Laboratory 2: Quality control, transformations, filtering, univariate testing, multiple comparison

Objectives:

- manipulate microbiome data in phyloseq
- calculate alpha diversity;
- run univariate testing analyses;
- adjust for multiple comparisons.

1. Load the data from Nature 488, pp. 621-626: STAT.RData.
2. Merge `phenotypes` variable with the phyloseq data.
3. Use estimate observed species, Chao1 and Shannon diversity using `estimate_richness(…)` function
4. Estimate rarified diversities in #3 averaged over 20 replicates, and compare the two estimates.
5. Summarize the data at order level.
6. Normalize the data using CLR, relative abundance, and DESeq2.
7. Compute appropriate univariate tests on the normalized data. TIP: When is `wilcox.test()` applicable as opposed to `t.test()`?
8. Adjust the p-values using False Discovery Rate. TIP: `p.adjust()`.
9. Plot the abundances using `plot_heatmap`, `plot_bar`, or produce box and whiskers plots. Which representation do you think is most useful?
10. Subset the order level dataset to only fecal samples.
11. Filter out taxa with low abundance mean abundance <0.1%.
12. Perform univariate analysis of the fecal subset with respect to the Treatment variable.
13. Perform univariate correlation analysis of the fecal taxa with respect to BMD and pFat variables. TIP: Make sure that you compute a global FDR adjustment for all comparisons combined.