Text, Supplemental Digital Content 2

Specimen Collection, Isolation of Bacterial DNA, and Sequence Analysis. Rectal swabs were obtained between 0930-1130 at 2, 4 or 8 weeks of age. A small quantity of fecal matter was sampled using the BBL™ CultureSwab™ Collection & Transport System swab/tube (Becton Dickinson) and stored at <-60 ºC. Extraction of genomic DNA was performed using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA). Purified genomic extracts were quantified using a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA), and stored at -20 ºC in 10 mM Tris buffer until sequenced. PCR amplification of the V4 variable region of the 16S rRNA gene using variable region-specific primers (515F-806R) and amplicon sequencing were performed on an Illumina MiSeq by the Argonne National Laboratory (Lamont, IL). Each sample generated an average 30,597 sequences. Sequences were de-multiplexed and quality filtered through QIIME default settings, and closed reference operational taxonomic unit (OTU) picking was conducted using the GreenGenes 13_8 reference database (19) to classify and group unique sequences into OTUs based on 97% nucleotide sequence identity. OTUs with fewer than 15 reads, and two samples with fewer than 1,000 filtered sequences, were excluded from further analysis.