered was 59/34%, serially diluted standard curve genome was *E. coli* at 81500000 copies in 2 µL nuclease-free water. F Primer: ATGGCTGTCGTCAGCTCGT; R Primer: CCTACTTCTTTTGCAACCCACTC.

Total Bacterial Density. Panbacteria was determined to calculate proportional shifts or relative abundances of individual order. Our 'universal' F/R primer has been referenced [1]: Tm was 65.5, amplicon size was 180 bp, 16S position was 334-514, covered was 68/71%, serially diluted standard curve genome was *E. coli* at 43000000 copies in 2 µL nuclease-free water. F primer: ACTCCTACGGGAGGCAGCAGT; R Primer: ATTACCGCGGCTGCTGGC.

DNA Isolation & Quantitative PCR Protocols. Microbial genomic DNA extraction and purification as well as real-time quantitative PCR (qPCR) BioRad machine protocol and materials prep are detailed in our Methods and extensively elsewhere [1]. A gut effluent specimen weighing on average 0.45 grams was harvested immediately post-mortem and transported on dry ice to be stored at -80 °C until processed. A 400 µL sample of effluent was combined with 400 µL of molecular biology-grade methyl-phenol (equilibrated at pH 8) and 300 µL of nuclease-free (DNase/RNase-free) water. Then, a 2 mL screw-top tube was filled half way with of 0.1-mm glass beads (100g, Bio-Spec Products) were added and the sample

homogenized in a Bead-Beater (Bio-Spec Products) for 3 min at full speed. The resulting homogenized slurry was transferred to a 2 mL Phase Lock Gel Light tube (Eppendorf) and spun for 10 min at 3,500 x g (12,000 rpm). The supernatant was transferred to a new Phase Lock Gel Light tube, mixed with 700 µL of phenol-chloroform (25:24:1), briefly vortexed, and centrifuged for 10 min at 13,500 x g (12,000 rpm). The supernatant was transferred to a new eppendorf tube and precipitated in 2.5 vol cold 100% ethanol and 0.1 vol 3 M NaOAc. This was vortexed and left to sit at -20 °C for 30 minutes. Afterwards the tube was spun at 14,000 rpm for 30 minutes, the supernatant was decanted, and the precipitate (DNA pellet) was washed in 500 µL 70% cold (-20 °C) ethanol by a brief vortex cycle, and spun at 14,000 rpm for 5 minutes to re-pellet the precipitate. The supernatant was again decanted and the stable genomic DNA pellet was dried in a fume hood for several hours after which it was re-suspended in 50 µL nuclease-free water.

All pipets, tube racks, well plates sat under uv light for several hours before use on a 'clean' PCR-only bench. Each qPCR-well was run in triplicates and contained 10 µL of 2x Takara Perfect Real Time master mix which was, 7.2 µL of water, 0.8 µL of a 10 µM F/R primer mix, and 2 µL of either an optimized dilution of 1:500 of extracted template DNA in DNase/RNase-free water for specimen analysis or a serial dilution series of bacterial reference genomic DNA for standard curves; all reactions were paralleled by a non-template water control analysis in every experiment. Cycling conditions: 95 °C for 20 sec; 40 repeats of the following steps: 95 °C for 4 sec, 30 sec annealing. SYBR green fluorescence was detected with a BioRad Chromo4 Real Time PCR Detector on a Dyad Disciple Peltier Thermal Cycler. Melting curves were obtained from 55 °C to 90 °C, with fluorescence measurements taken at every 1 °C increase in temperature. Mean triplicate numbers of 16S amplicons/µL effluent detected ≥ log-fold above background noise-control were considered signal using MJ Opticon Monitor Analysis Software, Version 3.1; bacterial orders were assessed as a proportion of total bacterial density. Quality control parameters included cycle threshold no higher than 1 and an R^2 value of 0.990 or higher.

References

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