The Human Microbiome Biodiversity in Health and Disease

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Focus: The students will analyze the human gut and vaginal microbiomes in healthy and diseased states using diversity of bacteria as determined by 16SrRNA sequence.

Overview: High-throughput sequencing of PCR-amplified taxonomic markers like the 16S rRNA gene has enabled a new level of analysis of the human body's complex microbial communities, which are collectively known as the human microbiome. Many tools exist to quantify and compare abundance levels of organisms via operational taxonomic units (OTUs) and to visualize the structure and composition of microbial communities in different conditions (e.g. healthy and diseased, or sick, states). In this introductory tutorial, we provide a simple workflow in R to perform basic analysis of gut and vaginal microbiome data in healthy and diseased states. This workflow is based on software packages from the open-source Bioconductor project (https://www.bioconductor.org/) and some CRAN packages.

Learning objectives:

1. Perform microbial ecology analysis of healthy and disease individuals based on the sequencing of the 16S rRNA gene.
2. Perform data analysis and visualization (barplots, boxplots, and dendrograms) using open source, statistical tools in R
3. Test (non-parametric) statistical differences in community composition and structure within and between samples using computational tools.

Lesson Sequence:

This lesson consists of 3 parts, within which an example is modeled for the student, the student the applies the techniques to new data, and then the student answers questions to interpret the data as processed. The three sections are as follows:

1. Part A: Taxonomic profiling of microbial communities in gut and vaginal cavity
2. Part B: Alpha-diversity analysis (within sample analysis)
3. Part C: Beta-diversity analysis (between samples and/or groups)

Pre-lesson activities: Students should complete a prelab reading on the human microbiome (pages 1-2 in the student handout).

Post-lesson activities: Students should complete the question sets, which include text answers for interpretation and the application of code to new data. Instructors should review and discuss the *biological meaning* of the results as a class to ensure students are using code merely as a tool for analysis alongside deeper interpretation.

Implementation notes: This course was originally designed to be done in a 3-hr laboratory period where students independently complete the three lessons, but has been run asynchronously online and is particularly useful in showing a medical application of ecological diversity topics as an alternative, or comparative example, to more traditional diversity measurements. Students should be familiar with the basics behind diversity indices prior to beginning, but instructors may wish to create a pre-reading quiz, particularly for the glossary terms, before the students begin the lesson, to aid learning. Encourage students to save their scripts, make notes, and answer the questions as they go (rather than at the end) to more efficiently complete the lesson.

Helpful References for Background:

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