Microbiome Associated with Severe Caries in Canadian First Nations Children

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Appendix

Materials and Methods

DNA extraction and sequencing analysis

DNA was extracted from each sample using the Epicentre MasterPure DNA Purification Kit (Madison, WI), following the manufacturer's instructions after mechanical digestion with glass beads and lysozyme treatment at 37 degrees for two hours. Total DNA was sent on ice to the Forsyth Institute (Cambridge, MA) for library prep and sequencing using the MiSeq (Illumina, San Diego, CA) platform. Samples were prepped for sequencing using a previously published protocol (Caporaso et al. 2011). Briefly, 10-50 ng of DNA was used in a PCR reaction with barcoded V3-V4 primers and purified using AMPure beads (Beckman Coulter). 100 ng of each library was then pooled, gel-purified, and quantified (Bioanalzer, Agilent), and 12 pM of the mixture, spiked with 20% PhiX, was run on the MiSeq. Reads were then de-multiplexed and adaptor sequences removed. Quality filtering removed bad reads and chimeric sequences prior to analysis.

Data were analyzed using QIIME (Quantitative Insights into Microbial Ecology) v. 1.9.1 (Caporaso et al. 2010). Sequences were clustered into operational taxonomic units (OTUs) using UCLUST (Edgar 2010) with a 20-sequence minimum for defining an OTU, and then aligned and taxonomy assigned with the HOMD database (Chen et al. 2010) as reference. For alpha (within-sample) diversity, OTU tables were rarefied to 30,000 reads and chao1 and Faith's phylogenetic diversity measures were calculated. For beta (between-sample) diversity, weighted and unweighted Unifrac distances (Lozupone and Knight 2005) were calculated, followed by principal coordinates analysis.