16S rRNA library preparation and next generation sequencing

16S rRNA library preparation for individual samples at Gut4Health (RRID:SCR_023673) was prepared similar to the method described in deWolfe and Wright (https://www.biorxiv.org/content/10.1101/2022.09.26.509576v1). Briefly, the V4 region of the 16S rRNA gene was amplified with barcode primers containing the index sequences using a KAPA HiFi HotStart Real-time PCR Master Mix (Roche). PCR product amplification and concentration was monitored on a QuantStudio 3 Real-Time PCR system (Applied Biosystems). Amplicon libraries were then purified using AMPure XP Beads (Beckman), normalized based on concentration, and then pooled equally. Library concentrations were verified using a Qubit™ dsDNA high sensitivity assay kit (Invitrogen) and KAPA Library Quantification Kit (Roche) following manufacturer details. The purified pooled libraries were submitted to the Bioinformatics + Sequencing Consortium at UBC which verifies the DNA quality and quantity using an Agilent high sensitivity DNA kit (Agilent) on an Agilent 2100 Bioanalyzer. Sequencing was performed on the Illumina MiSeq™ v2 platform with 2 x 250 paired end-read chemistry.