**­­Microbial Diversity and Ecology Lab, Fall 2020**

**Lab 14: DADA2**

**Due**: Friday October 16, 11:59 pm

**Task**: upload this document in the assignment on WyoCourse; Week 8 lab 14 DADA2, with your answers embedded and rename the file by adding your last name at the end of the file name, e.g. Week8\_Lab14\_DADA2\_Assignment\_vanDiepen. Please save your file as a Word document.

**Points**: 1

**Objective**: In this lab you will go through a tutorial dedicated to high throughput sequencing processing in DADA2. The goal of this exercise is to familiarize you with the basics of DADA2.

**Instructions:** Download the Rmd file “Sequence processing with DADA2” from WyoCourse Week 8 Module “Lab Materials”. Copy this file to your Desktop in a new folder that you will name DADA2. You will need to save all your files in this folder for this tutorial. Saving your work will allow you to revisit your code when you need it in the future.

Open the “Sequence data processing with DADA2.Rmd” file. This will open the program R studio and the tutorial.

You are provided a working environment which contains the products of the some of the longer steps in this tutorial. This will allow you to work through this tutorial during class time.

You can begin reading in the R tutorial and refer back to this document when you are ready to answer a question or complete one of the exercises.

**##Setting up your session**

Exercise 1 (Line 47-56):

Task 1: Copy the description from the DADA2 help page and record what version you have installed.

Answer:

**###Step 1: Remove primers and other non-biological nucleotides**

Exercise 2 (Line 69):

Task 1: Copy the path you entered to both the forward and reverse reads.

Answer:

###**Step 2: Filtering and Trimming**

Exercise 3 (Line 176)

Question 1: Where would you cut your forward and reverse reads?

Answer:

Question 2: What is the main difference between 16S and ITS reads?

Answer:

Exercise 4 (Lines 194): Within the filterAndTrim function, what would you set the trimLeft and trimRight arguments to for your reads?

Answer:

Exercise 5: (Line 240): how many samples and how many taxa are in your OTU table you created with the code above?

Exercise 6 (Line 249): After removing chimeras, view the first 5 columns of your sequence table (seqtab.nochim). Copy the code you used to do so here:

Exercise 7 (Line 267): Record the number of reads retained through the entire pipeline here:

What function did you use to get this number?

Answers:

Exercise 8 (Line 271): Download the Silva 138 database and move it to your desktop folder. Change the pathway in line 273 to the correct location. Paste the code you used for that here:

Exercise 9 (Line 281): View the first 10 lines of your taxonomy table. Record your code and what families are represented.

Answer:

Exercise 10 (Line 306-309): Install the Phyloseq package. What code did you run to accomplish this?

Answer: