JOURNAL OF MICROBIOLOGY & BIOLOGY EDUCATION
DOI: https://doi.org/10.1128/jmbe.v21i1.1991

JMBE Journal of Microbiology & Biology Education

Supplemental Materials

for

Fiber Force: A Fiber Diet Intervention in an Advanced Course-Based Undergraduate Research Experience (CURE) Course

Julia Massimelli Sewall[1]*, Andrew Oliver[1], Kameryn Denaro[2], Alexander B. Chase[3,4], Claudia Weihe[3], Mi Lay[1], Jennifer B. H. Martiny[3], and Katrine Whiteson[1]

[1]Department of Moleuclar Biology and Biochemistry, University of California, Irvine, CA 92697;
[2]Teaching and Learning Research Center, University of California, Irvine, CA 92697;
[3]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697;
[4]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, La Jolla, CA 92037

Table of Contents
(Total pages 87)

*Corresponding author. Present address: Faculty of Science and Engineering, Maastricht University, Kapoenstraat 2, 6211 KW, Maastricht, Netherlands. Phone: +31 (0)63 82 02 735.
E-mail: julia.sewall@maastrichtuniversity.nl.
Received: 4 October 2019, Accepted: 5 February 2020,
Published: 30 April 2020

Appendix 1: R workshop material

Instructions: display the following instructions and R materials in a screen and walk students through each task. Breaks are added as needed. Discussion questions can be found after most exercises.

```
##        R workshop for M130L Day 1        ##

# open-source statistical software

# large number of "packages" for R freely downloadable from [CRAN](http://cran.us.r-project.org/) (Comprehensive R Archive Network)

# individual packages do pretty much everything!

# R basics

3+12

3 + 12

# R (unlike other languages) does not care about spaces between functions

2*8                    # multiplication

5 - pi                 # subtraction

14/5                   # division

14 %/% 5               # integer division

sqrt(25)          # square root

# assign variables into the R environment

z = 5

z

z <- 3

z

y = sqrt(36)

y
```

The number [1] before the answers just means that this item is the first element of a vector (vectors can be thought of as a collection of related values, such as a column in a data table).

some more basic functions

```
10^2                # power function
log(10)             # natural logarithm (i.e. ln)
log10(10)           # log base 10
log2(10)        # log base 2
factorial(10)   # factorial
```

Questions with your partner

1/ How does R represent when the output of a function is not a number? Try an example.

2/ What is the difference between log and log10?

3/ Given a right triangle with sides x=5 and y=13, calculate the length of the hypotenuse in R (reminder: a^2 + b^2 = c^2)

4/ If a population starts with a density of 5 individuals/hectare and grows exponentially at a growth rate r=0.04/year, what is the size of the population in π years? (reminder: intrinsic rate of growth == dN/dt = rN)

```
# x = 5
# y = 13
# c = sqrt((x^2) + (y^2))
```

```
# Nt = No * e^(rt)
# Nt <- 5 * exp(0.04 * pi)
```

BREAK 1

open a new R script (workplace)

this provides a record of what you did and can save this for later use!

VECTORS!

most functions in R can be applied to vectors of data, not just individual data points

many only make sense when applied to vectors, such as the following that calculate sums, first differences, and cumulative sums.

```
X = 1:10
x
```

vector of ten values from 1 to 10, demonstrates the basic R syntax for creating a sequence of numbers

```
seq(1, 10, by = 0.5)
seq(1, by = 0.5, length = 10)
rep(1, 10)
x = seq(0, 3, by = 0.01)
```

```
sum(1:10)          # sum up all values in a vector
diff(1:10)         # calculate the differences between adjacent values in a vector
cumsum(1:10)   # cumulative sum of values in a vector
prod(1:10)         # product of values in a vector
```

Questions with your partner

1/ Describe the difference in output between sum and cumsum.

2/ Generate a sequence of even numbers from -6 to 6

3/ Generate a sequence of values from -4.8 to -3.43 that is length 8 (show code)

a. What is the difference between values in this sequence?

b. What is the sum of the exponential of this sequence?

BREAK 2

Combining vectors

simple function "c( )"" in R that “combines” vectors or numbers into a single vector

x = c(1,7)

x

y = c(10:15,3,9)

y

c(x,y)

Vectors can also be used for indexing other vectors

y[x] # return the 1st and 7th element of y

combine vectors to build up data frames by “binding” them together either are rows or as columns

p = 1:10

q = 10:1

cbind(p,q) # bind as columns

rbind(q,p) # bind as rows

Logical operators and indexing

R can perform standard logical comparisons, syntax for the different logical operators, some of which are odd:

````

> greater than

< less than

>= greater than or equal to

<= less than or equal to

== equal to (TWO equals signs...you were very close!)

!= not equal

````

1 > 3

5 < 7

4 >= 4

-11 <= pi

```
log(1) == 0

exp(0) != 1


# combine multiple logical operators using the symbols for ‘and’ (&) and ‘or’ ( | )

w = 4

w > 0 & w < 10

w < 0 | w > 10


# "logical" expression like "y > x" in R you get a TRUE/FALSE

z = y>13

z


# logical operations are performed element-by-element.

# If you want to apply a logical test to a whole vector at a time you can use the function "any" to test if any of the values are true and

# "all" to test if all values are true

any(y>13)

all(y>13)


# Questions with your partner

# 1/	Create a vector that contains the names of 4 superheroes.

# 2/	What is the difference between = and == ?


## BREAK 3


# working and saving data


setwd('path/to/directory')


frogs = c(1.1, 1.3, 1.7, 1.8, 1.9, 2.1, 2.3, 2.4,

2.5, 2.8, 3.1, 3.3, 3.6, 3.7, 3.9, 4.1, 4.5,

4.8, 5.1, 5.3)
```

```
tadpoles = rnorm(n = 20, mean = 2 * frogs, sd = 0.5)


dat <- cbind(tadpoles, frogs)


# working with data

# One of the first things you'll do with any data set when you first load it up is some basic checks to see what you are dealing with.

# Typing the variable name will show you its contents, but if you just loaded up something with a million entries then you'll sit for a long time as R lists every number on the screen.

# The function class will tell you the type of data you've just loaded.


class(dat)


# helpful TOOL

# Be aware that RStudio has the capacity to auto-complete function names, function arguments, and file names


# So, for example, if you type 'read.t' and then hit TAB, RStudio will finish typing read.table and it would also show what information you can specify for the read.table function.


# If you type read.table( and then hit TAB, RStudio will allow you to select the function argument that you want to fill in.

# If you type read.table(" and then hit TAB, RStudio will show you the files in your current working directory and allow you to select one.


# If there are a lot of files in the directory, you can start typing the file name you want and then hit TAB again and RStudio will limit what it shows to just those files that match what you've typed so far




# save the R environment and variables to use later

save(dat, x, y, c, file = "Lab1.RData")


# have students close and exit R, open a new window and:

setwd('path/to/directory')
```

```
load("Lab1.RData")

# or save EVERYTHING so far:
save.image("Lab1_all.RData")

# not sure what variables you have defined,
ls()



# visualize dataframe in R environment

# save as .csv file (like Excel format)
write.table(dat, "my_frogs.csv", row.names = FALSE, sep = ",")



# character data in R is usually displayed in double quotes to indicate that it is character data (e.g. the character “1” rather than the number 1)
# Note that when your data is characters, you'll need double quotes in your comparison. e.g.
a = c("north","south","east","west")
# also do logical comparisons with characters as well
a == "east"

# get the basic structure of the data

dat <- read.table("frogs.txt", header = TRUE, sep = '\t')

class(dat)

# dat is in a “data.frame”, which is like a matrix but can also contain non-numeric data.
# basic (or atomic) data types in R are integer, numeric (decimal), logical (TRUE/FALSE), factors, and character

str(dat)
```

```
# from this we learn that there are four columns of data named “frogs”, “tadpoles”, “color”, “spots” and
# that there are 20 rows of data, and
# that the data is numeric for the first two, a factor for the third, and logical for the fourth.

names(dat)
# get the names of the columns (remember we used header = TRUE!!!)

dim(dat)
# get dimensions of dataframe
nrow(dat)
ncol(dat)

# We can refer to specific columns of data by name using the $ syntax
# useful with auto-complete TAB function!

dat$frogs                          # show just the ‘frogs’ column
dat$color[6:10]          # show the 6th though 10th elements of the color column

# for a single vector, use length
length(dat$frogs)

# preview the data (useful if working with really large files!)
head(dat)
tail(dat)

# get quick statistical summary of each vector in the dataframe
summary(dat)

# Questions with your partner
# 1/ Try a few of these functions out and discuss with your partner. What else do you notice about these?
```

BREAK 4

Organizing data for analysis!

simple comparisons can provide a powerful means for subsetting data

the "," is needed to identify subsetting from where (e.g. columns or rows)

```
dat[dat$frogs >= 3,]
```

or use the R built-in functions

```
subset(dat,frogs >= 3)
```

or if you only want a specific column in the output

```
subset(dat, frogs >= 3, c("tadpoles","spots"))
```

Questions with your partner

1/ For the frog data set:

a. display just the rows where frogs have spots

b. display just the rows where frogs are blue

c. how many blue tadpoles are there?

d. create a new object containing just the rows where there are between 3 and 5 tadpoles

e. display just the rows where there are less than 2.5 red frogs

f. display where either frogs do not have spots or there are more than 5 frogs

BREAK 5

Analyzing data and basic statistical inference

want the ability to summarize and visualize data

```
table(dat$color)
table(dat$color,dat$spots)
```

basic statistical measurements - expanding on summary() function

```
mean(dat$frogs)

median(dat$tadpoles)

var(dat$frogs)                                          ## variance

sd(dat$frogs)                                                   ## standard deviation

cov(dat$frogs, dat$tadpoles)                    ## covariance

cor(dat$frogs, dat$tadpoles)                    ## correllation

quantile(dat$tadpoles, c(0.05,0.90))    ## 5% and 90% quantiles

min(dat$frogs)                                                  ## smallest value

max(dat$frogs)                                                  ## largest value


# R also has a set of apply functions for applying any function to sets of values within a data structure.

apply(dat[,1:2], 1, sum)        # calculate sum of frogs & tadpoles by row (1st dimension)

apply(dat[,1:2], 2, sum)        # calculate sum of frogs & tadpoles by column (2nd dimension)


# function "apply" will apply a function to either every row (dimension 1) or every column (dimension 2) of a matrix or data.frame.

# In this example the commands apply the “sum” function to the first two columns of the data (frogs & tadpoles) first calculated by

# row (the total number of individuals in each population) and

# second by column (the total number of frogs and tadpoles)


tapply(dat$frogs, dat$color, mean)              # calculate mean of frogs by color

tapply(dat$frogs, dat[, c("color","spots")], mean)      # calculate mean of frogs by color & spots


# function "tapply" will apply a function to an R data object, grouping data according to a second variable or set of variables.

# The first example applies the “mean” function to frogs grouping them by color.

# The second shows that tapply can be used to apply a function over multiple groups, in this case color X spots.


# PLOT DATA


plot(dat$frogs, dat$tadpoles)                   ## x-y scatter plot
```

```
abline(a = 0, b = 1)                                    ## add a 1:1 line (intercept=0,
slope=1)


plot.new()


hist(dat$tadpoles)                                      ## histogram

abline(v = mean(dat$tadpoles))                          ## add a vertical line at the mean


pairs(dat)                                                          ## all pairwise
scatter plots


plot.new()


barplot(tapply(dat$frogs, dat$color, mean))             ## barplot of frogs by color

abline(h = 3)                                                       ## add a horizontal line at
3


# Assignment for Friday - show all your work and stored variables

# Submit to Andrew the .Rdata file or .R script through Canvas

# 1/      Plot the following lines from 0 to 3 (hint: define x as a sequence with a small step size). Make sure to make
the resolution of x sufficiently small to see the curves

#       a.      ln(x)

#       b.      e^{-x}

# 2/      Make a barplot of the median number of frogs grouped by whether they have spots or not.

# 3/      Plot a histogram of only blue frogs. Please include the code to subset data as well.

# 4/      Use apply to calculate the across-population standard deviations in the numbers of frogs and tadpoles

#

#
```

Appendix 2: Qiime2 workshop

QIIME2 Analysis

The main website (https://docs.qiime2.org/2019.7/tutorials/) for Qiime2 contains all the necessary information for installing and running QIIME2 from a computer. This is meant to more directly reflect what was conveyed to the students. It is advisable to go through the Moving Pictures tutorial prior to leading a workshop, of which this is a subset of that excellent tutorial.

1. Students brought their own, or shared, laptops to class for the two days during this QIIME workshop session. The instructor should check that all students have access to a Linux environment. For Macintosh computers, the terminal is sufficient. For PC computers with Windows 10 or greater, Windows Subsystem for Linux is necessary.

2. To install QIIME2, follow the instructions located on this website: https://docs.qiime2.org/2019.7/install/native/ or follow below.

a. install miniconda for your system (choose the correct version based on the version of python you have installed)

i. website for miniconda: https://docs.conda.io/en/latest/miniconda.html

ii. to test for python, run the code below in a mac or linux terminal:

```
python
quit()
```

Your terminal should show something similar to Figure 1.

b. Next in the terminal (not in python), install the most recent version of QIIME2

i. this part will take some time, so in part 3 we will talk about vocabulary of sequence analysis and also basic navigation of the command-line.

```
wget https://data.qiime2.org/distro/core/qiime2-2019.7-py36-osx-conda.yml
conda env create -n qiime2-2019.7 --file qiime2-2019.7-py36-osx-conda.yml
# OPTIONAL CLEANUP
rm qiime2-2019.7-py36-osx-conda.yml
```

3. Navigating the command-line. In the time Qiime2 is installing, have the student's practice navigating the command-line.

a. open up a new terminal window

b. let's see what directory (folder) we are in:

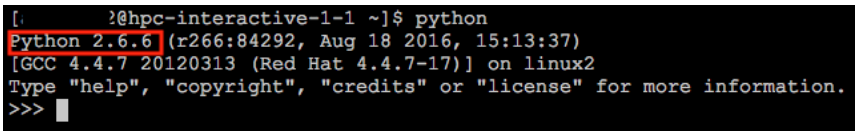


Figure 1: picture of terminal

```
print working directory
pwd
# the output should say the directory you are in, for example
# /Users/student or /Users/student/Downloads
```

c. to see what is in the current directory (folder), use the list command. This will give you a list of all the files and folders in the current directory

```
ls
```

d. next, to change to a new folder, we use the change directory command:

```
# for instance, if we wanted to go to this student's Downloads folder:
cd /Users/student/Downloads
# or we wanted to go to the previous directory, two periods will allow us to jump back
# to the "higher" directory
cd ..
```

e. next, to make a directory, we use the make directory command:

```
# it is always good practice to not have spaces in the folder names
mkdir qiime_practice
```

These are the basic tools to navigate the command-line. The internet is host to many tutorials. A very good and entertaining tutorial for these tools and more is the MIT terminus game:
http://web.mit.edu/mprat/Public/web/Terminus/Web/main.html

4. Learning the vocabulary of sequence analysis.

a. For the scope of this course, it was important for students to learn the following concepts:

i. How QIIME pipeline works

1. Import data: what does raw data off the sequencer look like? What does a fastq file look like? What does QIIME need to import the files (barcode file, forward reads, reverse reads)

2. Demultiplex data: What is a multiplexed library? Why do we multiplex? What is the purpose of barcodes?

3. Quality filter data: What does good sequence data look like, and what are the metrics we use to define good sequence data?

4. Denoise data: although slightly beyond the scope of this class, the idea of reducing the amount and complexity of the data for downstream analysis should be emphasized here. Denoise data can take hours and days, but it allows use to analyze the resulting "clean" data much more quickly.

5. Calculate diversity and other statistical metrics: see the next section

ii. Diversity metrics

1. alpha diversity

2. beta diversity

3. Operational taxonomic unit, exact sequence variant

4. Principle coordinates

5. Getting a feel for QIIME2: running example commands for less computationally tough tasks

a. For this part, students ran parts of the "Moving Pictures" tutorial, found on this website: https:

//docs.qiime2.org/2019.7/tutorials/moving-pictures/

i. care was taken to avoid steps such as demultiplexing and denoising.

```
first make a directory and navigate into it!
mkdir qiime_practice
cd qiime_practice

# next, download the sample metadata
wget -O "sample-metadata.tsv" \
https://data.qiime2.org/2019.7/tutorials/moving-pictures/sample_metadata.tsv

# next, make a new, sub-directory for the raw sequences:
mkdir emp-single-end-sequences
cd emp-single-end-sequences

# download the raw sequences:
wget -O "barcodes.fastq.gz" \
"https://data.qiime2.org/2019.7/tutorials/moving-pictures\
/emp-single-end-sequences/barcodes.fastq.gz"
wget -O "sequences.fastq.gz" \
"https://data.qiime2.org/2019.7/tutorials/moving-pictures\
/emp-single-end-sequences/sequences.fastq.gz"

# next, run the command to import all the data:
cd ..
qiime tools import \
--type EMPSingleEndSequences \
--input-path emp-single-end-sequences \
--output-path emp-single-end-sequences.qza
```

```
# the next step is too tough usually for most laptop computers, so it makes more sense
# to download the pre-made files on the QIIME2 Moving Pictures tutorial website. Download:
# 1. demux-details.qza
# 2. demux.qza
# 3. demux.qzv

wget -O "demux-details.qza" \
"https://docs.qiime2.org/2019.7/data/tutorials/moving-pictures/demux-details.qza"

wget -O "demux.qza" \
"https://docs.qiime2.org/2019.7/data/tutorials/moving-pictures/demux.qza"

wget -O "demux.qzv" \
"https://docs.qiime2.org/2019.7/data/tutorials/moving-pictures/demux.qzv"

# Next, have the students visualize the demux.qzv file on the qiime viewer, located
# here: view.qiime2.org. Simply drag and drop the demux.qzv file onto the website.
# the result should look like Figure 2.
# The next step, denoising, is also too tough for laptop computers. At this point we
# downloaded what was necessary to run the core-metrics command, several steps
# lower in the Moving Pictures tutorial:
```

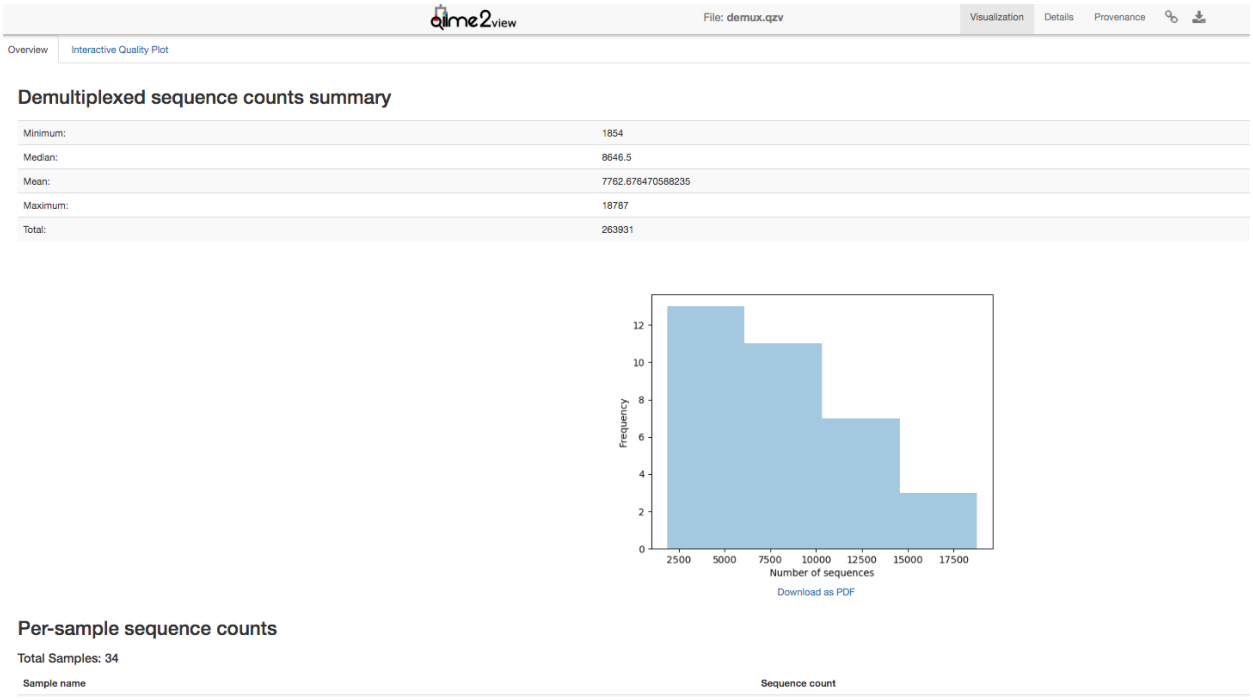


Figure 2: Screenshot of demux.qzv file. Have students discuss what sequence depth to rarefy to and what quality scores to trim to.

```
wget -O "table.qza" \
"https://docs.qiime2.org/2019.7/data/tutorials/moving-pictures/table-dada2.qza"

wget -O "rooted-tree.qza" \
"https://docs.qiime2.org/2019.7/data/tutorials/moving-pictures/rooted-tree.qza"

wget -O "rooted-tree.qza" \
"https://docs.qiime2.org/2019.7/data/tutorials/moving-pictures/rooted-tree.qza"

# the core-metrics command is below. What sampling depth did your students
# choose?

qiime diversity core-metrics-phylogenetic \
--i-phylogeny rooted-tree.qza \
--i-table table.qza \
--p-sampling-depth ???? \
--m-metadata-file sample-metadata
--output-dir core-metrics-results
```

b. After the core metrics analysis runs, a folder called core-metrics-results will be created, and there will be several visualization files. These are great plots for students to look at and discuss. An example of an emperor plot (a PCoA plot basically), is below as figure 3.

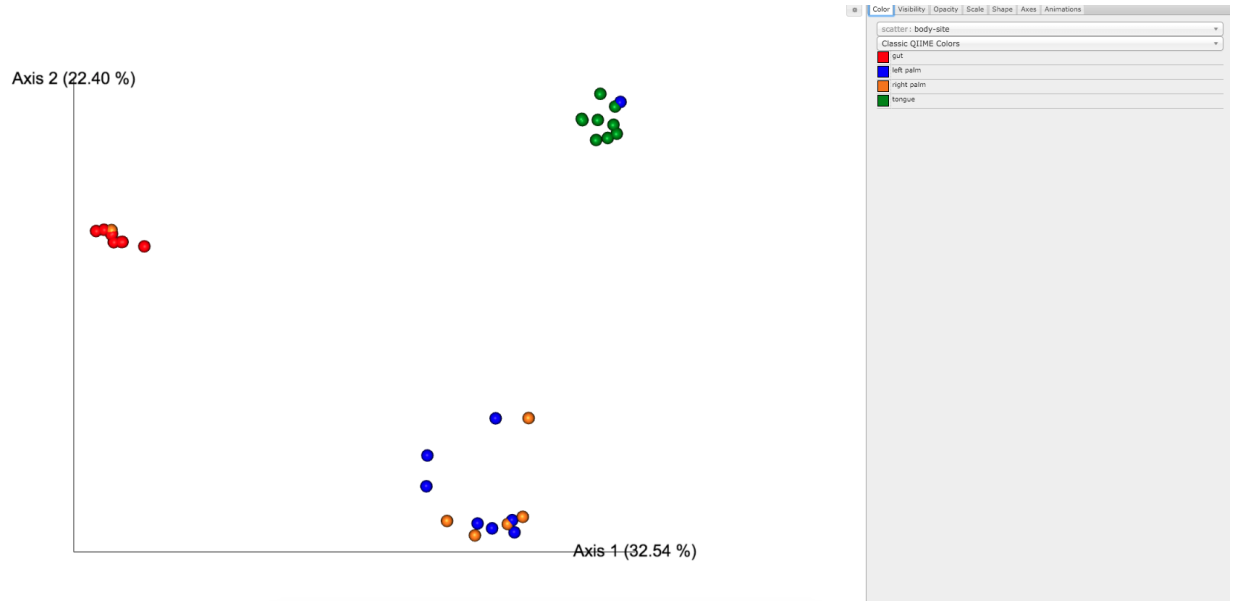
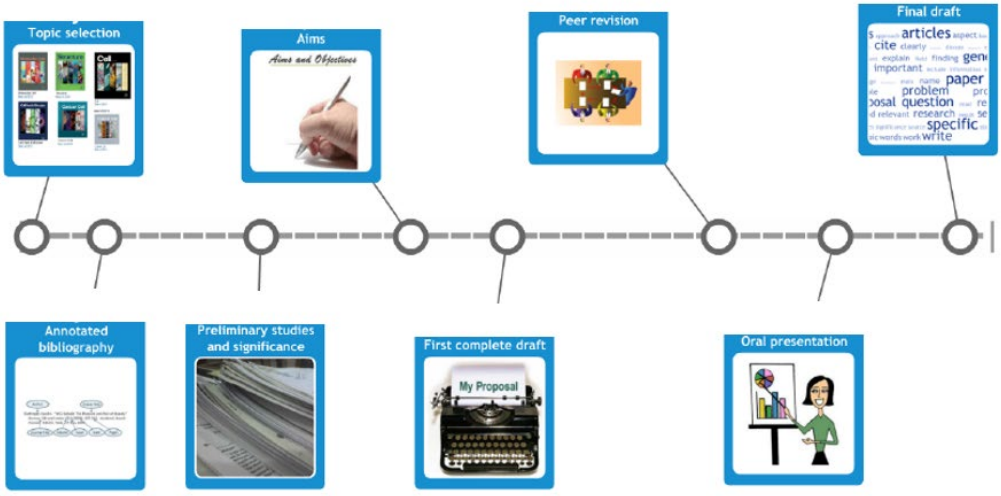


Figure 3: Screenshot of bray_curtis_emperor.qzv file. This is a good plot to talk about how sample relate to each other and what goes into making a plot like this. What do the points represent?

The second day of the workshop was more self-paced. Students joined small groups and discussed how the Qiime2 output could help them answer questions they would eventually have about their own data. Specifically, is there a difference between high fiber and low fiber microbiomes? In that case, perhaps they will stumble on the "qiime diversity beta-groupsignificance" command and learn about PERMANOVAs in the process.

Appendix 3: Grant proposal writing timeline



http://www.readwritethink.org/timeline

Topic selection: Students choose a topic and a big picture research question to answer from a pool of papers. Top-5 articles rank submitted for participation points.

Annotated bibliography: students find 10 papers to cite. Papers are listed as a formatted bibliography. For each paper, students also write a summary, including the relevance to their proposal topic, and a citation statement. **10 points**.

Preliminary studies and significance: the students take 3 weeks to draft this section. Detailed feedback is provided. **10 points**. Graded mainly based on effort.

Aims: students write their question or global aim and 2-3 specific aims. Grant proposal examples (available online) are discussed during class to exemplify the wording and details required to write specific aims. **5 points**. Feedback of aims by email.

First Draft: The draft includes all the sections they worked on until now (polished) plus the experimental design and methods they will use to answer each specific aim. Grant models are once again provided and discussed to serve as a model. **20 points**.

Peer revision: students re-write the first graft based on the feedback received by the instructor and prepare a new draft for peer revision. Here, they read each other proposal drafts, providing comments regarding writing, ideas and approaches. Extra credit.

Oral presentation: each student gives a 10 min-PowerPoint talk explaining their proposals to an audience of students and faculty, answering questions about their topic, aims and approaches. **30 points**.

Final Proposal: the students write a polished final proposal that addresses all the criticisms and questions raised by readers and the oral communication audience. **60 points**.

Appendix 4: 10 weeks course schedule

WEEKLY SCHEDULE:

Project: Fiber Force

Lectures held Wednesday 12-1.50 pm. Modality: active discussions.					
Laboratories (Lab) held from Wednesday 2-3.50 and Friday 12-3.50 SH288 Sometimes lab time is used for discussions.					
	M	**T**	**Wednesday**	**Th**	**Friday**
Lecture W1	Class intro, projects. Lab skills (serial dilutions). Introduction to R				
Lab **W1**			Project 2 ☐ Go over microbiome papers about diet. Discuss intervention. ☐ Download R Studio ☐ Download MyfitnessPal Log food into MyFitnessPal	**Proposal Topics Due (ranked list)** Upload on canvas Log food into MyFitnessPal	**Q1 (30 min)** ☐ Go over microbiome papers about diet. Discuss intervention. ☐ Take a bag with elements needed to collect stool sample (FMS). ☐ Download R Studio. Practice uploading a file. ☐ Finish diet intervention survey Log food into MyFitnessPal
Lecture W2	Combined lecture and lab. R tutorial part 1. Log MyFitnessPal data into Excel file				

Lab **W2** Log food into MyFitnessPal	Log food into MyFitnessPal Drop off FMS1	Log food into MyFitnessPal	☐ R tutorial part 1 ☐ Enter week 1 and 2 My Fitness pal data into an Excel file (online doc, can be done/finished outside class) Log food into MyFitnessPal. On your own time Mon to Wed: Drop off FMS2	Log food into MyFitnessPal	**Q2 (30 min)** ☐ R tutorial part 2 ☐ Plot graphs on R Studio ☐ Log food into MyFitnessPal. ☐ Receive Diet intervention instructions. Start diet intervention today. On your own time Wed to Fri: Drop off FMS3 **Annotated bibliography for Research Proposal Due** (check Canvas)
Lecture **W3**	General: proposal writing discussion. Log MyFitnessPal data into Excel file				
Lab **W3** Log food into MyFitnessPal on weekend.	Log food into MyFitnessPal	Log food into MyFitnessPal	☐ Enter week 2 and 3 My Fitness pal data into an Excel file (online doc, can be done/finished outside class) Log food into MyFitnessPal.	Log food into MyFitnessPal	**Q3 (30 min)** Log food into MyFitnessPal
Lecture **W4**	Discussion: proposal writing, aims. Wrap up R tutorial work. Log MyFitnessPal data into Excel file				

Lab **W4** Log food into MyFitnessPal on weekend.	Log food into MyFitnessPal Drop off FMS 4	Log food into MyFitnessPal	Project 2 (1 hour) ☐ Practice pipetting skills (check canvas) ☐ Enter week 3-4 My Fitness pal data into an Excel file (online doc, can be done/finished outside class) Log food into MyFitnessPal Mon to Wed: Drop off FMS5	Log food into MyFitnessPal	**Q4 (30 min)** Drop off FMS6. Log food into MyFitnessPal. **Lab meeting** Groups present R Data. **Specific Background (preliminary studies) and significance section of proposal due, paper copy at the beginning of lab.**
Lecture **W5**	**Aims due, paper copy at the beginning of lecture.** DNA extraction, PCR and electrophoresis discussion. We will have lecture discussion during incubation times.				
Lab **W5**			☐ Enter week 4-5 My Fitness pal data into an Excel file.		**Q5** ☐ FMS DNA extraction
Lecture **W6**	First proposal draft discussion. PCR and electrophoresis discussion.				
Lab **W6**			☐ 16S PCR for fecal samples.		**Q6 (30 min)** **Lab manual due** ☐ Gel electrophoresis, submit for sequencing.
Lecture **W7**	Discussion of 16S sequencing. Discussion of public microbiome data analysis.				

Lab **W7**	14	15	16 May ☐ QIIME2 workshop part 1	17 May	18 May **Q7 (30 min)** ☐ QIIME2 workshop part 2 **Proposal draft due: all sections (1st draft),** check Canvas. Please bring paper copy to lab meeting and upload the same file to Canvas.
Lecture **W8**	Guidance and discussion of microbiome data analysis.				
Lab **Week 8**			☐ We should get fecal sample sequences back here. Start analysis.		**Q8** ☐ R workshop part 3
Lecture **W9**	Lab meeting. Groups discuss quality of DNA extraction, 16S PCR and practices done with QIIME2				
Lab **W9**			☐ R work with own data		☐ R work with own data Proposal Peer revision
Lecture **W10**					
Lab **W10**	**Peer revision of proposals due**		**Lab manual due**		**Microbiome Poster presentation** **Written report of all data collected due.** **Final Proposal due**

Appendix 5: Fiber Force course Syllabus

Wednesday 12-1.50 pm (lecture)
Wednesday 2-3.50 (lab)
Friday 12-3.50 (lab)

COURSE DESCRIPTION:
Discovery-driven experimentation in the fields of molecular biology, microbiology/microbiome. The goal of this course is for you to gain a hands-on and advance experience on molecular techniques. The goal of this project is to study the responses of healthy human microbiomes to normal lifestyle perturbations. You will participate in the experimental design, data collection and data analysis in a microbiome research project.
This course also involves other aspects of the lab experience including group discussion of results, scientific paper analysis, writing and student presentations. Throughout the quarter, you will gain advanced understanding of what it is like to work, think, and learn in an actual laboratory setting.

CONTENT LEARNING OUTCOMES:

Scientific Skills to develop in this class:

- *To interpret data and graphs.*
- *To critically evaluate the work of others.*
- *To calculate and interpret numbers and statistical quantities.*
- *To plan and carry out an experiment and to accurately and methodically document hypotheses, experiments and initial analysis or interpretation of these experiments.*
- *To present data in graphs/tables.*
- *To write a grant proposal.*
- *To give an oral presentation.*
- *To work in collaboration with others.*
- *To learn microbiome research methodology and sequence analysis skills*

You will accomplish these in numerous ways:

- ✓ You will perform modern microbiology and molecular biology techniques in lab experiments and become proficient in numerous lab skills. You will present your results in the form of data meetings and poster presentation. Your reading, writing and proficiency in lab will be assessed through data meeting reports, lab points and poster presentation.
- ✓ We will discuss a number of molecular biology and microbiology techniques and experiments and concepts during discussions. This part of your learning will be assessed by your performance in lab and in weekly quizzes.
- ✓ You will then apply these core concepts to solve experimental problems. This part of your learning will be assessed by your performance in lab and in weekly quizzes.
- ✓ You will read various journal articles, answering and discussing high-level cognitive questions about the author's findings. These articles will present you with examples on how molecular biology concepts can be applied to research. Real life applications will help you make broader connections in the field. Your reading and participation in these activities will be assessed by article discussion handouts.
- ✓ You will practice the scientific method in different ways, including writing hypothesis, analyzing results, reporting data and proposing further experiments. This part of your learning will be assessed by poster presentation, lab meeting reports and grant proposal writing.

SCIENTIFIC SKILLS LEARNING OUTCOMES:

By the end of this course, you will be able to…

1) Effectively communicate science through discussions of research articles and written exams, lab reports and proposals
2) Interpret and evaluate results from lab experiments and primary research articles
3) Gain critical thinking and analytical skills through working in lab activities, problems and analysis of research articles.
4) Design, collect and analyze data regarding two research projects.

ASSESSMENTS & GRADES:

Lab Quizzes (9, one per week)	90 points
Scientific paper discussion	20 points
Data meeting reports	30 points
Lab and notebook points*	40 points
Group poster presentation	30 points
Individual grant proposal writing**	100 points
Peer revision of grant proposal	10 points
	Total: 350 points

Lab and notebook points 40
- Notebook* check 1: 10 points
- Notebook and figures check 2: 20 points
- Lab points 10 points

Research Proposal** 100
- Annotated Bibliography: 5 points
- Preliminary Studies/Significance: 10 points
- Global and 2-3 specific aims: 5 points
- Rough draft of entire proposal: 20 points
- Final draft: 60 points

Quizzes will be held during lab (once a week). The quizzes will cover specifics concerning the experimental protocols you perform during the lab, especially principles and applications of those lab protocols.

Scientific Paper discussions

An important part of your college and laboratory experience is being able to read and understand primary scientific literature. We will cover many papers during the quarter, I will post them on Canvas. We will work on handouts during lecture, and/or have group discussions on them (**Scientific paper discussions**).

Lab and notebook points evaluate your overall performance in lab. Performance will be based on whether you follow lab policies, come prepared for class, and dispose of your lab waste appropriately. In addition, you will be required to actively participate in experimental design, discuss experiments, come up with hypotheses, troubleshoot and come outside class time to complete lab tasks.

The points also include lab notebook keeping (20/30). We will collect the notebooks twice a quarter to check your writing in detail. We will grade these notebooks following a rubric that we will post on Canvas.

Lab meeting reports

We will have 2-3 lab meetings during the quarter. For these meetings, I will expect all groups to present professional looking figures on a PowerPoint (including slides as introduction, methods, results and conclusions) and turn in a short report containing figures and figure legends a short explanation of the findings and conclusions. It is important that you collect high quality images during your experiments and record all the data collected in an organized way. You will need to keep this data in your notebook, but also in digital files (*JPEG, *TIFF, *doc, *xls, etc) that can be used to process the data for lab meetings. For this we will share a Dropbox folder. This will teach you how to gather and organize your data so you can create high quality figures. This will also teach you to organize your thoughts and data in a way that can be shared with others (common in university, industry and clinical labs) and how to process data in a way to teach others about what you have accomplished in the lab.

Poster presentation day is on week 10. In this presentation you and your group will share your results and also difficulties and successes you faced during the process of obtaining your data. The poster should include title, authors, abstract, introduction, results with figures and complete legends, conclusions, citations and acknowledgements.

Lab proposal writing

Being able to write (a good) research plan, fellowship proposal and/or grant application is an essential aspect of your scientific formation. Even if you end up following a career outside academia, writing a grant proposal will help you understand how the scientific method works. It will also help you understand how science is funded. This assignment is split in smaller tasks due at different dates across the entire quarter. Proposals that are turned in late (either the hard copy or online copy) will be subject to a late penalty. This consists of a 15% penalty (off of the total points possible) for one day late, 30% for two days, and so on. I highly recommend that you turn in your file on time. Taking extra time to polish your proposal does not guarantee more points but turning it in late guarantees a loss of points.

LABORATORY SAFETY STATEMENT
M130L Advanced molecular techniques laboratory

The lab exercises in this course involve handling samples with living organisms. A**ll microorganisms should be treated as potential pathogens** (organisms capable of causing disease).

The following rules must be observed at all times to prevent accidental injury to and infection of yourself and others and to minimize contamination of the lab environment:

1. **Never place books, backpacks, purses, etc., on bench tops.** Always place these in the floor, under your working area. Keep manuals and pens on the areas designated as "note taking areas". Buy a pen/pencil for this class and leave it in the lab. You will not take these items home.

2. **Use Electronic devices responsibly**. Never use any electronic device while wearing gloves. In case of needing your phone camera or calculator when wearing gloves, we will provide Ziploc bags to protect your devices. You will wash your hands, get your phone and insert it in a Ziploc bag. Make sure you do this in the clean "note-taking" area. Once you are done with your phone, wipe the Ziploc bag with 70% ethanol and discard it in the appropriated trash area. When suing laptops in bench tops, make sure to disinfect the area.

3. Clean your work area with quaternary ammonium disinfectant or 70% ethanol solution at the **beginning AND end** of each lab.

4. **Wash your hands** with soap and dry with paper towels when entering and leaving the lab.

5. Wear a **lab coat** at all times while working in the lab to prevent contamination or accidental staining of your clothing.

   A. **Closed-toe shoes** (no sandals) are to be worn in the lab.

   B. **Long hair must be tied back** to prevent exposure to flame and contamination of cultures.

   C. **Gloves** should be worn when staining microbes and handling hazardous chemicals.

6. **Do not place anything in your mouth or eyes while in the lab.** This includes pencils, food, and fingers. Keep your hands away from your mouth and eyes.

   A. Eating and drinking are **prohibited** in the lab at all times.

   B. This includes gum, cough drops, and candy.

   C. Do not apply cosmetics in the lab. This includes Chapstick.

   D. **Never pipet by mouth.** Use a mechanical pipetting device.

7. **Do not remove media, equipment, or bacterial cultures from the laboratory.** This is absolutely prohibited and unnecessary.

8. Do not place contaminated instruments such as inoculating loops, needles, and pipettes on bench tops. Loops and needles should be sterilized by incineration, and pipettes should be disposed of in designated receptacles of bleach solution.

9. Carry cultures in a test tube rack when moving around the lab or when keeping cultures on bench tops for use. This prevents accidents and contamination of your person or belongings.

10. **Immediately cover spilled cultures or broken culture tubes with paper towels and then saturate them with disinfectant solution.** Notify your TA or instructor that there has been a spill. After 15 minutes, dispose of the towels and broken items as indicated by your TA or instructor.

11. **Report accidental cuts or burns to the TA or instructor immediately**. The incident must also be reported to UCI EHS within 24 hours: https://www.ehs.uci.edu/apps/hr/index.jsp. We will help you with this.

12. At the end of each lab session, place all cultures and materials in the proper disposal area.

13. Persons who are immune-compromised (including those who are pregnant or may become pregnant) and students

living with or caring for an immune-compromised individual are advised to avoid handling fecal samples and consult with your physician to determine the appropriate level of participation in the lab. Should your physician determine that you should not participate in this lab, please have him or her write a note stating the concerns. Alternative accommodations may be indicated.

OSHA INFORMATION
Material Safety Data Sheets (MSDS) are located _____.
The first aid kit is located _____.
The eyewash station is located _____.
The shower is located _____.

The fire extinguisher is located _____.

STUDENT AGREEMENT ON LABORATORY SAFETY

I agree to abide by all laboratory rules set forth by the instructor. I understand that my safety is entirely my own responsibility and that I may be putting myself and others in danger if I do not abide by all the rules set forth by the instructor.

COURSE: M130L SP 2018

NAME OF STUDENT (PRINT): _____

STUDENT ID: _____

SIGNATURE OF STUDENT: _____

DATE: _____

Adapted from: Guidelines for Biosafety in Teaching Laboratories, https://www.asm.org/images/Education/FINAL_Biosafety_Guidelines_Appendix_Only.pdf

Information about personal protective equipment for M130L

Safety goggles or safety glasses when handling liquids or when plating. Bring your own goggles. Please note that the goggles cannot be shared, and they must be sanitized with 70% ethanol before leaving the lab. Use soft wipes for wiping to minimize scratching the items.

Lab coats. Bring your own lab coats. Protective coats or covering must be worn in the lab. Please purchase cloth coats. Please note they should fit you properly, be made of flame-resistant cloth and be at least three-quarters in length to cover your lap when you are sitting. Lab coats must be used by individual students and never shared. The lab coat you use for a microbiology class cannot be used for other classes. Your lab coat should be stored in a Ziploc bag. You will remove the coat from the Ziploc bag in lab. At the end of lab, you will put the coat back inside the bag. The Ziploc bag is meant to protect your backpack/bags and other personal items from cross-contamination. At the end of the quarter, your lab coat will be properly decontaminated by us before we return them to you. If you spill a bacterial culture in your lab coat, please talk to us immediately. Your lab coat might need to be autoclaved before you can wash it or washed by the UCI EHS laundry system.

Closed-toe shoes. Hard-sole shoes without open toes are required in all laboratories to protect against heavy objects, hot liquids, or broken glass. The closed-toe style is necessary due to the additional risk of contamination in the microbiology lab (see "Lab Coats" above).

Gloves when handling hazardous and/or infectious materials. We will provide gloves. Gloves should be worn when microbial cultures are handled. Gloves are recommended for all procedures if a student has any open wounds such as a fresh cut. We recommend you put gloves on at all times to be safe. Gloves must be discarded in a waste container for biohazardous materials.

Note-taking area. You will need to bring your own notebook, lab manual and a microbiology lab pen/pencil. The area for culturing and working with microorganisms will be separate from the area for taking notes. We will designate and mark areas in the lab as "Note-taking" areas. You should also make sure to wash your hands before taking any notes. Your M118L lab manual/notebook and pen/pencil can only be used in the Note-taking areas. Always minimize the number of notebooks and/or lab manuals on the lab bench. The pen/pencil you use for this lab will remain in the lab during the entire quarter. Please bring one pen and one pencil and leave them in your microbiology tools container. You will not take these items home. You can take your notebook and lab manual home, but you will be asked to wipe the outside of your notebook and lab manual with 70% ethanol before leaving the lab. All other personal belongings, e.g., backpacks, purses, books, etc., should be stored way from the work area in spaces approved by the instructor.

Lab Clean-Up policy

At the start of each session, bench tops should be wiped down with disinfectant.

At the end of each session, each student should clean up the bench area by tidying up his/her equipment bin, moving all cultures to the refrigerator, incubator, or cabinet, and swabbing the bench top with disinfectant again. **Always wash your hands with disinfectant soap before leaving the classroom.**

Appendix 6 discussion handouts.

M130L week 1 Handout

Name: _____ ID:_____

Microbiome

1) Why most microbiome studies rely on sequencing rather than culturing of environmental/human microorganisms in the lab?

99% of bacteria cannot be grown in media/in the lab but sequencing methods can detect all

2) *Insert here Figure 1 from Michael J. Cox, William O.C.M. Cookson, Miriam F. Moffatt, Sequencing the human microbiome in health and disease, Human Molecular Genetics, Volume 22, Issue R1, 15 October 2013, Pages R88–R94, https://doi.org/10.1093/hmg/ddt398*

Adapted Legend: A schematic demonstrating the processes for 16S rRNA gene sequencing, Whole-Genome Sequencing and metagenomics. Sample collection, DNA extraction, sequencing and sequence analysis are required in all three techniques. 16S rRNA gene sequencing and WGS involve additional steps.

What is the difference between 16S sequencing and metagenomics sequencing in terms of the information provided?

16S =taxonomic info, sequences one gen that provides taxonomic and phylogenetic info

Metagenomics=sequences all DNA, so it provides taxonomic and functional (potential genes) information

3) Understanding the concept of diversity:

Insert here Figure 3 (and legend) from Michael J. Cox et al. Sequencing the human microbiome in health and disease, Human Molecular Genetics, Volume 22, Issue R1, 15 October 2013, Pages R88–R94, https://doi.org/10.1093/hmg/ddt398

Richness is the total number of organisms in a sample. Evenness whether they are evenly distributed, e.g. some more abundant than others

Diversity is combination of richness and evenness—can be considered to be a summary statistic for community structure as membership, abundance and evenness are taken into account. Is the number and abundance distribution of distinct types of organisms.

Insert here Figure 1 (and legend) from Elizabeth K. Costello et al. 2012.The Application of Ecological Theory Toward an Understanding of the Human Microbiome. Science 336, 1255. DOI: 10.1126/science.1224203

Alpha diversity: the organismal diversity within sample. Ecologically rich or not?

Beta: the organismal diversity shared between two or more communities, for example, from two or more different people at the same body site.

Gamma-diversity refers to the "regional" species pool.

Now to understand diversity, you also need to understand the concept of **species and OTU**

Species: A species is often defined as the largest group of organisms capable of interbreeding and producing fertile offspring. While in many cases this definition is adequate, the difficulty of defining species is known as the species problem. Source: Wikipedia

a. What is the problem with defining bacterial and archaeal species?

Asexual reproduction

Generally, when 16S sequences are clustered at 97% identity they belong to the same species. Therefore, when comparing our 16S sequence results with BLAST, a percentage of identity of ≥ 95% means they belong to the same GENUS. To assign species it is recommended that the identity is ≥ 97%. But this 97% *is arbitrary*. One example is the species of the families *Enterobacteriaceae*, *Clostridiaceae*, and *Peptostreptococcaceae* which can share up to 99% sequence similarity across the full 16S gene. As a result, reference databases are unable to reliably classify these bacteria at lower taxonomic levels like species.

OTUs (Operational Taxonomic unit): refers to clusters of (uncultivated or unknown) organisms, grouped by DNA sequence similarity of a specific taxonomic marker gene. In other words, OTUs are pragmatic proxies for microbial "species" at different taxonomic levels, in the absence of traditional systems of biological classification as are available for macroscopic organisms. For several years, OTUs have been the most commonly used units of microbial diversity, especially when analyzing small subunit 16S or 18S rRNA marker gene sequence datasets. Source: Wikipedia

b. The following is a curve called "rarefaction curve" that plots the different phyla, order, genus or species found in a sample as a function of increasing sample size (number of reads). Rarefaction curves show alpha diversity and also help researchers determine whether or not they have seen all the diversity in a particular sample.

For the following examples

Insert here figure 2 (and legend) from Dea Shahinas et al. Toward an Understanding of Changes in Diversity Associated with Fecal Microbiome Transplantation Based on 16S rRNA Gene Deep Sequencing. mBio Oct 2012, 3 (5) e00338-12; DOI: 10.1128/mBio.00338-12

Discuss the diversity in different samples and whether or not the researchers sequenced enough.

Compare samples by diversity (Y axis scale)

If you sequence deep enough you would see the curve leveling up, indicating that if you sequence more samples you wouldn't be seeing any more alpha diversity.

4) Diet

   a. Look at the following graphic summary:

Insert here Figure 1 (and legend) of Kristina M. Utzschneideret al. Mechanisms Linking the Gut Microbiome and Glucose Metabolism J Clin Endocrinol Metab. 2016 Apr; 101(4): 1445–1454.

doi: 10.1210/jc.2015-4251

Based on this, what conclusion can you extrapolate about nutrient availability for the microbiota in the colon?

Undigestible fiber feeds the microbiota

   b. Insert here Figure 1 (sand legend) from David et al., 2013. Nature. 2014 Jan 23;505(7484):559-63. doi: 10.1038/nature12820.

Using what you have learned so far, compare and contrast alpha and beta diversity for the subjects eating a plant-base diet vs. animal-based diet. Which diet showed the largest change?

Within-sample species diversity (α-diversity), did not significantly change during either diet. When comparing beta diversity in terms of similarity of each individual's gut microbiota to their baseline communities, significant changes were observed on the animal-based diet

M130L week 2-3 Handout

Name: _____ ID:_____

You will work with your lab group on this handout. Each group will be randomly assigned to one of these challenges and will have 30 min to prepare. After the 30 min, all groups will present their writing and findings in the front. 5 min per group.

1. Research the bibliography in relation to high fiber (50 gr) diet/interventions and bacterial diversity. Write a 3-4 sentence summary of what is known, including observed bacterial diversity shifts and types of fiber interventions.

2. Research the bibliography in relation to high fiber (50 gr) diet/interventions and bacterial diversity. Write a hypothesis for our fiber intervention experiment supported with literature (make predictions that are based on previous research). Your hypothesis must discuss bacterial diversity and abundance shifts you expect to observe.

3. Research the bibliography in relation to high fiber (50 gr) diet/interventions and bacterial diversity. Find 2 data figures that show changes in relative abundance and alpha/beta diversity in a fiber intervention publication. Summarize the results of each one in 2-3 sentences each. The summary sentence must make reference to the data (not conjectures or assumptions) in the figure.

No unique answer key.

M130L week 4 Handout

Name: _____ ID:_____

5) Let's analyze the Figure below from Holscher et al., 2015. In this study, healthy adult men (n = 21) consumed bars containing **no supplemental fiber (placebo; NFC)**, **polydextrose (21 g/d)**, and **soluble corn fiber (SCF; 21 g/d)** for 21 days. Fecal specimens were collected between days 16 and 21 for fermentative end-product analysis and 16S ribosomal RNA bacterial gene amplification for bacterial taxa identification.

Objective: The objective was to perform whole-genome shotgun 454 pyrosequencing on the same fecal specimens collected in the study to explore the full range of bacterial genetic information in the fecal microbiome.

Insert here Figure 21 from Hannah D Holscher et al. Fiber supplementation influences phylogenetic structure and functional capacity of the human intestinal microbiome: follow-up of a randomized controlled trial, The American Journal of Clinical Nutrition, 2015101 (1): 55–64, https://doi.org/10.3945/ajcn.114.092064.

Why did the authors run a correlation analysis in the figure above? What does the correlation show?

To (graphically) show what is the effect of fiber consumption on bacteria diversity and to determine whether a correlation between these two variables exists.

There is a positive linear correlation between high fiber consumption with higher bacteriodetes/firmicutes ratio, but there is no correlation between BMI and bacteriodetes/firmicutes ratio.

M130L week 5 Handout

Name: _____ ID:_____

1. You have a DNA solution that is 19.4 µg/µL and you want 75 µl that is 30 ng/µL. How do you make this solution? include volume from stock and water.

Stock 19.4 µg/µL = 19400 ng/µL Ci x Vi = Cf x Vf [75 µl x 30 ng/µL]/ 19400 ng/µL = 0.11 µL + 74.89 µL of water.

2. This week we will be running a PCR reaction. For this, we need to add each primer (U1 and U2) in the recommended concentration of 350 nM.
   a. How many pmol/µl are in 350 nM?

      350 nM= 350 nmol/1000 ml = 350 pmol/1 ml = 0.350 pmol/µl

   b. Based on the pmol/µl calculated above, how many picomols of each primer should be add for a 25 µl of PCR reaction?

      0.35 pmol/µl x 25 µl of PCR reaction = 8.75 pmol

   c. Our primer stocks were prepared as 10 µM or 10 pmol/µl solutions.
      To make the 15 µl primer mix that your pipet today into your 25 µl PCR reaction, we mixed the recommended amount of each primer (calculated in b) and water to complete 15 µl. How did we make this solution?

      0.35 pmol/µl x 25 µl of PCR reaction = 8.75 pmol for our 10 pmol/µl primers that is 0.875 µl each. Primer mix was made mix by adding 0.875 µl of each primer plus 13.25 µl of water.

   d. To make enough primer mix for all students running PCR today we mixed (complete volumes):
      _29__ µl of U1 10 µM
      _29__ µl of U2 10 µM
      _442__ µl of water (sterile, molecular biology grade).
      total: 500 µl.

3. List all the components needed for a PCR reaction and explain why each one is added/needed.

- Template DNA: Contains the region that needs to be amplified
- Primers: Complementary to sequences at the ends of the DNA fragment to be amplified, provide a 3'OH end the polymerase can extend.
- Deoxynucleoside triphosphates (dNTPs): Provide the precursors for DNA synthesis
- thermostable DNA *Taq* polymerase: catalyze polymerization of DNA. Must clarify the enzyme is thermostable for full credit, as PCR involves heating steps that inactivate most other DNA polymerases
- Buffer: provides stable pH optimal for DNA synthesis and provides ions necessary for enzyme function.

M130L week 6 Handout

Name: _____ ID: _____

1. Find the handout shared with your last week with the principle of the DNA extraction protocol. It is on Canvas (protocols folder).
   Answer:

   a. How does the kit accomplish cell lysis? List all treatments/ingredients involved in this process and action.
      we use a **buffer** (chemical) and **bead-beating** (mechanical) lysis.
      - **Chaotropic salts** – break up hydrogen bonds, van der Waals forces, and hydrophobic interactions
        - Destabilize proteins
        - Helps step two (transferring DNA to the silica membrane)
        - Includes: guanidine HCl, guanidine thiocyanate, urea, and lithium perchlorate
      - **Detergents** – solubilize proteins and lyse cells
      - **Enzymes** – lysis, protein removal
        - Proteinase K – digests cell walls/proteins
          - Should be added to target a virome
        - Lysozyme – digests cell walls

      Great for fungi and gram-positive bacteria

   b. Step 2 of the protocol is binding to silica, how is this accomplished? List all treatments/ingredients involved in this step and mode of action.
      Ingredients:
      a. **Chaotropic salts** (from lysis step)
      b. **Alcohol** – enhance the binding of nucleic acids to silica
         i. Usually ethanol, but can be isopropanol
         ii.

   c. Explain the principle of the washing steps.

      remove impurities from DNA (e.g. Residual proteins and salt are attached to the silica membrane)
      Typically, two washes:
      (1) Low amount of chaotropic salts to remove proteins and other impurities
      (2) Ethanol to wash off the salt

   d. What is the importance of the dry centrifugation step?
      - removes ethanol
      - Ethanol contamination: Inhibits PCR success; DNA will not stay in well (gel electrophoresis)
      - Centrifuging step: Combination of pulling ethanol out of the column and evaporation

   e. How was the DNA eluted in the protocol you run last Friday? With water, that solubilizes DNA
2. Today we will assess the quality of your DNA extraction in two ways.
   a. By electrophoresis
   b. By quantification of concentration and absorbance ratios.

What you expect to see in a good quality DNA extraction

Tight band, high molecular weight, minimal to no smearing, no RNA contamination and no impurities in the well.

Read the attached sheet describing concentration and DNA absorbance.

- Absorbance:
    - 230nm – guanidinium salts (lysis and purification buffer)
    - 260nm – DNA (calculate DNA concentration)
    - 280nm – protein (tyrosine and tryptophan)
    - 320nm – measure of turbidity (calculate DNA concentration)
- Concentration (µg/ml) = (A_{260} reading – A_{320} reading) x 50

Based on this, describe the importance and expected values of the following rations:

- 260/230 (> 1.5-1.7) – measure of salt contamination
- 260/280 (1.7 – 2.0) – measure of protein contamination

M130L week 8 Handout (post QIIME2)

Name: _____ ID: _____

Go to the visualization files from Qiime2's "Moving Picture's Tutorial".
https://docs.qiime2.org/2018.4/tutorials/moving-pictures/#sample-metadata

There you will find several files that can be downloaded and opened/viewed with the Qiime2 viewer:

https://view.qiime2.org/

Using these files, and this viewer, please answer these following questions. To be clear, you may have to open up these files and look around!

1. How many subjects are present in this study, and how many sites were sampled in this study? What are the sites?

2. At a sequencing depth of 4000, what is the difference in the observed OTUs between antibiotic users and non-antibiotic users (rounded to the nearest 10)

3. At the genus level (L6), what is the highest bacteria by relative abundance for subject L3S242.

4. Attach a Emperor (Qiime's version of a principle coordinates plot) plot showing different body sites (beta-diversity).

5. Using UniFrac comparisons across body sites, what is the only comparison that shows the microbial composition is not statistically significant?
    a. What is this test called (what does the acronym stand for?)
    b. What does this test do?
    c. What is the non-significant p-value?

PS: Here is the tutorial from where the data came from:

https://docs.qiime2.org/2018.4/tutorials/moving-pictures/#sample-metadata

M130L week 9 Handout

Name: _____ ID:_____

You will work with your lab group on this handout. Each group will be randomly assigned to one of these challenges and will have 30 min to prepare. After the 30 min, all groups will present their writing and findings in the front. 5 min per group.

1. Research literature on the microbiome analysis. Write a summary on what data and calculations will be necessary to create: A) a figure showing relative abundance of different bacteria phyla in each sample (will you do it per student/average?) B) a Principal Coordinates Analysis (PCoA) graph

2. Research literature on the microbiome analysis. Design two correlations to test with our fiber intervention data. Describe all methodological steps and coding and analyses required to obtain a final plot.

No unique answer key.

Appendix 7: stool collection instructions

Materials: gloves, plastic caps, wax paper (if you prefer plastic wrap let us know), tape, spoons, eppendorfs, tubes rack, marker, stickers with sample code.

Sample collection schedule

Week 2: 3 stool samples. We will call these **samples 1, 2 and 3** (pre intervention)
Week 3 and 4: diet intervention. Eat 40-50 grs of fiber a day
Week 4: 3 stool samples. We will call these **samples 4, 5 and 6** (post intervention)

Plan:
Try to collect 3 stool samples per collection period. For each bowel movement, you will fill up three eppendorf tubes, **A**, **B** and **C**. For example, sample 1-A, sample 1-B, sample 1-C. Since you will collect 6 samples (1-6) in triplicate, you will provide a total of 18 fecal samples in Eppendorf tubes. Now, since the samples must be stored properly, you will freeze samples in your home freezer immediately after each collection. You should keep a frozen ice pack in the freezer as well. Frozen samples can be dropped off to the lab on the indicated periods (class schedule). For drop off, take the eppendorfs out of your freezer, place them on an ice box, cover the tubes with a frozen ice pack and make sure they make it to our -20 freezer within one hour.

SAMPLE COLLECTION

Sample collection materials

Per student:
Eppendorfs (18-20)
6-8 pairs of gloves
6 plastic caps
Wax paper/plastic wrap (one roll)
Eppendorf storing box (cardboard -80 freezer box work well)
Marker
Stickers with anonymous codes
Spoon or spatula (disposable or washable) small enough to push fecal sample inside eppendorf tube.
Ice pack
Styrofoam insulated box

1. Place on the provided pair of gloves
2. Label **3** eppendorf tubes like this:

Anonymous Sample code (provided on your stickers). – sample number (1-6) --- triplicate code A, B, C (use one for each eppendorf tube). --- date and time.

Please label **CLEARLY**.

3. Urinate before collecting stool to prevent urine and stool from mixing
4. Stool collection. To collect stool, we will provide a series of materials and you can decide what is the way you personally find the easiest. Here are some options:

a. Plastic cup method: Pass stool directly into a plastic cap. **Do not urinate on the specimen.**

b. Wax paper/plastic wrap method: before using the toilet, lift up the toilet seat and place a piece of wax paper or plastic wrap over the toilet bowl secured by adhesive tape to prevent the sample from falling into the toilet bowl. Make a depression in the plastic wrap or wax paper to aid specimen collection. Lower the toilet seat and proceed with expelling bowel movement (stool specimen). Do not expel the specimen into the toilet. **Do not urinate on the specimen.**

5. Using the provided spoon, transfer enough stool sample to your 3 1.5 ml tubes[1], enough to fill the tube at least 1/2 capacity.
6. Dispose the remaining contents (plastic cap or wrap and left over stool) in the trash.
7. Wash your thoroughly hands with soap before continuing
8. Place your three eppendorfs on your freezer. Use the provided tube rack to keep your samples organized and upright. Make sure you keep an ice pack on the freezer.

DROP OFF

Whenever you are ready for drop off, plan on doing it within one hour, so the samples do not spend a long time outside the freezer. When you are ready, put the ice pack you have in the freezer (keep a pack there at all times), place it on the provided cooler. Take the samples out of your freezer and place them on the cooler, making sure the ice pack keeps them cool. Drop off on the arranged location within one hour.

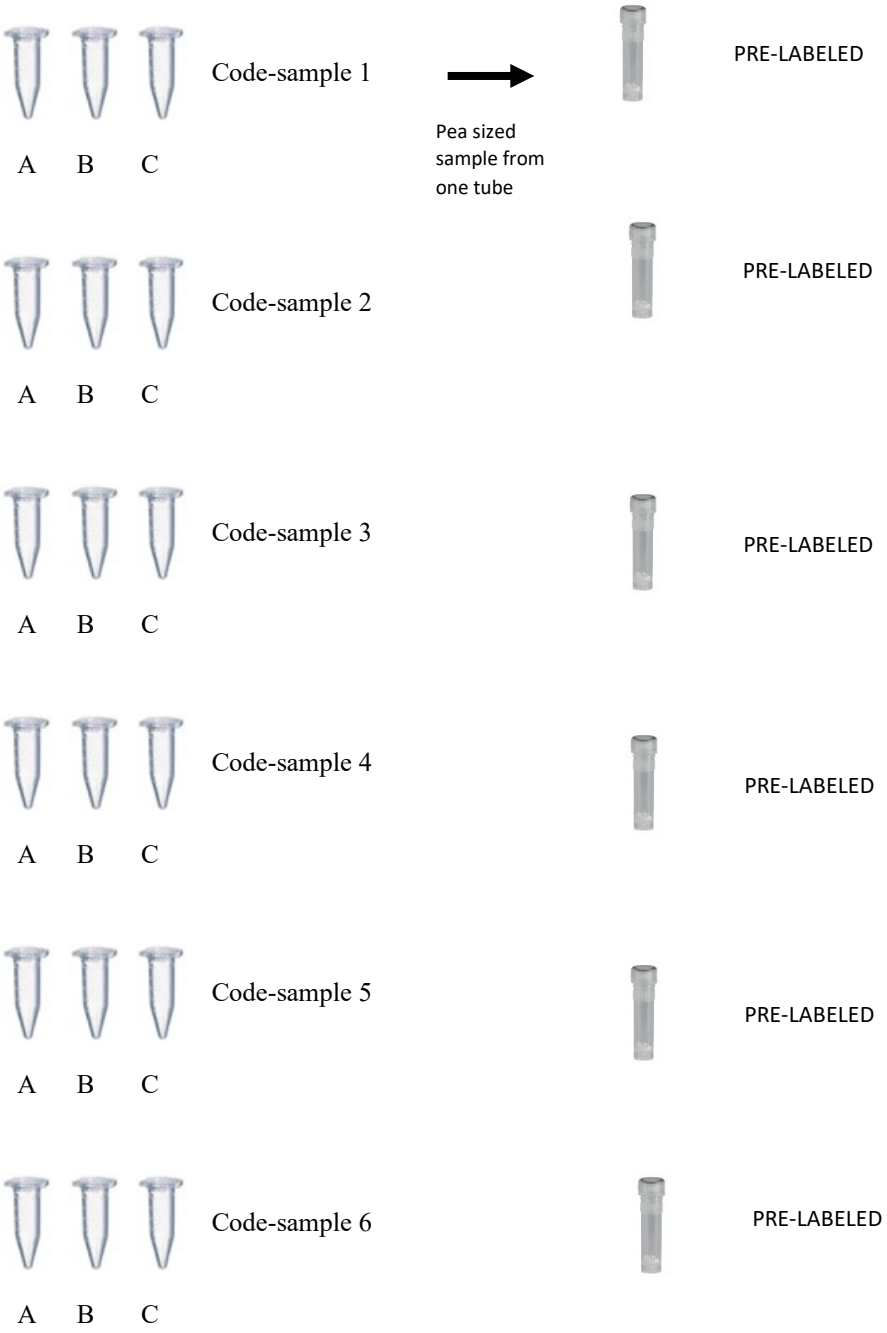
[1] Note from the authors: after the experience we realized that scooping fecal samples in 1.5 ml Eppendorf tubes is very hard, so we recommend using larger tubes.

Appendix 8: Fiber Force Protocols

Aliquoting fecal samples

Purpose: Each student has 3 pre-intervention (1-3) and 3 post-intervention (4-6) fecal samples collected in triplicates (A, B, C). Before DNA extraction, each sample needs to be aliquoted in a bead beating tube (Zymo Research, Zymobiomics DNA Mini kit).

Visual summary



Summary of instructions: take ONE of your replica tubes (A, B **or** C) for each sample (1-6) and aliquote a **pea sized** (0.15-0.199 grs) amount of FMS into 6 pre-labelled bead tubes.

Materials (per student)

Frozen fecal samples
6 bead tubes (Zymo kit, D4300)
Scale
Scoop
Ice bucket
Marker
Cleaning supplied (tissue, ethanol)

Detailed instructions:

A. You will aliquot your samples at Biosafety level 2 laboratory (safety cabinet). Sign up for a time slot and make sure you are punctual, as appointments are back to back from each other.
B. Find an assigned bench with a scale in the lab.
C. Before you start, read carefully the visual summary and all the steps listed below.
D. Make sure you have all you need: 6 bead tubes, loops to scoop the sample, marker, ice bucket.
E. Get some ice. if your ice bucket is empty ask where the ice is and fill it up.
F. Find the frozen fecal matter samples (FMS).
G. Place FMS tubes on ice.
H. Bring the tubes to the biosafety cabinet on ice.
I. Wait 2-4 minutes to thaw your samples enough to break a chunk off. DO NOT THAW COMPLETELY. Use a disposable loop to test for sample consistency, and as soon as you can break a piece with the loop, start aliquoting.
J. Place your first bead tube on a scale and hit TARE. The scale should now read 0.
K. Add a small amount of your first sample to your first bead tube until the scale reads 0.180 (0.150-0.199 is fine).
L. Repeat this until all bead tubes are filled.
M. Return your samples to the freezer. Place the bead tubes into a box labeled bead tubes (also in the freezer).
N. Make sure you leave the station clean for the next person.

DNA extraction

Purpose: fecal sample DNA extraction

Materials:

Bead tubes

FastPrep Classic (MP Bio)

Zymo kit D4300 https://www.zymoresearch.com/collections/zymobiomics-dna-kits/products/zymobiomics-dna-miniprep-kit

Get the complete protocol from the manufacture's manual:
https://www.zymoresearch.com/collections/zymobiomics-dna-kits/products/zymobiomics-dna-miniprep-kit

Amplicon Sequencing library prep protocol (core facility or sequencing unit)

Purpose: PCR amplification for 16S sequencing

Summary: PCR of the 16S rDNA was performed using Nuclease free water, Accustart II PCR tough mix (Quantabio, Beverly, MA), primers (515FB Forward Primer with barcode and 926R Reverse Primer (39). The 25 µl PCR reactions were run in a BioRad C1000 PCR machine (BioRad, Hercules, CA) with and initial denaturing cycle of 94ºC for 3 min and 30 cycles of 94ºC, 45 seconds; 55ºC, 30 seconds; and 72ºC, 60 seconds and the final extension at 72ºC for 10 minutes. To verify successful amplification, we loaded the libraries onto a 1% Agarose gel for gel electrophoresis with 1x SB (sodium borate) buffer. Libraries were pooled based on visual quantification of the amplification: 1 µl for strong products, 2 µl for medium products and 3 µl for weak bands. The pooled library was cleaned using Speed Beads (1:1 v/v ratio beads to pool) and washed twice with 200 µl 80% Ethanol. After drying, the pooled library was eluted with 50 µl of water and submitted for Miseq sequencing, paired end reads for 300 base pairs.

Detailed step by step protocol:

We recommend that the following protocols (A-E) are performed by a core facility or specialized microbiome unit.

4. Setting up car coding PCR
5. Checking for positive amplification by agarose gel electrophoresis
6. Pooling of products
7. Clean up of library pool
8. Run a gel to confirm clean pool and size of pool (instead of bioanalyzer)

A. Setting up the PCR

Materials:

- Genomic DNA (Sample 1-6), Water will be used for the negative.
- Nuclease free water
- PCR mix (dNTPs, Taq Polymerase, buffer, PCR enhancers)
- Bovine Serume Albumine (BSA)
- CAAGCAGAAGACGGCATACGAGATAGTCAGCCAGGGCCGTCAATTCCTTTRAGTTT (Illumina adapter, primer pad, primer linker and gene specific primer)
- AATGATACGGCGACCACCGAGATCTACACGCTxxxxxxxxxxxxTATGGTAATTGTGTGYCAGCMG CCGCGGTAA (Illumina adapter, 12bp Golay barcode, primer pad, primer linker, gene specific primer)
- PCR machine (we used BioRad C1000)
- PCR set up—-meaning which sample is connected to which barcode. If a well plate is used also record the position on the plate

1. Label PCR tubes/plate,
2. All reagents need to be spun down before use
3. Prepare Mastermix with all reagents **but** genomic DNA and barcoded primers. Follow the order of ingredients to set up your PCR reaction
4. Distribute 19 µL of master mix into each PCR tube
5. Add 1 µL of genomic DNA into the according PCR tube, record PCR tube ID in the table
6. Add 5 µL of barcoded primer to the according PCR tube, **record in table barcode ID for which sample**
7. Centrifuge PCR tubes
8. Transfer tubes into PCR machine and choose the program 16S

PCR program 16S

Cycles	**Temperature**	**Time**
1 X	94°C	3 minutes
30 X	94°C 55°C 72°C	45 seconds 30 seconds 60 seconds
1 X	72°C	10 minutes
1 X	12°C	Forever

B. Checking for positive amplification by agarose gel electrophoresis

Materials:

- Agarose
- Buffer, 1x SB (sodium borate) buffer
- Gel casting apparatus
- Power supply
- Gel chamber
- Comb

To prepare the gel:

1. take 0.4g agarose into 40 ml of SB buffer
2. Microwave for 1 min at 70% power
3. Make sure all the agarose is melted. It needs to start boiling a little. **Be careful** when shaking the agarose it can flash boil over
4. Let the agarose cool a little
5. Set up gel casting by locking gel chamber into gel casting frame, add the comb/s
6. Add 4 µL of a 1% Ethidium bromide solution to the 40 ml of liquid agarose gel
7. Mix well and pour into gel chamber
8. Wait until the gel is set ~20 min

Loading PCR products on agarose gel

1. After PCR is done take tubes out and spin them down
2. Take comb carefully and slowly out of hardened agarose gel
3. Open PCR tube and take 3 µL (approx. 10% of your PCR reaction) and load them carefully into the well
4. Record which well you loaded which sample
5. repeat this step for all the samples using a new well each time
6. Load 3 µL of molecular weight marker
7. Put gel in gel chamber and fill it with SB buffer to the rim of the gel
8. Run gel for 2 min at 60 V
9. Open gel chamber and add more SB buffer to submerge the gel
10. Run gel for 15 min at 150 V

C. Pooling of PCR products

1. According to gel picture write in the table that contains sample name, (position in well plate, which PCR plate if applicable) and tube #
2. Record in the table if the amplicon was weak on the gel, you will add 3 µL, if medium 2 µl and if strong 1 µL to the pool

3. With that table line up the tubes and pipet the amounts you determined per sample based on the gel picture into a new tube (ideally low binding tube)

D. Clean up of library pool

This step can also be used for concentrating the pool

Materials:

- Library pool
- Magnetic bead solution (has to be brought to RT)
- Molecular grade ethanol (prepare 80% Ethanol, same day as used)
- Magnetic holder
- Nuclease free water

Procedure:

1. Add 17 µL of water to your pool (this is only done because the pool is such low volume)
2. Add 1:1 v/v library pool to bead solution mix well by pipetting up and down 10 x.
3. Put mix on magnet, wait two minutes or until all the beads are pulled down
4. Pipet out supernatant, while still on the magnet and discard
5. Add 200 µL 80% Ethanol to the bead pellet, wait 30 sec and pipet out
6. Add 200 µL of 80% Ethanol again, wait 30 sec and pipet out, make sure all the Ethanol is removed
7. Let pellet air dry for 10-15 min
8. Remove sample from magnet and add water, (add the same volume you had in the original pool)

E. Confirm clean pool

Follow B. See above

16S PCR protocol and electrophoresis for the classroom practice

Purpose: PCR amplification and electrophoresis for discussion and troubleshooting.
This is a simplified 16S PCR reaction that can easily be performed by students of all levels. This procotol is not suitable for preparing sequencing libraries.

Materials: Eppendorf with water, eppendorf with PCR bead, primer mix.

Procedure

1. Quantify the concentration of your fecal sample DNA (nanodrop). If needed, make a dilution of the DNA (use water) so that the final concentration is below 50 μg/ml.
2. Meanwhile, obtain PCR bead tubes, which contain Taq polymerase (heat-resistant enzyme) and other necessary reagents. The tubes are pre-labeled with a code. Write it down. Do not add any other labels to your tube (no tape!).
3. Add 15 μL of PCR primer mix into the PCR bead tube. The bead will start to dissolve and slightly effervesce. Make sure the bead dissolves fully. Mix until it does.
4. Add 10 μL of your DNA to the PCR bead tube.
5. Cap the PCR tube, mix gently. If bubbles are observed on the walls of the tube, tap it until they come down.
6. The program used for PCR is described below.

Table 1. The conditions for PCR amplification

Cycles	**Temperature**	**Time**
1 X	94°C	10 minutes
36 X	94°C 58°C 72°C	30 seconds 30 seconds 1.5 minutes
1 X	72°C	10 minutes
1 X	4°C	Forever

Checking for positive amplification by agarose gel electrophoresis

Same as B above.

Appendix 9: laboratory preparation instructions

Week 1 Fecal sample collection material

Set up bag for fecal sample collection: stickers, tape, wax paper, plastic cups, spoons, eppendorfs, box. One set of instructions.

Per student:

Eppendorfs (18-20)

6-8 pairs of gloves

6 plastic caps

Wax paper/plastic wrap (one roll)

Eppendorf storing box (cardboard -80 freezer box work well)

Marker

Stickers with anonymous codes

Spoon or spatula (disposable or washable) small enough to push fecal sample inside eppendorf tube.

Ice pack

Styrofoam insulated box

Week 5: aliquoting fecal samples and DNA extraction

Aliquotation Materials (per student)

Frozen fecal samples

6 bead tubes (Zymo kit, D4300)

Scale

Scoop

Ice bucket

Marker

Cleaning supplied (tissue, ethanol)

DNA extraction materials

Bead tubes

Beat beater

Zymo kit D4300 https://www.zymoresearch.com/collections/zymobiomics-dna-kits/products/zymobiomics-dna-miniprep-kit

Notes for instructors: it is very important that the aliquoting process is done correctly to avoid confusion with anonymized coded samples. In addition, fecal sample weighting, aliquoting and bead beating should be done in a

biosafety cabinet and subsequent DNA extraction steps in a BSL-2 laboratory. For samples that will be used for sequencing we recommend that one person does all the aliquoting to minimize error.

Week 6: 16S sequencing (in the classroom) protocol

We recommend that the following protocols (A-E) are performed by a core facility or specialized microbiome unit.

9. Setting up car coding PCR
10. Checking for positive amplification by agarose gel electrophoresis
11. Pooling of products
12. Clean-up of library pool
13. Run a gel to confirm clean pool and size of pool (instead of bioanalyzer)

In class, have students use 2 out of the 6 fecal samples for a PCR practice and electrophoresis. For the classroom, we recommend using PCR beads but primer mix is also ok. Below is the detailed preparation for classroom protocols.

16S PCR

Note: this is the preparation for the 16S protocol suitable for the classroom.

MATERIALS

- Tips, pipettors. They really only need a P20 to pipet 10 ul of the DNA and 15 of the primer mix
- A big cooler with ice for the front of each room.
- 0.2 ml tube racks (empty pipette boxes), one every two students.
- Ice for each 2 students, this can be provided in small buckets or inside the pipette tip boxes.
- 200-500µl of sterile dH_2O (dnase free) aliquoted in sterile 1.5 ml microfuge tubes.
- **illustra PuReTaq Ready-To-Go PCR Beads (27-9559-02, GE).** 2-6 per student. Note: You can let students do PCR of all 6 samples of just of 2 to practice. We recommend that the bar-coding PCR for high throughput sequencing, cleaning and sequencing is performed by a core facility or specialized lab.
- Make **primer mix** ahead of time, aliquot in tubes of 20 µl. Student will retrieve their tubes from the ice. MAKE FRESH (right before each lab), see instructions below.

Concentration information:

Primers

U1: 5'-ACGCGTCGACAGAGTTTGATCCTGGCT-3'

U2: 5'-CGCGGATCCGCTACCTTGTTACGACTT-3'

Or same primers shown in appendix 8 without barcode.

The recommended amount of primer to add to each reaction is 350 nM= 350 nmol/1000 ml = 350 pmol/1 ml = 0.350 pmol/µl. For one PCR tube we need to make the primer mix by adding 0.875 µl of each primer plus 13.25 µl of water. Below is the calculation to make the mix for all students.

Preparation of primer mix: one per LAB SECTION. MAKE FRESH BEFORE LAB
29 µl of U1 10 µM
29 µl of U2 10 µM
442 µl of water (sterile, molecular biology grade).
total: 500 µl. Keep all tubes on ice.

Electrophoresis.

- A big cooler with ice for the front of each room
- 0.2 ml tube racks (empty pipette boxes), one every two students
- Ice for each 2 students, this can be provided in buckets or inside the pipette tip boxes.
- PCR Product(s) stored in freezer, bring on ice to the front of the room
- 2-6 Clean and sterile 1.5 ml tubes per student (to prepare sample for electrophoresis). Two students can share a rack with these tubes.
- 10x Loading dye aliquoted in 10 µl in 1.5 ml tubes, one per student.
- DNA ladder/Marker. Aliquot (5-10 ul, depending on the volume to use recommended by the manufacturer) of marker.
- Agarose
- Ethidium bromide solution (add to gel or to buffer, as preferred)
- 1X TAE (tris-acetate EDTA) or SB (sodium borate)
- 1 electrophoresis chamber per student (or per group depending on the number of PCR samples). You can prepare the gels 24-48 hours before use, but please add the 1X TAE or SB buffer right before each lab.
- Two extra gels submerged in water in a plastic container PER ROOM. Have these in the back of each room for students to practice loading.
- One power source to run gel per room.
- Tips, pipettors. Students will need a P20 and P20 tips in their stations. We will load the gels with P10, so have two P10s (one per gel) and two boxes of P10 tips in the front of each room.
- UV light box to photograph gels.
- Colored dots/stickers to label tube caps. Different colors for different lab sections.

Appendix 10 Guide to laboratory notebooks

SPECIFIC REQUIREMENTS FOR EACH LABORATORY WRITE-UP

- **Date:** The date on which the laboratory was performed appears in the upper left-hand corner of the first page.

- **Title:** An appropriate title for the laboratory is written at the top of the page. Write this title and the page on which it appears in the table of contents.

- **Objective:** This section should include 1-2 sentences explaining the purpose or reason behind the current experiment. Use your own words!

- **Results: Raw data and observations from the experiment.** Graphs and calculations appear in this section. **You must record your data directly into your lab notebook. Digital files can be saves in our shared Dropbox and location listed in your notebook.** You need not show all your calculations but include one example for each type of calculation. Include words of explanation and the appropriate mathematical formulas so that the calculations are intelligible. Always show units! Summarize your results and calculations in neatly prepared graphs and tables (prepare them before lab if necessary). Clearly label all tables and data (figure legends go below a figure and must be descriptive; table titles go above the table). Label tables as Table 1: Title of Table; Table 2: Title of Table, etc. and graphs as Figure 1: Title of Figure; Figure 2: Title of Figure, etc. Attach extra material such as computer printouts or graphs to clean left- or right-hand pages. For gel runs, make a clear reference for your loading order.

- **Discussion:** Summarize and explain your results. You are also expected to discuss whether your data makes sense, and if not, why you think it is not correct, where are possible places for error, and any other pertinent comments about the lab. Address questions from the "analysis of data and points for discussion" part of the lab handout.

- **Conclusion:** This section should only be a few sentences long (maximum of five). Address all of your objectives and how you accomplished each of them.

Appendix 11: Lab meeting guidelines

For each lab meeting you will be required to present your background, hypothesis, hypothesis support, preliminary data and data (if available) in a PowerPoint and in a short report file.

The written report should include

- Short background, hypothesis, hypothesis support
- Figures and legends
- Brief description of results in text (half a page max)
- One paragraph with conclusions.

The oral (PowerPoint) presentation should include the same data described above, however make sure you are telling a compelling story as you go.

For oral presentations:

- Short background, hypothesis, hypothesis support
- The oral (PowerPoint) presentation should include the same data described above, however make sure you are telling a compelling story as you go.
- All figures and tables should have legends.
- If during the oral presentation you want to discuss methods, keep in simple and make outlines.
- Conclusions can be listed as bullet points in alignment with the purpose of each experiment.
- We encourage you to discuss problems and troubleshooting as well.
- No results text required

Tips for oral communication:

- Present a hypothesis of what you expect to find before and after the diet intervention.
- Describe the results by discussing purpose of the experiment, results on figures and tables, and take-home message.
- Make connections, apply what you learned in this class:
- Close your presentation with your bullet point conclusions and share ideas of what you wish you could do next with your isolate.

Appendix 12: Poster Presentation guidelines

Poster presentation day is on week 10. In this presentation, you and your group will share your microbiome hypothesis, results, conclusions and also difficulties and successes you faced during the process of obtaining your data. You will judge each other's posters and I will assign you a grade that incorporates my comments and other students' comments.

The poster should include:

- Title
- Authors
- Abstract
- Introduction (can be visual instead of text)
- Results with figures and complete legends
- Conclusions
- Citations
- Acknowledgements.

The same information should be used to put together a final Word file report. This is the exact same writing and figures used for the poster.

Examples of posters can be found on Canvas

Make your poster on PowerPoint (size 4 feet by 4 feet or so) and print full scale on letter pages.

Ensemble your poster with tape and bring it to class ready to use.

Appendix 13. Grading rubrics

Lab Notebook checkup 1

Section	Section Rubric	Possible	Earned
Lab notebook and Tables (2 points)	1. Uses a **bound** notebook with sequentially numbered pages 2. Notebook is written legibly, numbered pages, no pages removed 3. Table of content is complete and clear. References (citations for all tools included)	0.25 0.5 0.5 0.75	
Experiments and Protocols (2 points)	1. Date, title, objective/goals written for each lab 2. Protocols are thorough and complete, calculations are shown 3. Changes to the protocols clearly addressed	0.5 1 0.5	
Results (3.5 points)	1. Clear description of antibiotic testing results. 2. Gels for 16S PCR and DNA extraction. Wells are labeled. 3. Data is organized and all lanes/photos are labeled and have units (if needed) 4. Includes complete and APPROPIATED figure legends. Legend described concentrations, strains, abbreviations and basic procedures	1 1 0.5 1	
Discussion (2.5 points)	1. Summarizes and explains results. Short conclusion included after each lab 2. Discuss whether data makes sense, and if not, where are possible places for error. Discussion ok to have one at the end of each project milestone.	1.5 1	

Lab Notebook checkup 2.

Section	Section Rubric	Possible	Earned
Lab notebook and Tables (1.75 points)	1. Notebook is written legibly, numbered pages, no pages removed 2. Table of content is complete and clear	0.5 0.62	
Experiments and Protocols (**second part)** (2.25 points)	4. Date, title, objective/goals written for each lab 5. Protocols are thorough and complete 6. Changes to the protocols clearly addressed	1.5 0.375	
Results: Raw data **(second part**) (3 points)	1. Contains raw data as tables for data collection, gel pictures with loading order, plate pictures or colony description, calculations (2. Raw data is clear, easy to read and interpret 3. Raw data includes description below (e.g. legend) that allows any person to read the data even if not plotted. 4. All calculations are shown, including media, concentrations, dilutions and data processing	0.75 0.75 0.75 0.75	
Discussion (second part) (3 points)	1. Summarizes and explains results for each experiment 2. Discussed whether data makes sense, and if not, where are possible places for error 3. Includes a conclusion statement for each lab	1 1 1	
Graphs (6 points) Processed data	1. Produce images near to the desired size of the printed version. (~1/4 of a letter/A5 page, as the provided examples) 2. Graph labels are clear and represent well the experiment 3. Graph labels are aligned and have uniform lettering and sizing (Arial, Courier, Times, Symbol) 4. Quality of images is good 5. PCR gel includes marker band sizes and explains band sizes 6. Producer photos and microbiome graphs (if finished) are clear, labeled and include units when appropriated	1 1 1 0.5 0.5 2	
Legends (4 points)	1. Provides captions (legends) to illustrations (not just title) 2. Legend comprises a brief title 3. Legend describes the illustration and how the experiment was run 4. Information is complete and accurate and includes n value. 5. Explains all symbols and abbreviations used in the Figure.	0.5 1 1 1 0.5	

Microbiome written report

NP = No points At: Attempted Av: Average E: Excellent

		NP	At	Av	E	
1.5	**Prediction** of expected diet intervention outcome is supported by primary literature **Prediction** is directly aligned with the experiment that we run		0.53 0.53	0.6 0.6	0.75 0.75	
0.5	**Graphs** Produce images near to the desired size of the printed version. (~1/4 of a letter/A5 page, as the provided examples). All media, graphs labels and conditions are easy to read and understand		0.18 0.18	0.2 0.2	0.25 0.25	
1	**Legends** Legend comprises a brief title Legend describes the figure and how the experiment was done (including population, diet, n value, description of how variables were calculated e.g. relative abundance). Explains all labels used in the Figure.		0.18 0.35 0.18	0.2 0.4 0.2	0.25 0.5 0.25	
1	**Brief paragraph with results** Describes and compares data in graphs using well elaborated and specific sentences Results sentences uses scientific vocabulary and refers to the figures		0.35 0.35	0.4 0.4	0.5 0.5	
1	**Conclusion** Summarizes main findings in alignment with prediction Demonstrates clear understanding of the results and conclusions that can be drawn from them		0.35 0.35	0.4 0.4	0.5 0.5	

Group Presentation

NP = No points At: Attempted Av: Average E: Excellent

			NP	At	Av	E	
1	**Short background**:						
	Effectively summarizes relevant background			0.35	0.4	0.5	
	Includes brief description of bacteria, promoter, metabolic conditions tested, type of regulation			0.35	0.4	0.5	
1	**Hypothesis**:						
	States hypothesis			0.35	0.4	0.5	
	provides reasoning and support for it (specific background)			0.35	0.4	0.5	
1	**Data presentation**						
	Describes how the experiment was run			0.18	0.2	0.25	
	Describes variables tested connecting this part with the hypothesis			0.18	0.2	0.25	
	Highlights and compares data from graphs			0.18	0.2	0.25	
	Points at data clearly so we can see the data as the presenter describes it			0.18	0.2	0.25	
0.5	**Graphs**						
	Graphs are a good size in the PowerPoint file, easy to read and see			0.14	0.16	0.2	
	Variables and axes are clearly labeled			0.07	0.08	0.1	
	Graphs have a (shortened) version of the legend or title. (Long full legend is in written report)			0.14	0.17	0.2	
1	**Conclusion**						
	Describes whether results support or refute hypothesis			0.35	0.4	0.5	
	Demonstrates clear understanding of the results and conclusions that can be drawn from them			0.35	0.4	0.5	

Poster Presentation

NP = No points At: Attempted Av: Average E: Excellent

		NP	At	Av	E	
4	**Content** Content is accurate and all required information is presented in a logical order. No spelling errors. No grammar errors. Text is in authors' own words.		1.4 1.4	1.6 1.6	2 2	
5	**Background and rationale** Describe background studies in relation to diet intervention. Rationale for work: why this research is interesting or important		1.4 2.1	1.6 2.4	2 3	
5	**Prediction** of expected diet intervention outcome is supported by primary literature **Prediction** is directly aligned with the experiment that we run		2.1 1.4	2.4 1.6	3 2	
3	**Graphs** Produce images near to the desired size of the printed version. (~1/4 of a letter/A5 page, as the provided examples) Graph labels are clear and represent well the experiment All variables, conditions and strains are easy to read and understand		0.7 0.7 0.7	0.8 0.8 0.8	1 1 1	
2	**Legends** Legend comprises a brief title Legend describes the figure and how the experiment was done. Information is complete and accurate, it includes n value,		0.7 0.7	0.8 0.8	1 1	
6	**Data presentation** Describes variables presented in relation to prediction Graphs/Tables/figures clearly explained		1.4 1.4	1.6 1.6	2 2	

	Points at data clearly so we can see the data as the presenter describes it		0.7	0.8	1	
	All presenters refrain from reading directly from poster or notes and have a dynamic interaction with the audience		0.7	0.8	1	
3	**Project results and conclusions**					
	Describes whether results support or refute prediction		1.4	1.6	2	
	Demonstrates clear understanding of the results and conclusions that can be drawn from them		0.7	0.8	1	

(up to 2 points) Individuals points for organization, clarity and preparation

Presenter's Names: _____ Points:
_____ Points:

_____ Points:
_____ Points:

Appendix 14: Diet definitions and resources

What is Fiber?

“Carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the human small intestine"

Figure 1. In 2008, the Codex Alimentarius Commission reached a consensus on this definition for dietary fiber. Two footnotes were added to the definition. One footnote state that the decision whether to include carbohydrates from three to nine monomeric units should be left to national authorities. The other footnote clarified the terms for inclusion of lignin and other noncarbohydrate plant compounds closely associated with dietary fiber polysaccharides. Dietary “fiber” is not really an accurate term, as many of its components are not fibrous. Photo from Wikipedia commons.

Dietary fiber is a good source of “microbiota accessible carbohydrates” (MACs) used for fermentation. As such, dietary fiber has been labeled a prebiotic, which by definition consists of low-, or non-digestible dietary components that benefit the host’s health via selective stimulation of the growth and/or activity of certain microorganisms.

Dietary sources for these prebiotics include raw oats, beans, lentils unrefined wheat and barley, soybeans, inulins, and non-digestible oligosaccharides (Pandey et al., 2015).

Soluble fiber

Soluble fiber dissolves in water and is fermented in the colon into short-chain fatty acids. It also slows the rate of glucose digestion and absorption and increases stool volume and water content. These include pectin, raffinose, xylose, polydextrose, lactulose

Figure 2. Soluble fiber is found in oats, fruits, grains (e.g. barley), legumes and psyllium seeds. Photos from Wikipedia commons.

Insoluble fiber

Insoluble fiber does not dissolve in water. It accelerates intestinal transit and increases fecal weight. These include cellulose, hemicellulose, lignin, xanthan, resistant starch.

Figure 3. Insoluble fiber is found in cereals, fruit, vegetables (in all plants in general, especially in stalks, peels and seeds)

High fiber diet resources

Meals supplied by Thistle (https://www.thistle.co/menu/) are pretty high on fiber, with salads having between 10-20 grs of fiber.

Breakfast

- Berries (frozen or fresh), one cup will also get you ~ 7-8 grams of fiber. Raspberries are the highest in fiber, with 8 grs per cup.
- If you use berries on a shake, consider adding flaxseed, chia or wheat germ for extra fiber, one tablespoon will add 5 to 6 grams of fiber
- Some cereal brands (Like Trader Joes high fiber Os) have 9 grs of fiber per serving.
- Oatmeal, 4 grs per cup

Meals

- Wraps with Mission Carb balance fiber wraps (e.g. form Target). These wraps have 11-15 grs of fiber (depending on the ones you get).
- Sandwiches with Nature's Own Double Fiber Wheat Bread (Target), 4 grs of fiber per slice
- Dave's Killer Bread (website), with at least 5 grs of fiber per slice
- Beans: Split peas, lentils, lima beans, black beans, chickpeas etc. 12-15 gr of fiber per cup. Lentils and split peas are the highest in fiber.
- Broccoli, 5 grs per cup
- Brussel Sprouts, 4 grs per cup
- Avocados, 6 grs, half.
- Whole wheat or chickpea pasta (check a brand called Banza, 13 grs fiber per serving)
- Barley, 6 grs
- Quinoa, 5 grs per cup

Snacks

- Fiber one bars ~9 grs of fiber.
- Kashi Go Lean Bars ~5 gr of fiber
- Fruit: apples, bananas, oranges, and grapes are a good source of fiber. One large apple will get you around 4 grams, a pear 5 grs (Asian pears even higher), a banana will get you around 3 grams, and 1 cup of grapes have just over 1 gram.
- Almonds ~ 11 grs per cup, walnuts ~ 8 gr

High fiber diet examples

http://www.eatingwell.com/article/289462/7-day-high-fiber-meal-plan-1200-calories/

http://www.eatthis.com/high-fiber-foods/

https://www.mayoclinic.org/healthy-lifestyle/nutrition-and-healthy-eating/in-depth/high-fiber-foods/art-20050948

https://www.google.com/amp/s/www.redbookmag.com/food-recipes/gmp2813/fiber-foods/

https://www.webmd.com/diet/eat-this-fiber-chart

Intervention instructions

In this intervention we will collect three types of data

1) MyFitnessPal information including macros (protein, total fat, protein), fiber and total calories. We will keep track for 3 weeks.
2) Answers to a health survey that includes questions about you, your health and eating habits.
3) 3 fecal samples before the diet intervention and 3 fecal samples after the intervention.

Instructions:

If you are interested in participating:

- Contact the lab coordinator to obtain your fecal sample collection kit and sample code. Students taking M130L will receive the kit during lab.
- Sign it and drop the consent form.
- Download MyFitnessPal to your Smartphone and start keeping track of what you eat. MUST start full tracking on April 6th to qualify for the study. Keep track every day from April 6th to April 27th. Every 2-3 days, you will need to enter the data in a common class excel file using your anonymous code.
- Diet intervention should last the two weeks outlines in the schedule and aim for 40-50 gr of fiber per day. We recommend aiming for 40 gr on week 1 and 50 grs on week 2.
- Instructions to collect your fecal sample are found (Appendix 7). Read the file carefully. You will collect a total of 6 samples.
- As indicated in the fecal sample collection instructions (Appendix 7), daily samples will be transferred to 3 duplicated Eppendorf tubes which must be stored in your freezer. To drop the samples off, place the tubes on an insulated box with an ice pack and drop the samples off within one hour.
- Sample drop will happen in a private room with a freezer.
- Finally, you will be required to answer a Health survey that will be shared with you by email.

Appendix 15: UCI IRB consent form

UNIVERSITY OF CALIFORNIA, IRVINE

CONSENT TO ACT AS A HUMAN RESEARCH SUBJECT

Responses of healthy human microbiomes to normal lifestyle perturbations.

You are being asked to participate in a research study. Participation is completely voluntary. Please read the information below and ask questions about anything that you do not understand. A researcher listed below will be available to answer your questions.

RESEARCH TEAM

Lead Researcher

Katrine Whiteson

Assistant Professor

Department of Molecular Biology and Biochemistry

(949) 824-9032 katrine@uci.edu

Co-researcher

Jennifer Martiny

Professor

Ecology & Evolutionary Biology

949.824.0487 jmartiny@uci.edu

Staff

Mi Lay

Laboratory coordinator

Department of Molecular Biology and Biochemistry

949-824-6952 mlay@uci.edu

STUDY LOCATION(S):

Mcgaugh Hall, UCI

STUDY SPONSOR(S):

UCI Microbiome Initiative

WHY IS THIS RESEARCH STUDY BEING DONE?

The purpose of this research study is to *study the effect of normal lifestyle changes (e.g. diet change, students moving to a new house) of the gut microbiota of healthy individuals.*

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

Approximately 100 participants will take part in the research at UCI. A total of 100 participants will be asked to participate across all study sites.

AM I ELIGIBLE TO PARTICIPATE IN THIS STUDY?

Please note this may not be a complete list of eligibility criteria. We have included a few examples of study criteria to help you better understand how your eligibility in the study will be determined; your study team will go through the study eligibility criteria with you to verify if you qualify for participation in this study.

If you are a healthy individual donating a sample for educational purposes on the courses M130L and M118L, you are eligible to participate by donating your sample for future research use.

Inclusion Requirements

You can participate in this study if you are at least 18 years of age or older.

Exclusion Requirements

You cannot participate in this study if you are taking medications (e.g. antibiotics) or suffer of any digestive or intestinal condition, like diarrhea or chronic digestive issues.

HOW LONG WILL THE STUDY GO ON?

Except for diet changes that will take place as intervention, other lifestyle changes are normal things you do your normal daily life (like moving to a new house). For diet interventions you will be asked to eat more fiber or more vegetables for the duration of 2 weeks. You will be asked to donate three fecal samples one week before a lifestyle change and three samples 1-2 weeks after the lifestyle change. These samples will be used in M130L, M118L or other similar courses for educational purposes. You will be asked to donate these samples for future research use.

WHAT PROCEDURES ARE INVOLVED WITH THIS STUDY?

Before you can participate in the main part of the study...

The screening procedures include a health survey. The survey will have simple questions about lifestyle. For example, for diet studies the survey will have questions about food habits and general health (in case samples need to be removed from the pool due to health concerns). For cohousing /moving studies the survey will ask about geography, climate, type of house, daily habits, etc. This screening will be done in the context of a course for educational purposes, but the survey will also serve to select consented samples for later research.

During the main part of the study...

The main study tests are part of an educational course and the procedures include:

1. You will be asked to complete a survey using an anonymous sample code to answer it. The survey will have simple questions about lifestyle.

2. For the diet intervention studies, you will be given a list of foods for a balanced diet (e.g. vegetarian and/or high fiber). You will be asked to eat a certain amount of fiber (sourced from natural food), a particular balanced diet or a food type for the duration of two weeks. For studies involving location, you will be given a sample code based on whether you are moving to a new location, with a roommate, or not moving.

3. Fecal samples will be collected three times before and three times after the lifestyle change. You will be asked to produce fecal samples at your convenience using a kit provided by the study team and drop them off at a designated private location that only one person can access at a time.

4. Samples will be labeled with an anonymous sample code and used for educational purposes. If you decide to donate your samples for future research, these will be stored in our research laboratories and will be used for sequencing and microbiome analysis purposes. We will study how the lifestyle change you went through changed (or not) your normal healthy gut microbiota.

5. Mi Lay will be in charge of collecting and storing the consent forms. Mi lay will destroy the samples that were not donated for research (no consent from). Once this is done, the key linking your name to your sample code will be destroyed.

6. Refusal to consent to the future research use of samples will not affect your grades in the course, as the instructor will only know who consented/not consented after the course grades are posted/released.

The time commitment to participate in this study will include 15-20 min to answer a survey (lifestyle/diet questions), 1-2 hours for collection and drop off of each fecal sample and two weeks for the diet intervention (e.g. high fiber).

After you complete the main part of the study

You will be asked to donate your fecal samples for future research use.

WHAT ARE THE POSSIBLE SIDE EFFECTS OR RISKS RELATED TO THE STUDY?

We will require some of your time to answer anonymous surveys and collect fecal samples. Collecting fecal samples might be embarrassing to you.

Some of the procedures may cause embarrassment or anxiety, or the questions the researchers ask you may be upsetting or make you uncomfortable. If you do not wish to answer a question, you can skip it and go to the next question. If you do not wish to participate you can stop.

Breach of confidentiality might occur. If you are concerned that your sample might be associated to your name, you are free to opt out from participation or from donating your sample for future research.

ARE THERE BENEFITS TO PARTICIPATING IN THIS STUDY?

Participant Benefits

There are no direct benefits from participation in the study.

Benefits to Others or Society

This study will help researchers learn more about normal lifestyle perturbations on the gut microbiota of healthy individuals and it is hoped that this information will help gain better understanding of normal fluctuations in the gut microbiota of healthy individuals.

WHAT OTHER CHOICES DO I HAVE IF I DON'T WANT TO PARTICIPATE?

If you decide not to participate, or if you withdraw from this study before it is completed, your other choices may include:

- Donating fecal samples during the class is optional, you can opt out without consequences to your grade and still participate in all course work.
- Donating fecal samples for future research is optional, you can decide to not donate your sample.

WILL I BE PAID FOR TAKING PART IN THIS STUDY?

You will not be compensated nor is there any cost to you for your participation in this research. In case of expensive diet interventions, we will provide meal coupons to offset the costs of the diet transition for a week.

WHAT ARE THE COSTS OF TAKING PART IN THIS STUDY?

There is no cost to you or your insurer for participation in this study. However, there may be out-of-pocket expenses such as cost of food that is not supplied by us for the diet intervention and the cost of transportation to drop samples off.

WHAT HAPPENS IF I AM INJURED BECAUSE I TOOK PART IN THIS STUDY?

It is important that you promptly tell the researchers if you believe that you have been injured because of taking part in this study. You can tell the researcher in person or call him/her at the number listed at the top of this form.

If you are injured as a result of being in this study, UCI will provide necessary medical treatment. The costs of the treatment may be covered by the University of California or billed to you or your insurer just like other medical costs, depending on a number of factors. The University and the study sponsor do not normally provide any other form of compensation for injury. For more information about this, you may call UCI Human Research Protections (949) 824-6068 or (949) 824-2125 or by e-mail at IRB@research.uci.edu

WHAT HAPPENS IF I WANT TO STOP TAKING PART IN THIS STUDY?

The key linking your sample code and name will be destroyed after the sample collection and consent period is over. If you donated a fecal sample for course work but do not wish to let us use the sample for future research, simply do not sign the consent form and your sample will be destroyed. The main researcher, Dr. Massimelli will not know who donated/not donated or consented/not consented to donate the sample for future research use until after the course grades are posted/released.

HOW WILL INFORMATION ABOUT ME AND MY PARTICIPATION BE KEPT?

Subject Identifiable Data

Your responses to surveys will be collected anonymously. To do this we will randomly assign you a sample code, which you will use to answer the survey. The same sample code will be used to label your fecal samples, which you will collect on your own and drop off at location consisting of an empty room with a cooler. The key linking your sample code and name will be destroyed after the sample collection period is over. However, we will not save any identifiers that link your sample code to your real name, making the entire process completely anonymous.

Data Storage

Research data will be maintained in paper format (consent forms) in a secure location at UCI. Only authorized individuals will have access to it. Survey data will be stored electronically on

a secure *computer with password protection.*

Data Retention

The researchers intend to keep the research data for approximately 5 years.

WHO WILL HAVE ACCESS TO MY STUDY DATA?

The research team, authorized UCI personnel, and regulatory entities such as the Office of Human Research Protections (OHRP), may have access to your study records to protect your safety and welfare.

Any information derived from this research project that personally identifies you will not be released or disclosed by these entities without your separate written consent, except as specifically required by law. Research records provided to authorized, non-UCI entities will not contain identifiable information about you. Publications and/or presentations resulting from this study will not include identifiable information about you.

While the research team will make every effort to keep your personal information confidential, it is possible that an unauthorized person might see it. We cannot guarantee total privacy.

ARE THERE OTHER ISSUES TO CONSIDER IN DECIDING WHETHER TO PARTICIPATE IN THIS STUDY?

Use of Specimens

Biospecimens (fecal samples collected from you for this study and/or information obtained from your biospecimens may be used in this research or other research. You will not share in any commercial value or profit derived from the use of your biospecimens and/or information obtained from them.

Request for Donation of Specimens and/or Data for Future Use

This is a request for donation of your *fecal sample* for medical research. By signing bellow, you are agreeing to let us collect, store and share your coded fecal sample and coded survey responses. We will keep your fecal sample for future research. This research will study how normal lifestyle changes alter the gut microbiota of healthy individuals. Your sample will be labeled with an anonymous code in lab freezers and will be used for sequencing to study bacterial diversity. We will ask you to complete the health survey online and the answers will not be linked to your student ID. These survey responses will be stored in a secure computer protected with a password. Your coded fecal sample and surveys answers will be shared with other researchers. Your fecal sample will be stored and shared in a way that does not directly identify you.

WHO CAN ANSWER MY QUESTIONS ABOUT THE STUDY?

If you have any comments, concerns, or questions regarding the conduct of this research, please contact the research team listed at the top of this form.

What is an IRB? An Institutional Review Board (IRB) is a committee made up of scientists and non-scientists. The IRB's role is to protect the rights and welfare of human subjects involved in research. The IRB also assures that the research complies with applicable regulations, laws, and institutional policies.

HOW DO I AGREE TO PARTICIPATE IN THIS STUDY?

You should not sign and date this consent form until all of your questions about this study have been answered by a member of the research team listed at the top of this form. You will be given a copy of this signed and dated consent form, and the attached "Experimental Subject's Bill of Rights" to keep. **Participation in this study is voluntary.** You may refuse to answer any question or discontinue your involvement at any time without penalty or loss of benefits to which you might otherwise be entitled. Your decision will not affect your future relationship with UCI or your quality of care at the UCI Medical Center.

If, during the course of this study, significant new information becomes available that may relate to your willingness to continue to participate, this information will be provided to you by the research team listed at the top of the form.

Your signature below indicates you have read the information in this consent form and have had a chance to ask any questions you have about this study.

I agree to participate in the study.

_____ _____

Subject Signature **Date**

_____

Printed Name of Subject

_____ _____

Name and Signature of Person Obtaining Informed Consent **Date**

(Individual must be listed on Page 1 of this consent)

Appendix 16: Health Questionnaire

1. This questionnaire is anonymous and will not be linked to your student ID. However, for research purposes we need to link your questionnaire answers to your fecal sample anonymous code. What is your sample code?
2. What is your age?
    a. 17 or under
    b. 18-20
    c. 21-24
    d. 25-30
    e. 30-35
    f. 35-40
    g. 40-50
    h. 50-60
    i. Over 60
3. Sex
    a. male
    b. female
    c. prefer not to answer
4. Please choose one that best describes you:
    a. American Indian/Alaskan native
    b. Black or African American
    c. Asian or Pacific Islander
    d. Hispanic/Latino
    e. White
    f. Other _____
5. How would you describe your living situation?
    a. University accommodation/dorm/university
    b. family home
    c. rental room
    d. rented apartment on your own
    e. rented apartment with roommates
    f. Other _____
6. Do you currently cohabitate with other individuals? Explain
    a. Yes.
    b. No.

    Explain:

7. Have you moved to a new house or with a new person in the past two months?
    a. Yes.
    b. No.
    Explain:
8. Have you recently traveled abroad in the last three months?
    a. Yes.
    b. No.
    Explain (approximate Date & Duration):
9. Have you recently out of state in the last three months?
    a. Yes.
    b. No.
    Explain (approximate Date & Duration):
10. Do you have pets?
    a. Yes.
    b. No.
    Explain:
11. How would you rate your general physical health?
    a. Excellent
    b. Very good
    c. Good
    d. Fair
    e. Poor
12. Prescriptions and supplements
    a. Have you taken a course of antibiotic in the past 3 months?
        i. Yes
        ii. No
        If yes, what antibiotic and how many courses within the past 3 months?
    b. Are you currently taking any other prescription medication?
        i. Yes.
        ii. No.
        Explain:
    c. Do you take any form of supplements (i.e. Omega 3, Vitamin B, Fiber, etc.)?
        i. Yes.
        ii. No.
        Explain:

13. GI health
    a. Do you suffer from GI/digestive issues? (irritable bowel syndrome, inflammatory bowel disease, coeliac disease, constipation, diarrhea, excessive bloating)
        i. Yes
        ii. No

        If yes, explain:

    b. Have you taken laxatives, gastric motility medications or supplements within the past month?
        i. Yes
        ii. No

        If yes, explain

    c. Do you have any food allergies? (ie, lactose intolerance, gluten sensitivity)
        i. Yes
        ii. No
    d. Have you taken a probiotic in the last month?
        i. Yes
        ii. No
    e. Are you currently taking a probiotic?
        i. Yes
        ii. No

        If you are taking or have taken a probiotic in the last month, which one?

    f. Have you ever had a Fecal Matter Transplant?
        i. Yes
        ii. No
    g. Have you experienced any form of gastrointestinal sickness (e.g. food poisoning, travel diarrhea) in the last month?
        i. Yes
        ii. No

        If you have experienced gastrointestinal sickness, explain:

14. Have you been or are you currently pregnant?
    a. Yes
    b. No

    If yes, number of children (if any)

15. Diet
    a. Do you follow any type of restrictive diet? (organic, gluten-free, vegetarian, paleo, etc.)

i. Yes
ii. No

If follow any type of restrictive diet, explain:

b. How often do you eat at fast food restaurants?
   i. Never
   ii. Once a month
   iii. Once a week
   iv. 2-3 times a week
   v. 3+ times a week
c. Do you consume primarily organic foods?
   i. Yes
   ii. No
d. Do you consume diet beverages containing artificial sweeteners?
   i. 1 daily
   ii. 1+ daily
   iii. 1-2 week
   iv. 1-2 month
   v. rarely or never
e. Have you undergone a significant dietary change within the past year (i.e., has become vegetarian, removed gluten from their diet, actively trying to lose weight, etc.)?
   i. Yes
   ii. No
f. if you have undergone a significant dietary change within the past year explain:
g. How many portions of fruit and vegetables do you eat for breakfast? _____
h. How many portions of fruit and vegetables do you eat during lunch? ___
i. How many portions of fruit and vegetables do you eat during dinner? ____
j. How many portions of fruit and vegetables do eat as snack? __
k. How often do you eat yogurt?
   i. Every day
   ii. 2-3 times a week
   iii. once a week
   iv. occasionally
   v. never
l. What other fermented do you eat frequently? _____

16. Use the following tool https://www.smartbmicalculator.com/ calculate your BMI, your number. Write the number in the space provided below_____
17. Have you experienced significant weight loss or weight gain (>5% of total body weight) within the past year?
    a. Yes
    b. No

    if yes, explain
18. How many hours of sleep do you get on an average night?
    a. Less than 4
    b. 4 to 6
    c. 7 to 8
    d. 9 to 10
    e. More than 10
    f. Exercise
19. Which of the following best describes your activity level?
    a. Not very active
    b. Sitting most of the day (ex: desk job, bus driver)
    c. Light Active
    d. Occasionally on your feet throughout the day
    e. Active
    f. Some physical activity throughout the day (ex: cross-guard, waiter)
    g. Very Active
    h. Heavy physical activity the majority of the day (ex: construction worker, full time athlete)
20. Do you have a workout routine? e.g. running, going to the gym, etc.
    a. No
    b. Yes, I work out once a day
    c. Yes, I work out two or more times a day
    d. Yes, I work out 2-3 times a week
    e. Yes, I work out once a week
21. Your exercise/ workout routine is mostly
    a. Cardiovascular
    b. Muscle building
    c. None
22. On a given week, on how many days are you physically active (e.g. Workout out or walking) for a total of 30 minutes or more?
    a. 0 days
    b. 1-2 days

c. 3-4 days
d. 5 or more days

23. If 4 days or less, are you usually physically active for at least two and a half hours (150 minutes) over the course of 7 days?
24. 19. Do you have Type 1 or 2 diabetes, or have you been diagnosed as pre-diabetic?
    a. Yes
    b. No
    c. if yes, explain
25. How would you describe your dental hygiene?
    a. Brush and floss daily
    b. Only brush daily
    c. Only floss daily
    d. No daily routine
26. How much water you consume a day?
    a. 0.5 liters or less
    b. 0.5 -1 liter
    c. 1-2 liter
    d. 2 liters or more
27. Were you born via:
    a. natural birth
    b. C-section
28. How often do you wash your hands?
    a. Very frequently (mostly after toilet visits and when dirty or manipulating sticky or wet food/lab items)
    b. Frequently (e.g. after each toilet visit, before and after cooking, before and after working in the lab = 15+ times a day)
    c. rarely (mostly if going to the toilet outside my house)
29. How many alcoholic beverages do you consume in a given week?
    a. I do not drink
    a. drinks a week
    b. 2-3 drinks weekly
    c. 3+ drinks a week
30. How many cups of coffee do you consume a day?
    a. I do not drink coffee
    a. day
    b. 2-3 day
    c. 3+ a day

31. Which of the following best describe your smoking habit?
    a. I do not smoke
    b. I smoke every day
    c. I smoke 2-3 times a week
    d. I smoke of rare occasions

Appendix 17: Post Microbiome study survey

1. This survey is anonymous. For alignment purposes please enter your sample code:
2. Side effects
    a. Did your experience any symptoms or side effects? Yes/no
    b. If yes, select all the applies
        i. no symptoms
        ii. Flatulence
        iii. bloating
        iv. lack of appetite
        v. heartburn
        vi. abdominal pain or discomfort
        vii. diarrhea
        viii. constipation
        ix. other
    c. For your top 1-3 symptoms provide additional observations and describe duration and frequency
    d. Did you experience any weight change during those two weeks?
3. Traveling
    a. Did you travel during the diet intervention period? Yes/no
    b. If yes, describe duration and effect in your high fiber diet.
4. Other changes
    a. Did you experience any changes in your living situation (moving, new roommate, new pet) during the intervention period? Yes/no
    b. If yes, explain
5. Prescriptions and supplements
    a. Did you take any prescription or supplements during the duration of the intervention? Yes/no
    b. If yes, explain
    c. Did you take any laxatives, gastric motility medications or gas medication to ameliorate the symptoms you experienced from a high fiber intake? Yes/no
    d. If yes, explain
6. Diet
    a. Did you make any other changes to your diet (in addition to increasing fiber intake) during the intervention period? e.g. stopped/started eating vegetarian/vegan, gluten free, etc. yes/no
    b. If yes, explain
    c. How often did you eat at fast food restaurants during the intervention period? Explain
    d. Did you consume primarily organic foods? Explain

e. Did you consume diet beverages containing artificial sweeteners?
f. How many portions of fruit and vegetables did you eat for breakfast?
g. How many portions of fruit and vegetables did you eat as snack?
h. We didn't include lunch/dinner portions because you ate plenty with Thistle. Describe any other lunch/dinner portion/habit during days you did not have Thistle meals
i. How often did you eat yogurt during the intervention?
j. What other fermented did you eat during the intervention?
k. What was your "go to" or staple high fiber foods? name your top 3 and provide a description
l. How many cups of coffee did you consume a day during the intervention?

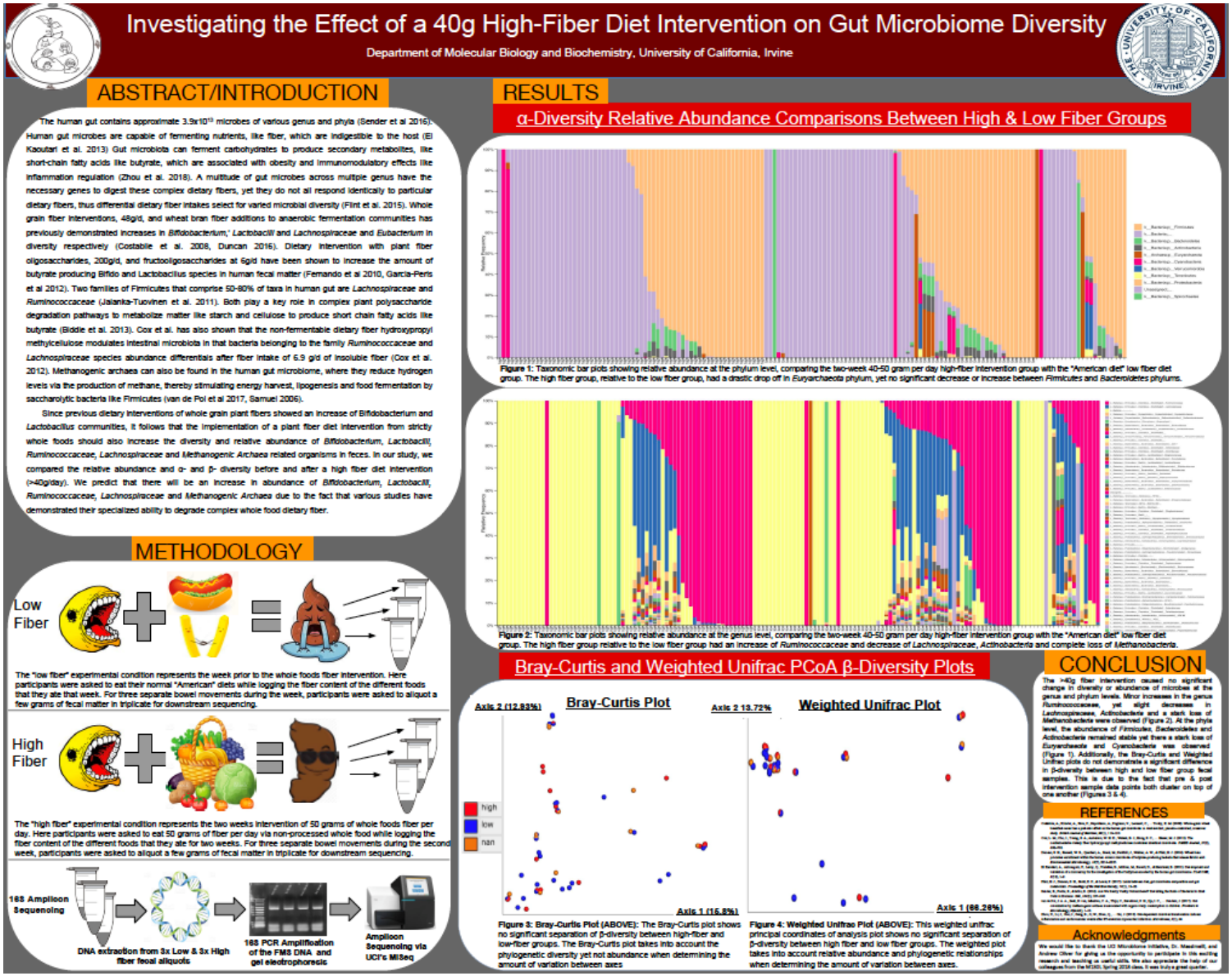
7. The intervention
    a. Was it difficult for you to make the fiber mark? Explain
    b. Did the intervention change any of your habits? e.g. do you eat more fiber now, check fiber of foods, etc. Provide detailed information.
    c. What was the biggest lesson your learned about the relationship between the microbiome and fiber? Describe
    d. Describe your opinion about the Thistle meals we provided
    e. tell us something funny or memorable related to the diet intervention
8. Time and cost commitment
    a. To eat more fiber, did you have to spend more time cooking? (compared to your pre-intervention routine)
    b. if yes, explain
    c. Taking out the Thistle meals, did you spend more money in food than you did before the intervention? Explain
    d. In addition to time and cost, what else is difficult/a limitation about a diet high in fiber?
    e. Are you eating more fiber now than you did in the weeks before the intervention? Explain
9. The class
    a. what misconceptions did you have about the microbiome and/or fiber before taking this class and how did this class address that misconception?
    b. Describe your favorite thing about his class (assignment or research)
    c. What skills did you cultivate the most in this class? name two and explain what you learned
    d. What can we do differently in the future? tell us how to make the class better
    e. if you dropped out or skipped data days, explain why
    f. Add here anything else you want to share:

Appendix 18: Post Microbiome Study Survey responses

Download data from Open Science Framework

Sewall, J. M. (2019, October 3). Appendix 18 Post Study survey responses. Retrieved from osf.io/zsqdb

Appendix 19: Sample Students' Posters
Poster 1



Poster 1 conclusion:
The >40g fiber intervention caused no significant change in diversity or abundance of microbes at the genus and phylum levels. Minor increases in the genus *Ruminococcaceae*, yet slight decreases in *Lachnospiraceae*, Actinobacteria and a stark loss of *Methanobacteria* were observed (Figure 2). At the phyla level, the abundance of Firmicutes, Bacteroidetes and Actinobacteria remained stable yet there a stark loss of *Euryarchaeota* and Cyanobacteria was observed (Figure 1). Additionally, the Bray-Curtis and Weighted Unifrac plots do not demonstrate a significant difference in β-diversity between high and low fiber group fecal samples. This is due to the fact that pre & post intervention sample data points both cluster on top of one another (Figures 3 & 4).

Poster 1 References

Costabile, A., Klinder, A., Fava, F., Napolitano, A., Fogliano, V., Leonard, C., … Tuohy, K. M. (2008). Whole-grain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: A double-blind, placebo-controlled, crossover study. British Journal of Nutrition, 99(1), 110–120.
Cox, L. M., Cho, I., Young, S. A., Anderson, W. H. K., Waters, B. J., Hung, S. C., … Blaser, M. J. (2013). The nonfermentable dietary fiber hydroxypropyl methylcellulose modulates intestinal microbiota. FASEB Journal, 27(2), 692–702.
Duncan, S. H., Russell, W. R., Quartieri, A., Rossi, M., Parkhill, J., Walker, A. W., & Flint, H. J. (2016). Wheat bran promotes enrichment within the human colonic microbiota of butyrate-producing bacteria that release ferulic acid. Environmental Microbiology, 18(7), 2214–2225.
El Kaoutari, A., Armougom, F., Leroy, Q., Vialettes, B., Million, M., Raoult, D., & Henrissat, B. (2013). Development and validation of a microarray for the investigation of the CAZymes encoded by the human gut microbiome. PLoS ONE, 8(12), 1–9.
Flint, H. J., Duncan, S. H., Scott, K. P., & Louis, P. (2015). Links between diet, gut microbiota composition and gut metabolism. Proceedings of the Nutrition Society, 74(1), 13–22.
Sender, R., Fuchs, S., & Milo, R. (2016). Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. Cell, 164(3), 337–340.
van de Pol, J. A. A., Best, N. van, Mbakwa, C. A., Thijs, C., Savelkoul, P. H., Ilja, I. C., … Penders, J. (2017). Gut colonization by methanogenic archaea is associated with organic dairy consumption in children. Frontiers in Microbiology, 8(MAR), 1–10.
Zhou, X., Li, J., Guo, J., Geng, B., Ji, W., Zhao, Q., … Cai, J. (2018). Gut-dependent microbial translocation induces inflammation and cardiovascular events after ST-elevation myocardial infarction. Microbiome, 6(1), 66.

The effect of a high fiber diet intervention on short chain fatty acid producing bacteria in human gut

Ayala School of Biological Sciences, University of California, Irvine
Irvine, CA 92697

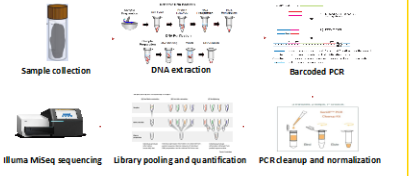
Abstract

- **Background and rational:** Dysbiosis connected to disease such as colon cancer (Dejea et al., 2014). Metabolism of the bacteria making up microbiome can affect host: in this study biofilms cause inflammation which induce colorectal cancer which is found in colonoscopy biopsies of human patients. Meta-analysis of case-control study of colorectal cancer indicates high fiber as prevention of disease (Levi, et al., 2001). Microbiota richness found to be associated with Short chain fatty acids (SCFA) during diet intervention (Tap et al., 2015). Group where fed either 10 or 40g of fiber for 5 days, two week washout, then switch the amount of fiber. As a result, alpha diversity not changed, based on OTUs. Individual changes were found. A high fiber diet in mice led to increase in *Bifidobacterium* and *Lachnospiraceae* (Bisheksari *et al.*, 2018). Groups of mice with and without colorectal tumors split into groups that would receive normal chow or 'high fiber' diet. High fiber based on 20% the mouse's weight. Contains 50% chitinglucan and 50% potato starch. After a 12 week intervention they found increase in SCFA which attributed to an increase in *Bifidobacterium* and *Lachnospiraceae*, seen by from Illumina.

Hypothesis

If fiber is known to increase gastrointestinal fermentation that produces short-chain fatty acids, then a 40-50 gram fiber intervention should exhibit increases in short-chain fatty acid producing, gram-positive, anaerobic bacteria such as *Bifidobacterium* and *Lactobacillus* during the second week.

Methods



Results

Figure 1. Relative Abundances of High vs Low Fiber groups. Taxa bar plots of 22 subjects with 6 samples each. 64 low fiber and 65 high fiber samples per subject. Relative abundances were run via two tailed two sample t test with equal distribution between high and low fiber groups. Genus *Methanobrevibacter* of the Archea kingdom was the only group to demonstrate a significant change amongst groups with a p-value of 0.045 (p-value<0.05).

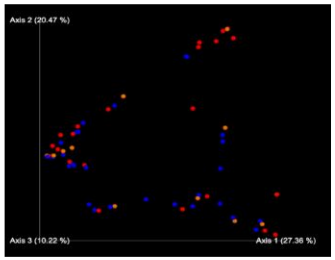


Figure 2. Unweighted Unifrac of High and Low Fiber Groups. Unweighted Unifrac distance is a qualitative measure of community dissimilarity that incorporates phylogenetic relationships between the features. Red markers depict high fiber groups while blue markers were low fiber groups. Orange markers represent the controls and other unmarked subjects. Skewing of both high and low fiber groups has random variances amongst groups which means beta-diversity did not change amongst groups.

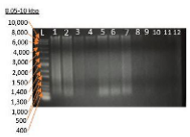


Figure 3. Assessing the quality of the fecal matter sample (FMS) DNA extraction by gel electrophoresis. The fecal matter DNA extraction was processed using the **ZymoBIOMICS DNA Miniprep Kit** recommended protocol. DNA was eluded using **100 µl ZymoBIOMICS DNase/RNase Free Water**. 6 µl of each sample was loaded on a 1.0% agarose gel. Lane L contains the 10 µl ladder and lanes 1-12 contain the class FMS data DNA extracts.

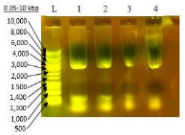


Figure 4. Assessing the fecal matter sample (FMS) 16S polymerase chain reaction (PCR) extract based quality and success by gel electrophoresis. The antibiotic producer isolate DNA extraction was processed using the SWI Research Protocols. The 16S rRNA gene of the isolated antibiotic producer was amplified by PCR. Lane 1 and 2 contains 2.5 µl of DNA and 1 µl of 10X DNA Gel Loading Dye on a 1.0% TBE agarose gel that ran with an electric voltage for 15 minutes. 6 µl of the 1 kilobase (Kb) ladder was loaded in lane L. Lane 1-4 contain the classmates 16S PCR extract band.

Conclusion

In conclusion, we found that there was no significant difference in relative abundance of short fatty chain acid producing bacteria such as *Bifidobacterium*, *Lactobacillus*, *Dorea* and *Coprococcus* after a 40-50 gram fiber diet in comparison to a normal diet. We also found that beta-diversity did not change among the group. For a future direction we would start with low fiber diet with 10g/day, follow by washout period with normal Western diet and end by high fiber diet with 50g/day.

Acknowledgements

We'd like to thank Dr. Julia Massimelli, Ph.D and Andrew Oliver for their guidance and the support that they provided on this project. And we would also like to thank Dr. Mi Lay and her staff for their assistance providing lab supplies for this project.

References

- Levi F, Pasche C, Lucchini F, et al. : Dietary fiber and the risk of colorectal cancer. Eur J Cancer 37:2091–2096, 2001
- Tap, J. , Furet, J. , Bensaada, M. , Philippe, C. , Roth, H. , Rabot, S. , Lakhdari, O. , Lombard, V. , Henrissat, B. , Corthier, G. , Fontaine, E. , Doré, J. and Leclerc, M. (2015). Gut microbiota richness and dietary fibre intake. Environ Microbiol, 17: 4954-4964.
- Bisheksari, F., Engen, P.A., Preite, N.Z., Tuncil, Y.E., Naqib, A., Shaikh, M., … Keshavarzian, A. (2018). Dietary Fiber Treatment Corrects the Composition of Gut Microbiota, Promotes SCFA Production, and Suppresses Colon Carcinogenesis. Genes, 9(2), 102. doi:10.3390/genes9020102

References poster 2

Levi F, Pasche C, Lucchini F, et al. : Dietary fiber and the risk of colorectal cancer. Eur J Cancer 37:2091–2096, 2001

Tap, J. , Furet, J. , Bensaada, M. , Philippe, C. , Roth, H. , Rabot, S. , Lakhdari, O. , Lombard, V. , Henrissat, B. , Corthier, G. , Fontaine, E. , Doré, J. and Leclerc, M. (2015), Gut microbiota richness and dietary fibre intake. Environ Microbiol, 17: 4954-4964.

Bishehsari, F., Engen, P. A., Preite, N. Z., Tuncil, Y. E., Naqib, A., Shaikh, M., … Keshavarzian, A. (2018). Dietary Fiber Treatment Corrects the Composition of Gut Microbiota, Promotes SCFA Production, and Suppresses Colon Carcinogenesis. Genes, 9(2), 102. doi:10.3390/genes9020102