



Supplemental Materials

for

Unique Down to Our Microbes—Assessment of an Inquiry-Based Metagenomics Activity

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Appendix 1: Student Handout

Unique Down to Our Microbes...

(AN INQUIRY-BASED METAGENOMICS CLASSROOM ACTIVITY)

INTRODUCTION

Microbes (i.e., bacteria) are around us everywhere, in the soil, in our homes and on our skin! We also use microbes in our everyday lives. They are important in making certain foods (e.g., cheese and bread), keeping our environment clean (e.g., waste water treatment and bioremediation), and producing pharmaceutical and industrial products (e.g., antibiotics, hormones, and enzymes).

“Each of us consists of about 10 trillion human cells, but we harbor as many as 100 trillion microbial cells. So they outnumber us 10 to one”

- Dr. Rob Knight, 2014 TED Talk

However, our current understanding only begins to scratch the surface of the complexity of microbes that share our world.

Case studies of the human microbiota

Effect of route of birth on our microbes

Infants born naturally versus via cesarean section have quite different microbiota. These differences correlate with increased incidence of diseases, such as obesity, asthma, and allergies in infants born via cesarean section (Dominguez-Bello et al., 2010; Neu & Rushing, 2011).

Microbes are a piece of the puzzle

Scientists have found a link between microbiota and autism spectrum disorders using a mouse model for the disease. Interestingly, they also found that specific probiotics may decrease the severity of autistic behavior (Hsiao et al., 2013).

Positive effects of microbes in our bodies

Certain gut microbiota in male mice promote high testosterone levels, which correlates with protection against type 1 diabetes. Further, transfer of these microbes to female mice resulted in increased testosterone levels and conferred similar protection against the disease (Markle et al., 2013).

With the help of new technologies, we are learning that the microbes that share our world and inhabit our bodies play a remarkable role in our personal health and perhaps our individuality. The microbes that live on or within our bodies make up our **microbiota**. Scientists and clinicians are working to understand how a person's microbiota impacts personal health and how disruptions of our microbiota may contribute to disease. In 2007, the National Institutes of Health launched an initiative, the Human Microbiome Project, to collect data and support the development of analysis tools that will help scientists uncover the connections between our microbiota and human health (Turnbaugh et al., 2007).

Identifying all of the microbes on a person's body and capturing a snapshot of their microbiota is a daunting task. The human body is made up of ~10 trillion cells and it's estimated that we are inhabited by 100 trillion microbes. Historically, this has been done by growing and identifying each bacteria, which is time consuming, expensive and perhaps impossible

for some species that are not easily cultured. However, scientists have found that by studying the **microbiome**, or the microbial DNA isolated from a person's microbiota, we can get closer to identifying all of the microbes present. This method of analyzing the DNA, or genomes, associated with a sample is referred to as **metagenomics**.

Dr. Rob Dunn, a researcher at North Carolina State University, uses a citizen science approach of metagenomics data collection to gather information on the diversity of microbial communities found on individuals. His research group has analyzed samples from hundreds of belly buttons, armpits, and homes ([http:// yourwildlife.org/](http://yourwildlife.org/)). By recruiting participation from people and classrooms across the United States, they have accumulated extensive data sets on the diversity of personal microbiomes. In this activity, we focus on the data set of swabs from belly buttons. Take a look at the project website (<http://navels.yourwildlife.org/>) and his research publication to learn more (Hulcr et al., 2012). Dr. Dunn's group found similarities and differences in the types and distribution of microbes across more than 250 individuals tested, but there is still much to learn. Through this activity, you will use the **metadata**, or data describing information about each of the samples within the data set (e.g., innie/outie, gender, age), to design and test your own hypothesis about what makes us unique down to our microbes.

Keywords

Microbiota: The microbial community present in or on a surface/person

Microbiome: The microbial DNA that is isolated from a surface/person

Metagenomics: A technique to study the microbial DNA present in a sample to identify and characterize the microbes present.

Metadata: Data that describes information about the samples within a data set.

REFERENCES

- Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A*, *107*(26), 11971-11975. doi: 10.1073/pnas.1002601107
- Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., . . . Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*, *155*(7), 1451-1463. doi: 10.1016/j.cell.2013.11.024
- Hulcr, J., Latimer, A. M., Henley, J. B., Rountree, N. R., Fierer, N., Lucky, A., . . . Dunn, R. R. (2012). A jungle in there: bacteria in belly buttons are highly diverse, but predictable. *PLoS One*, *7*(11), e47712. doi: 10.1371/journal.pone.0047712
- Markle, J. G., Frank, D. N., Mortin-Toth, S., Robertson, C. E., Feazel, L. M., Rolle-Kampczyk, U., . . . Danska, J. S. (2013). Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science*, *339*(6123), 1084-1088. doi: 10.1126/science.1233521
- Neu, J., & Rushing, J. (2011). Cesarean versus vaginal delivery: long-term infant outcomes and the hygiene hypothesis. *Clin Perinatol*, *38*(2), 321-331. doi: 10.1016/j.clp.2011.03.008
- Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, *449*(7164), 804-810. doi: 10.1038/nature06244

ACTIVITY OVERVIEW

LEARNING OUTCOMES

After completion of this activity you will be able to...

1. **Define** microbiota, microbiome, metagenomics, and metadata
2. **Describe** the biological impact of microbiota on human health
3. **Formulate** a hypothesis to address a specific question
4. **Analyze and interpret** metagenomics data to compare microbiota
5. **Evaluate** a specific hypothesis
6. **Synthesize** a conceptual model as to why microbial populations vary

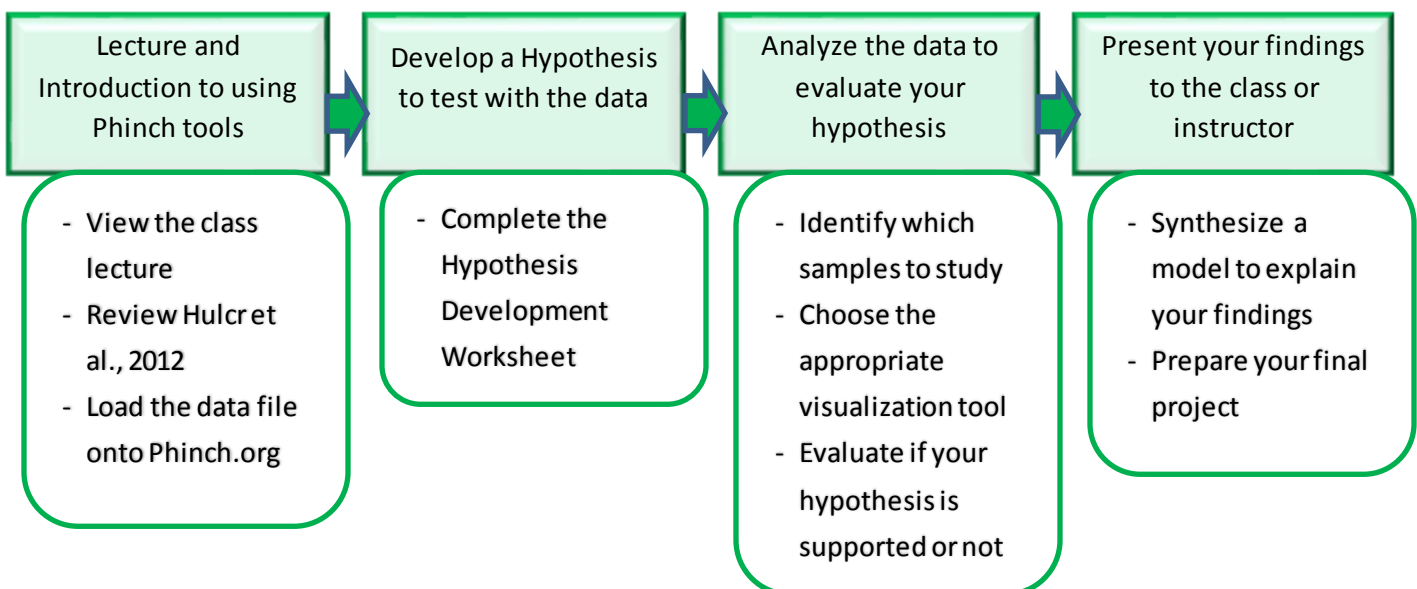
In this activity, you will work directly with Dr. Dunn's data to test your own hypothesis and discover differences in the microbial communities on people's belly buttons. This data set includes the identity of bacteria present in each subject's belly button and information on the subject's location, washing habits, and other variables (metadata). You will be guided through developing a hypothesis and using this data set to evaluate if your hypothesis is supported or refuted. This is a unique opportunity to work with *real data* and possibly uncover a novel scientific finding!

TASKS

1) Develop a hypothesis about the differences in microbiota from person to person by completing the Hypothesis Development Worksheet. *What types of bacteria are found on our skin? Where might they come from? What environmental and human factors affect these microbial populations?*

2) Analyze the Belly Button Biodiversity project data using Phinch.org to evaluate whether your hypothesis is supported or refuted by the microbial diversity found on individuals tested. Work with the different visualization tools and use your judgment in choosing which you think best renders the data to evaluate your hypothesis.

3) Present your findings and conclusion to the class or instructor. Your instructor will indicate whether the final project will be a presentation to the class or turning in a written report.



INSTRUCTIONS FOR USING PHINCH.ORG

Working with the Phinch.org metagenomics data visualization tool:

1. A tutorial video can be viewed at <https://vimeo.com/103012368>.
2. The tutorial video can also be reached through www.phinch.org. Follow the 'BLOG' link in the upper right hand corner. A link to the video is posted under the blog entry on August 10, 2014: *Narrated Screencast of Phinch Now Posted Online*.

Uploading the data file onto Phinch.org:

1. Obtain the Belly Button Biodiversity project metagenomics data file from your instructor (BellyButtonDunn.biom)
2. Open www.phinch.org (works with **Chrome** browser)
3. Drag-and-drop or Load the BellyButtonDunn.biom file when prompted on the website homepage
4. Once loaded, you should see a screen similar to the following:

The screenshot shows the Phinch.org interface for analyzing a file named 'BellyButtonDataFile_v5-26-15.biom'. The main area displays a table with the following data:

PHINCH NAME	BIOM SAMPLE ID	SAMPLE NAME	SEQUENCE READS
0	1	BBP1F7134	1000
1	2	BBP3A101496	1000
2	3	BBP4E6YWL133	1000
3	4	BBP2A11332	1000
4	5	BBP4G1CAL31005	1000
5	6	BBP1E1185	1000
6	7	BBP4B4YWL1084	1000
7	8	BBP2E1180	1000
8	9	BBP2H61270	1000
9	10	BBP2H81315	1000
10	11	BBP3C121571	1000
11	12	BBP1D1132	1000
12	13	BBP3H61412	1000
13	14	BBP3G21361	1000
14	15	RRP3C11353	1000

Exporting or capturing charts/graphs/figures from Phinch.org:

The website is enabled to export data as .png image files. Once you have the display tool you are interested in using, simply select the [Export] button at the top of the web page. Then click on the image in the pop-up window to automatically download a .png file.

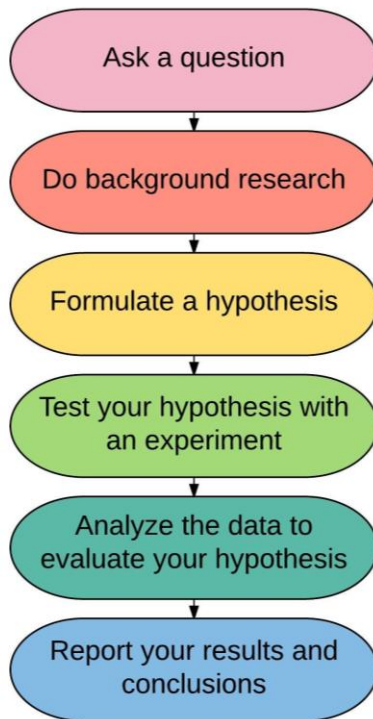
The following approach can be used for capturing charts/graphs/figures by screen capture on PCs.

1. Display the chart/graph/figure you would like to capture on your computer screen.
2. Find and press the 'Print Screen' key on your keyboard (this saves a copy of your screen to your computer's clipboard as an image).
 - a. This key may appear as [PrntScrn], [PrtScn], or other shortened version.
 - b. This key may be both Print Screen and another function (e.g., 'End' or 'SysRq') and may require holding the 'Function' key [Fn] when pressing the 'Print Screen' key.
3. In the program of choice, use the 'Paste' function ([Ctrl][v] will work on PCs).

HYPOTHESIS DEVELOPMENT WORKSHEET

Do you know what you would find if you could identify ALL of the bacteria on your belly button? Think about this for a minute. With advances in DNA sequencing technology and metagenomics data analysis, we can begin to answer questions like this.

Scientific Method



In this portion of the activity, use the scientific method to develop a hypothesis about the types of bacteria found on the human belly button or why they may be there. Consider what you have learned about microbiota and metagenomics data, as well as information about the subjects collected as metadata in the study (listed below).

Begin by identifying a question you have about the belly button microbiota. As you settle on a question that interests you, do some background research. What is already known? Have researchers asked similar questions before? What did they discover? Also, consider the data available to you.

Upload the data set to Phinch.org to see what type of information is available and how it can be visualized. Will you be able to answer your question with the data provided? Do you have to modify or make your question more specific to generate a testable hypothesis? Complete the guiding questions to reflect on your question throughout this process. Finally, formulate a specific hypothesis that you can test by analyzing the Belly Button Biodiversity study metagenomics data using Phinch.org.

METADATA:

Variable

- Gender
- Age
- Wash Frequency [times per week]
- Innie or Outie
- Location [City, State or Country]

Appearance in Phinch

Slide scale* (1 = male, 2 = female)

Slide scale

Slide scale (99 = unknown)

Slide scale* (innie = 1, outie =2)

Check boxes

* 0 = unreported

Your question:

REFLECTION QUESTIONS (short answer – 1-2 sentences):

Can your question be answered with the available metadata variables? How could you modify/specify your question so that it can be?

Does answering your question require finding information on how environmental and/or human factors affect the microbial population on the belly button? Which factors?

Does answering your question require finding information on a specific type of bacteria (e.g., bacteria with a specific function or potential pathogens)? Which type?

Drawing from your answers above, develop a hypothesis that is more specific than your initial question. Your hypothesis should be a statement. It should clearly communicate a prediction about the nature of microbiota on the belly button.

Your specific hypothesis:

A fun example: *The most common bacteria on belly buttons from Raleigh, NC are shaped like wolves.* To answer this hypothesis, you would need to use the data from the study to identify the most common bacteria in Raleigh, NC samples and then do background research to find the shape of this organism. *Spoiler – this hypothesis is refuted (not supported) by the data.*

STUDENT PROJECT

After developing a hypothesis to test with the Belly Button Biodiversity study, the next step is to analyze the data and determine if your hypothesis is supported or refuted. For this activity, analysis will be performed with the Phinch.org online tool. This tool enables visualization of metagenomics data in a number of different ways and will help you think about the data and assess the validity of your hypothesis. You should choose a visualization method (e.g., graph or diagram) that is relevant to evaluate your hypothesis. Use the resulting figures in preparing your presentation or report. See the 'Instructions for Using Phinch.org' document provided for help with this. Below is an outline of what should be included in your presentation or report. Expectations for each section are further defined in the rubric provided.

OUTLINE:

1. **Title.** Create a title that clearly describes your project.
2. **Introduction and Background.** Introduce why it is important to study the human microbiota. Discuss the data collected in this study and why you think it is interesting.
3. **Hypothesis.** Clearly indicate your hypothesis and the rationale behind it. What samples from the data set can you use to test your hypothesis?
4. **Data.** Show the data you used to evaluate your hypothesis. Include and label graphs/diagrams from Phinch.org as figures. Justify the graphs/diagrams you chose for visualizing the data.
5. **Conclusions and Limitations.** Explain whether your hypothesis is supported or refuted by the data. Discuss any limitations in the data that hindered your evaluation.
6. **Model and Future Directions.** Based on your conclusions, create a model (drawing, cartoon, diagram, flow chart, concept map, etc.) that illustrates how human microbial communities may develop, change, or impact human health. Be creative! Describe future directions you would pursue if this were your research.
7. **References.** Include citation of all documents and resources used.

PRESENTATION:

Prepare a presentation ~10 minutes in length (i.e., about 6-9 slides). Design your slides to cover each of the topics indicated in the outline above. Practice your presentation without an audience to gauge how much time it takes.

REPORT:

Prepare a paper of about 2-3 pages in length. Your paper should not be separated into sections based on the topics indicated in the outline above, but be sure to include the content from each topic. Your instructor will indicate the expected format.

Appendix 2: Sample Lesson Slides

Unique Down to Our Microbes...

An Inquiry-Based Metagenomics
Classroom Activity

Our world is full of microbes

In the environment...

<http://www.troutnut.com/underwater-pictures/of-rivers/2>

<http://ngm.nationalgeographic.com/2013/01/125-microbes/oeggerli-photography/#05-intestinal-bacteria-670.jpg>

In our homes...

http://ngm.nationalgeographic.com/wallpaper/img/2013/01/11-mouth-microbes_1600.jpg

<http://www.wilburhenry.com/save-a-service-call-how-to-avoid-grease-build-up-clogging-your-drain/>

On our kitchen tables...

http://www.nautiandco.com/p/1998/1_amazing-kitchen-table-dining-table-pool-table-table-centerpieces-round-cherry-kitchen-table-and-chairs-round-kitchen-table-and-chairs-with-leaf-large/

<http://phys.org/news/2013-04-crevices-coli-reveals-role-flagellum.html>

We are surrounded by microbes

...even on our bodies

<http://www.wellsphere.com/complementary-alternative-medicine-article/multivitamin-and-mineral-supplement-better-then-iron-and-folic-acid-alone-in-nbsp-pregnancy/863124>

http://www.huffingtonpost.com/alanna-collen/microbiome-health_b_7305128.html

Scientists estimate there are at least as many bacterial cells as human cells on/in your body

Microbiota - The microbial community present in or on a surface

We don't know what these Microbiota do

Are these microbes.

<http://www.wellsphere.com/complementary-alternative-medicine-article/multivitamin-and-mineral-supplement-better-than-iron-and-folic-acid-alone-in-nbsp-pregnancy/863124>

Harmful?
 Stable?
 Helpful?
 Unique?
 Changing?
 Common?

We wash our hands to remove them.
 We use antibiotics to kill them.
 - How do these practices affect our microbiota? -

Traditional method of studying microbiota

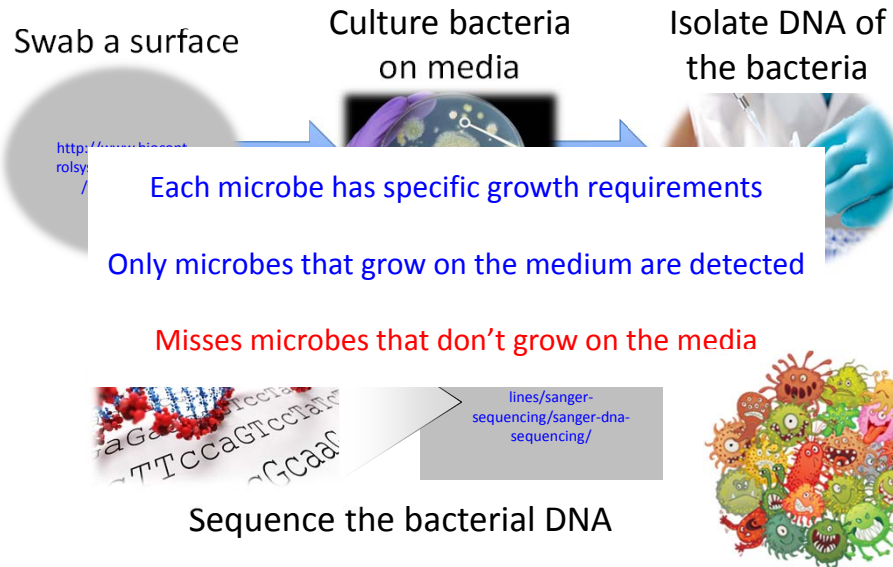
Swab a surface Culture bacteria on media Isolate DNA of the bacteria

<http://www.biocontrolsys.com/products/view/MVPS>

<http://vantage.vanderbilt.edu/vantage-services-lines/sanger-sequencing/sanger-dna-sequencing/>

Sequence the bacterial DNA

Traditional method of studying microbiota

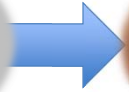


Metagenomics approach to studying microbiota

Metagenomics – A technique to study the microbial DNA present in a sample to identify and characterize the microbes present

Metagenomics approach to studying microbiota

Swab a surface



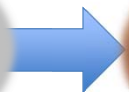
Isolate the DNA



Microbiome - The microbial DNA that is isolated from a surface/person

Metagenomics approach to studying microbiota

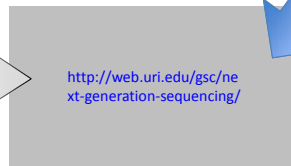
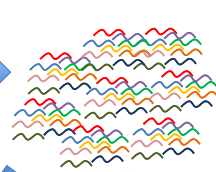
Swab a surface



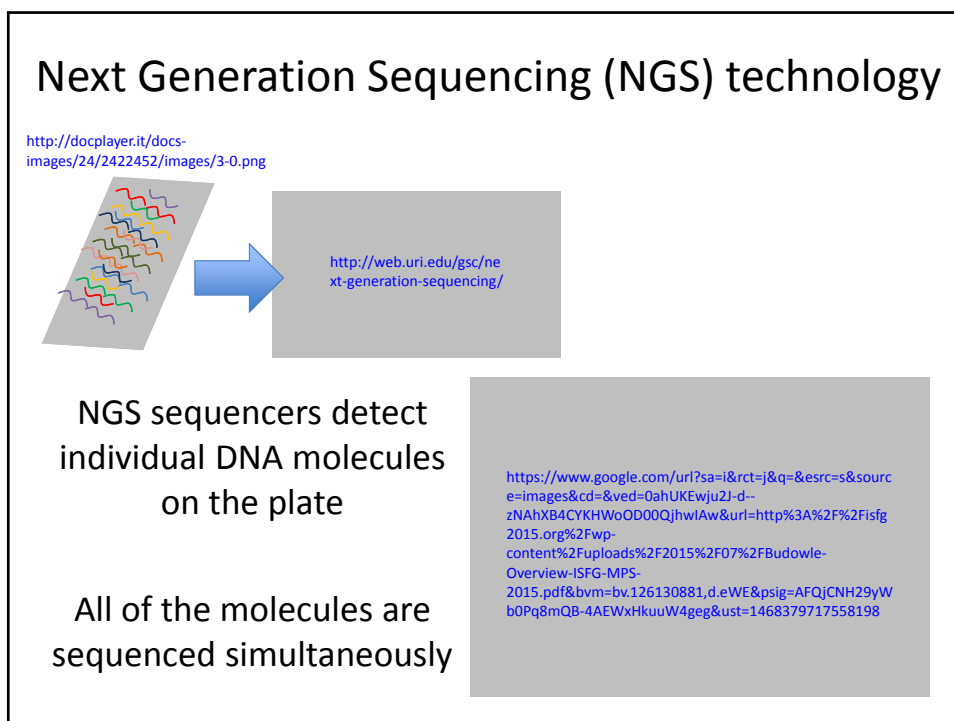
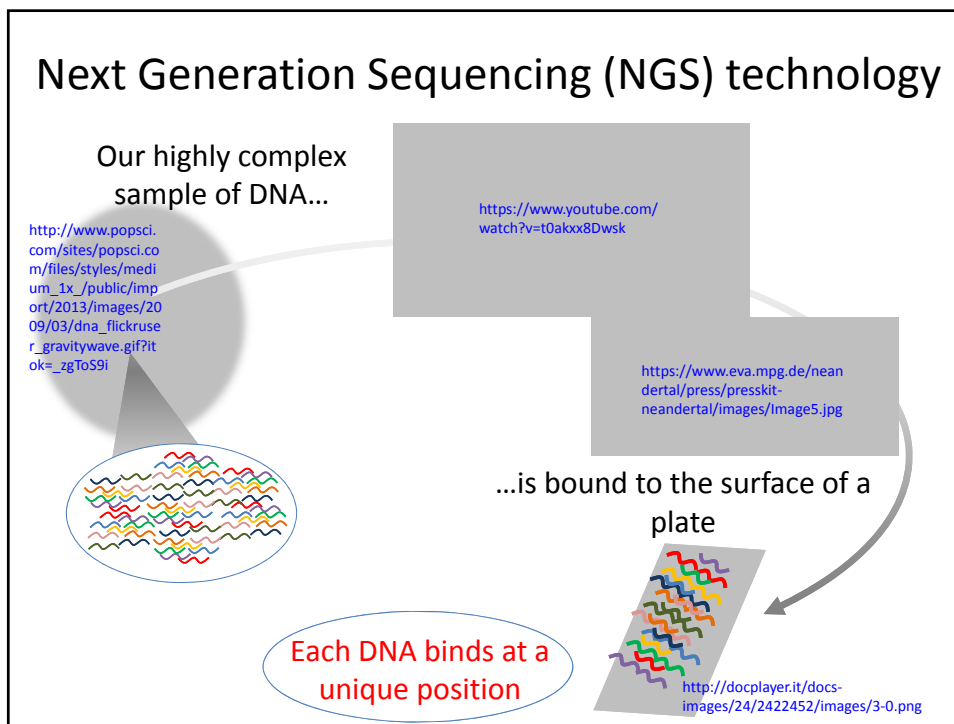
Isolate the DNA



Amplify bacterial DNA



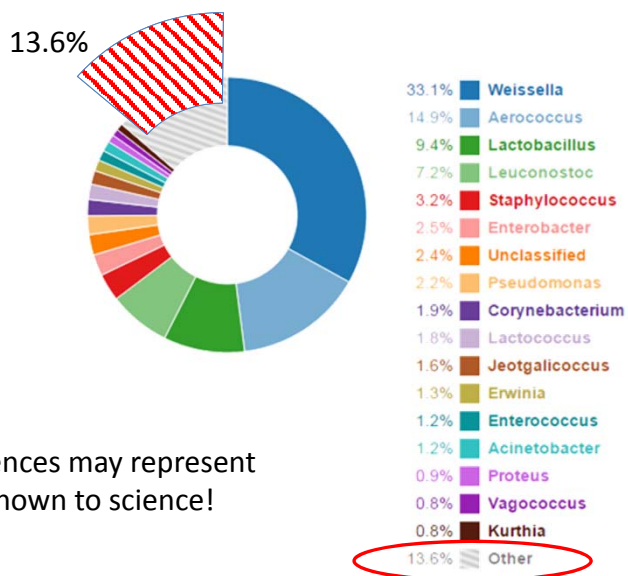
Next generation sequencer determines the bases of every DNA molecule



Identification of the Bacteria



Unknown species are out there



Unknown sequences may represent bacteria unknown to science!

Metagenomics can identify more than just bacteria

<http://www.wellsphere.com/complementary-alternative-medicine-article/multivitamin-and-mineral-supplement-better-than-iron-and-folic-acid-alone-in-nbsp-pregnancy/863124>



- Human DNA - from the person
- Dog or Cat DNA – from pets
- Vegetable or Meat DNA – from food
- Bacteria
- Molds/Fungi
- Viruses
- Any DNA-based organism the person has come in contact with

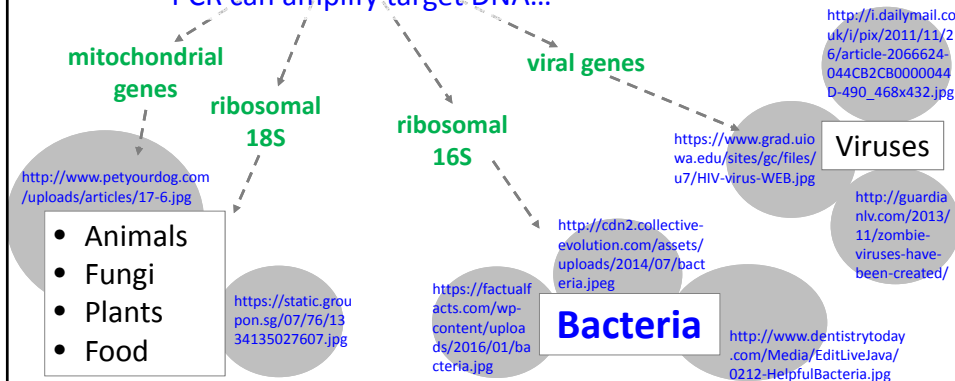
If you started with a swab from a person's skin?
What DNA would you find?

Targeting different organisms

If you sequenced everything...

- Human
- Pet
- Food
- Bacteria
- Fungi
- Viruses
- Anything

PCR can amplify target DNA...



Human Microbiome study

Characterized gut microbiota of children born vaginally vs cesarean section

<http://www.wellsphere.com/complementary-alternative-medicine/article/multivitamin-and-mineral-supplement-better-then-iron-and-folic-acid-alone-in-nbsp-pregnancy/863124>

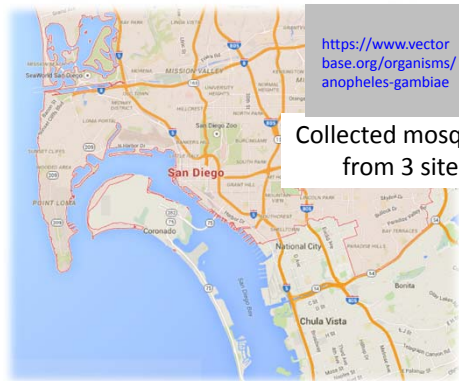
Figure 1B.
Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns – Dominguez-Bello et al. 2010, PNAS

- Birth via cesarean section leads to gut microbiota similar to the mother's skin

Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns – Dominguez-Bello et al. 2010, PNAS

Mosquito Virome study

Found many different viruses



<https://www.vectorbase.org/organisms/anopheles-gambiae>

Collected mosquitos from 3 sites

Tested for viral genomes

Figure 2.
Broad Surveys of DNA Viral Diversity Obtained through Viral Metagenomics of Mosquitos – Ng et al. 2011, PLOS ONE

Figure 1B.
Broad Surveys of DNA Viral Diversity Obtained through Viral Metagenomics of Mosquitos – Ng et al. 2011, PLOS ONE

Broad Surveys of DNA Viral Diversity Obtained through Viral Metagenomics of Mosquitos – Ng et al. 2011, PLOS ONE

Unique Down to Our Microbes Activity

Dr. Rob Dunn

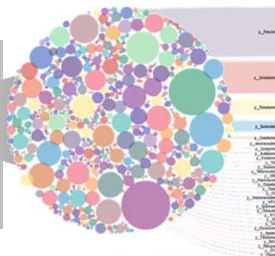


YOUR WILD LIFE

Exploring biodiversity in our daily lives

Explore bacteria present on people's belly buttons

https://s3.amazonaws.com/yoga-assets/assets/content/articles/ZID1110_L.jpg



- Over 200 individual's (belly buttons) were swabbed
- Total DNA was isolated
- PCR was used to amplify the Ribosomal 16S gene (BACTERIA)

Unique Down to Our Microbes Activity

Dr. Rob Dunn

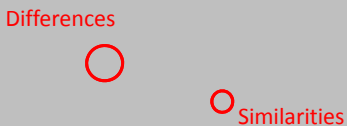


YOUR WILD LIFE

Exploring biodiversity in our daily lives

This citizen-science project found similarities and differences.

Figure 1.
A Jungle in There: Bacteria in Belly Buttons are Highly Diverse, but Predictable—Hulcr et al. 2012, PLOS ONE



People from
NC Museum of Nat. Sci.

- Develop a hypothesis of your own about the similarities or differences between people's belly buttons
- Use this data to test if it is supported or refuted.

People from
Science Online conference [A Jungle in There: Bacteria in Belly Buttons are Highly Diverse, but Predictable—Hulcr et al. 2012, PLOS ONE](#)

Slide Images

Slide 1

Slide 2

- Sink drain - <http://www.wilburhenry.com/save-a-service-call-how-to-avoid-grease-build-up-clogging-your-drain/>
- River bed - <http://www.troutnut.com/underwater-pictures/of-rivers/2>
- Table - http://www.nautiandco.com/p/1998/1_amazing-kitchen-table-dining-table-pool-table-table-centerpieces-round-cherry-kitchen-table-and-chairs-round-kitchen-table-and-chairs-with-leaf-large/
- Bacteria 1 - <http://ngm.nationalgeographic.com/2013/01/125-microbes/oeggerli-photography#/05-intestinal-bacteria-670.jpg>
- Bacteria 2 - http://ngm.nationalgeographic.com/wallpaper/img/2013/01/11-mouth-microbes_1600.jpg
- Bacteria 3 - <http://phys.org/news/2013-04-crevices-coli-reveals-role-flagellum.html>

Slide 3

- Bacteria - http://www.huffingtonpost.com/alanna-collen/microbiome-health_b_7305128.html
- Baby - <http://www.wellsphere.com/complementary-alternative-medicine-article/multivitamin-and-mineral-supplement-better-then-iron-and-folic-acid-alone-in-nbsp-pregnancy/863124>

Slide 4

- Baby - <http://www.wellsphere.com/complementary-alternative-medicine-article/multivitamin-and-mineral-supplement-better-then-iron-and-folic-acid-alone-in-nbsp-pregnancy/863124>

Slide 5

- Swab - <http://www.biocontrolsys.com/products/view/MVPS>
- Plate - shutterstock
- DNA test tube - shutterstock
- Capillary sequencer - <http://vantage.vanderbilt.edu/vantage-service-lines/sanger-sequencing/sanger-dna-sequencing/>
- DNA sequence and text - shutterstock

Slide 6

- Swab - <http://www.biocontrolsys.com/products/view/MVPS>
- Plate - shutterstock
- DNA test tube - shutterstock
- Capillary sequencer - <http://vantage.vanderbilt.edu/vantage-service-lines/sanger-sequencing/sanger-dna-sequencing/>
- DNA sequence and text - shutterstock
- Bacteria cartoon - shutterstock

Slide Images

Slide 7

Slide 8

- Swab - <http://www.biocontrolsys.com/products/view/MVPS>
- DNA test tube - shutterstock

Slide 9

- Swab - <http://www.biocontrolsys.com/products/view/MVPS>
- DNA test tube - shutterstock
- Next gen sequencer - <http://web.uri.edu/gsc/next-generation-sequencing/>
- DNA text - shutterstock
- Bacteria cartoon - shutterstock

Slide 10

- DNA in tube
http://www.popsci.com/sites/popsci.com/files/styles/medium_1x_/public/import/2013/images/2009/03/dna_flickruser_gravitywave.gif?itok=_zgToS9i
- Chip linkers - <https://www.youtube.com/watch?v=t0akxx8Dwsk>
- Chip in hand - <https://www.eva.mpg.de/neandertal/press/presskit-neandertal/images/Image5.jpg>
- Chip - <http://docplayer.it/docs-images/24/2422452/images/3-0.png>

Slide 11

- Chip - <http://docplayer.it/docs-images/24/2422452/images/3-0.png>
- Next gen sequencer - <http://web.uri.edu/gsc/next-generation-sequencing/>
- Fluorescence - <https://www.google.com/url?sa=i&rct=j&q=&esrc=s&source=images&cd=&ved=0ahUKewju21-d--zNAhXB4CYKHwOD00QjhwAw&url=http%3A%2F%2Fisfig2015.org%2Fwp-content%2Fuploads%2F2015%2F07%2FBudowle-Overview-ISFG-MPS-2015.pdf&bv=126130881,d.eWE&psig=AFQjCNH29yWb0Pq8mQB-4AEWxHkuuW4geg&ust=1468379717558198>

Slide 12

- Bacteria cartoon - Shutterstock
- DNA text - shutterstock
- Search button - Shutterstock

Slide 13

Slide Images

Slide 14

- Baby - <http://www.wellsphere.com/complementary-alternative-medicine-article/multivitamin-and-mineral-supplement-better-than-iron-and-folic-acid-alone-in-nbsp-pregnancy/863124>

Slide 15

- Virus 1 - http://i.dailymail.co.uk/i/pix/2011/11/26/article-2066624-044CB2CB0000044D-490_468x432.jpg
- Virus 2 - <http://guardianlv.com/2013/11/zombie-viruses-have-been-created/>
- Virus 3 - <https://www.grad.uiowa.edu/sites/gc/files/u7/HIV-virus-WEB.jpg>
- Bacteria 1 – <https://factualfacts.com/wp-content/uploads/2016/01/bacteria.jpg>
- Bacteria 2 – <http://cdn2.collective-evolution.com/assets/uploads/2014/07/bacteria.jpeg>
- Bacteria 3 – <http://www.dentistrytoday.com/Media/EditLiveJava/0212-HelpfulBacteria.jpg>
- Burger – <https://static.groupon.sg/07/76/1334135027607.jpg>
- Dog - <http://www.petyourdog.com/uploads/articles/17-6.jpg>

Slide 16

- Baby - <http://www.wellsphere.com/complementary-alternative-medicine-article/multivitamin-and-mineral-supplement-better-than-iron-and-folic-acid-alone-in-nbsp-pregnancy/863124>

Slide 17

- Mosquito - <https://www.vectorbase.org/organisms/anopheles-gambiae>

Slide 18

- Belly - https://s3.amazonaws.com/yoga-assets/assets/content/articles/ZID1110_L.jpg

Slide 19

Appendix 3: Final Project Rubric

Criteria <i>Learning outcomes addressed</i>	Inadequate (<D)	Adequate (C)	Effective (B)	Outstanding (A)	Points
Title (3 points)	1	1.5	2	3-Clearly, succinctly describes the topic. Students' names are listed. <i>How can I summarize my project in one sentence or phrase?</i>	/3
Introduction (10 points) <ul style="list-style-type: none"> Describe the biological impact of microbiota on human health Define microbiota, microbiome and metagenomic analysis 	2	3	4	5-Clearly discusses at least one reason for studying the human microbiota.	/5
	2	3	4	5-Introduces briefly the type of data collected. Defines and differentiates microbiota and microbiome. Provides all necessary background.	/5
Hypothesis (20 points) <ul style="list-style-type: none"> Formulate a hypothesis to address a specific question 	2	3	4	5-Clearly describes rationale that influenced the development of the hypothesis.	/5
	9	11	13	15-Hypothesis is specific and testable with the metadata variables available. Hypothesis is creative and unique. <i>Think back to your hypothesis worksheet. Can your hypothesis be addressed with the metadata variables available?</i>	/15
Data (20 points) <ul style="list-style-type: none"> Analyze and interpret metagenomics data to compare microbiota and evaluate a specific hypothesis 	9	11	13	15-Sufficient data is presented to enable the evaluation of the hypothesis. Multiple pieces of data are shown and fully described. <i>Can others follow your logic?</i>	/15
	2	3	4	5-Data visualization choice is the most appropriate for assessing the validity of the hypothesis. <i>Justify the visualization tool/chart you chose for this evaluation.</i>	/5
Conclusions & limitations (20 points) <ul style="list-style-type: none"> Analyze and interpret metagenomics data to compare microbiota and evaluate a specific hypothesis 	6	7	9	10-Draws a fair conclusion from the data and states if the hypothesis is supported or refuted.	/10
	6	7	9	10-Balanced discussion of the limitations of the data in drawing a conclusion. <i>What could have strengthened your conclusions?</i>	/10
Model & future directions (25 points) <ul style="list-style-type: none"> Synthesize a conceptual model as to why microbial populations vary 	9	11	13	15-Creative and original model is made by the student (drawing, cartoon, diagram, etc.). Proposes how microbial communities may develop with respect to the student's project and identifies potential influential environmental factors.	/15
	6	7	9	10-Discussion of a single potential future direction of study. Clearly discusses what type of samples or data could be collected to test a component of your model.	/10
References (2 points)	1	1.5	2	2-References included (at a minimum, Hulcr et al. <i>PLoS ONE</i> 2012). <i>What other sources did I use for my project?</i>	/2

Appendix 4: Pre- and Post- Activity Surveys

PRE-ACTIVITY ASSESSMENT

1. Match the term with its corresponding definition:

Metagenomics	A. The microbial community present in or on a surface/person
Microbiome	B. Data that describes information about the samples within a data set
Microbiota	C. A technique to study the microbial DNA present in a sample to identify and characterize the microbes present.
Metadata	D. The microbial DNA that is isolated from a surface/person

2. How are microbes identified in a metagenomics approach?

- Microbes are identified by morphology (e.g., shape, color, size)
- Microbes are identified by sequence of their DNA
- Microbes are identified by growth/culture requirements
- Microbes are identified by visualizing their metagenome

3. Indicate whether the statement below is true or false:

Using a metagenomics approach recovers living organisms from the samples.	True	False
Bacterial species can be identified by the sequence of their ribosomal 16S gene.	True	False
The DNA of many organisms (e.g., animals, plants, bacteria) may be present in the swab sample from a person's skin (e.g., belly button).	True	False

4. Which of the following do you think best describes the role(s) of microbiota in human health?

- Microbes are always pathogens and cause disease
- Microbes are incidental passengers and do not contribute to human health
- Microbes contribute to human health and can be pathogenic, beneficial or both
- Microbes are always beneficial to the body

POST-ACTIVITY ASSESSMENT

By participating in this activity, I gained the ability to...

1. ...define the terms microbiota, microbiome, metagenomics, and metadata.
 - a. Strongly disagree
 - b. Disagree
 - c. Neither agree nor disagree
 - d. Agree
 - e. Strongly agree
2. ...describe the biological impact of microbiota on human health.
 - a. Strongly disagree
 - b. Disagree
 - c. Neither agree nor disagree
 - d. Agree
 - e. Strongly agree
3. ...formulate a hypothesis to address a specific question.
 - a. Strongly disagree
 - b. Disagree
 - c. Neither agree nor disagree
 - d. Agree
 - e. Strongly agree
4. ...analyze metagenomics data.
 - a. Strongly disagree
 - b. Disagree
 - c. Neither agree nor disagree
 - d. Agree
 - e. Strongly agree
5. ...interpret metagenomics data to compare microbiota.
 - a. Strongly disagree
 - b. Disagree
 - c. Neither agree nor disagree
 - d. Agree
 - e. Strongly agree
6. ...evaluate a specific hypothesis.
 - a. Strongly disagree
 - b. Disagree
 - c. Neither agree nor disagree
 - d. Agree
 - e. Strongly agree
7. ...synthesize a model on why microbial populations vary.
 - a. Strongly disagree
 - b. Disagree
 - c. Neither agree nor disagree
 - d. Agree
 - e. Strongly agree

8. Match the term with its corresponding definition:

- | | |
|--------------|--|
| Metagenomics | E. The microbial community present in or on a surface/person |
| Microbiome | F. Data that describes information about the samples within a data set |
| Microbiota | G. A technique to study the microbial DNA present in a sample to identify and characterize the microbes present. |
| Metadata | H. The microbial DNA that is isolated from a surface/person |

9. How are microbes identified in a metagenomics approach?

- a. Microbes are identified by morphology (e.g., shape, color, size)
- b. Microbes are identified by the sequence of their DNA
- c. Microbes are identified by growth/culture requirements
- d. Microbes are identified by visualizing their metagenome

10. Indicate whether the statement below is true or false:

- | | | |
|--|------|-------|
| Using a metagenomics approach recovers living organisms from the samples. | True | False |
| Bacterial species can be identified by the sequence of their ribosomal 16S gene. | True | False |
| The DNA of many organisms (e.g., animals, plants, bacteria) may be present in the swab sample from a belly button. | True | False |

11. Which of the following do you think best describes the role(s) of microbiota in human health?

- e. Microbes are always pathogens and cause disease
- f. Microbes are incidental passengers and do not contribute to human health
- g. Microbes contribute to human health and can be pathogenic, beneficial or both
- h. Microbes are always beneficial to the body

12. Overall, I enjoyed participating in this activity.

- a. Strongly disagree
- b. Disagree
- c. Neither agree nor disagree
- d. Agree
- e. Strongly agree

13. In two sentences, which aspect of this activity was the most challenging and/or frustrating?


Pre- and Post- activity quiz results by question.

Student Survey Question		Number of Correct Responses													
		UMBC (n=8)		NCSU Fall'15 (n=8)		NCSU Spring '16 (n=12)		NCCU (n=20)		WLU (n=18)		KC (n=17)		Total (n=83)	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Define Terms Match SLO 1	1) Microbiota ¹	3	6	1	4	2	8	5	8	2	7	2	13	15	46
	2) Metadata ²	7	8	7	8	12	10	18	15	18	18	17	17	79	76
	3) Metagenomics ³	6	7	7	8	11	12	16	16	18	18	17	16	75	77
	4) Microbiome ⁴	1	5	1	4	1	7	6	6	2	8	2	13	13	43
5) How are microbes Ident. ⁵ Mult. Choice SLO 1		4	6	6	7	9	10	12	12	13	14	13	17	57	66
6) Recovery of organisms ⁶ T/F SLO 1		3	4	2	3	5	3	6	3	6	8	14	11	36	32
7) Identification by 16s ⁷ T/F SLO 1		7	8	5	5	11	11	15	13	15	15	16	17	69	69
8) DNA of many organisms ⁸ T/F SLO 1		7	8	7	8	9	12	15	18	18	17	17	17	73	80
9) Impact on Human health ⁹ Mult. Choice SLO 2		7	7	7	8	12	12	20	19	17	18	17	17	80	81

¹Two-tailed t-test values $p = 0.567$, $t = 0.575$; ² $p < 0.001$, $t = 5.177$; ³ $p = 0.369$, $t = -0.904$; ⁴ $p = 0.530$, $t = 0.630$; ⁵ $p < 0.001$, $t = 5.029$; ⁶ $p = 0.095$, $t = 1.690$; ⁷ $p = 0.469$, $t = -0.728$; ⁸ $p = 1.000$, $t = 0.000$; ⁹ $p = 0.034$, $t = 2.156$.

**Appendix 5: Example of Student Final
Project A**

**Metagenomics: Relationship
between Wash Frequency and
Bacterial Diversity**



Microbiota , Microbiomes, and Metagenomics

What is a microbiota?

the “community” of bacteria living on or in our body

What is a microbiome?

the combined genetic material from bacteria in a microbiota

What is Metagenomics?

the study of a collection of DNA (the genome) from a microbiota

Microbiota and Human Health

Usually we think of germs as being bad, but the bacteria living on our skin or in our body can be beneficial or don't cause harm.

- Example: we have bacteria in our gut to help digestion. The bacteria have formed a mutualistic relationship with us.
- Studying microbiota is important because they can affect aspects of our health such as immunity or our weight.

<https://truweight.in/blog/nutrition/probiotics-the-bacterial-army-to-the-health-rescue.html>

Hypothesis: People that wash more often will have more variety of bacteria in their belly button.

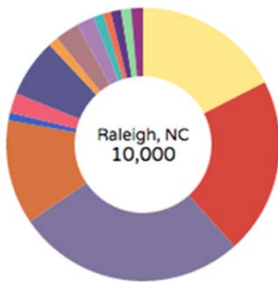
Reasoning: The more you wash, the less bacteria you'll have in your belly button. The less bacteria, the more "food" available. The more food available, the less competition between types for nutrients. The less competition for nutrients, the more types of bacteria can thrive together.

<http://animals-universe.info/dog/page1352/>

<http://now.tufts.edu/articles/app-your-pets>

0-1 washes per week

Kingdom — Phylum —



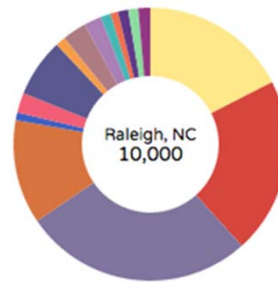
16 Taxonomy in Total

about 15 different phyla of bacteria

Data

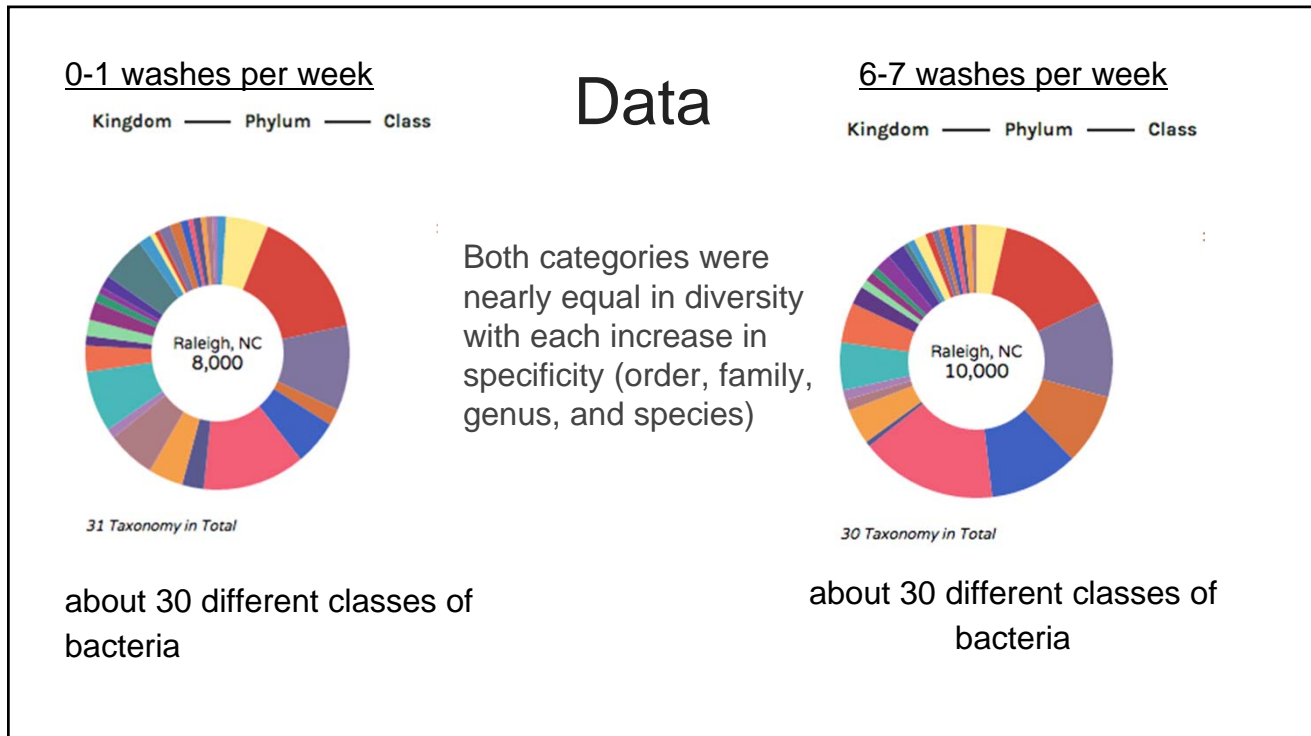
6-7 washes per week

Kingdom — Phylum —



16 Taxonomy in Total

about 15 different phyla of bacteria



Conclusions and Limitations

Hypothesis restated: People that wash more often will have more variety of bacteria in their belly button.

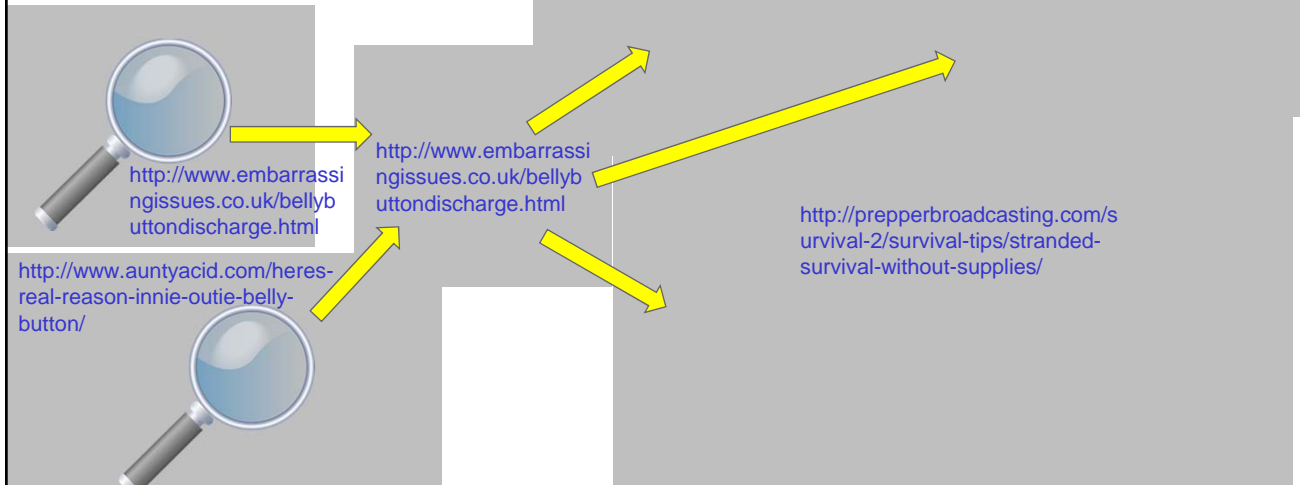
Data refutes this hypothesis because variety of bacteria was equal in both high and low wash frequencies.

Limitations: I only recorded results from Raleigh, NC. In general, the results were about the same in other cities, but I didn't look at all the cities available.

Improvement: I could average quantitative amount of diversity in several cities instead of just Raleigh.

Proposed Explanation:

Bacteria hide out in deep crevices and do not get washed out with soap & water



Future Study

Take samples with measures from various types of washes, such as antibacterial soap, regular soap, wash with just water, how vigorously they washed, how long they washed, and how often.

References

Role of the Microbiota in Immunity and Inflammation

Belkaid, Yasmine et al.

Cell , Volume 157 , Issue 1 , 121 - 141

Hulcr et al. PLoS ONE 2012

Am J Gastroenterol Suppl (2012) 1:22–27; doi:10.1038/ajgsup.2012.5

Example of Student Final Project B

Belly Button Diversity and Age

— The effect of age on bacterial composition —

Why is studying microbiota important?

Microbiota provides insight of the environments in or on the human body-- bacteria is everywhere and this allows to see what bacteria lives where

Provides information about bacteria that is unique to certain age groups, areas, gender, or possible the morphology of the belly button

The data collected in this study--belly buttons

Hypothesis

Individuals between the ages of 40 and 60 have less pathogenic bacterial composition than individuals between the ages of 0 and 20.

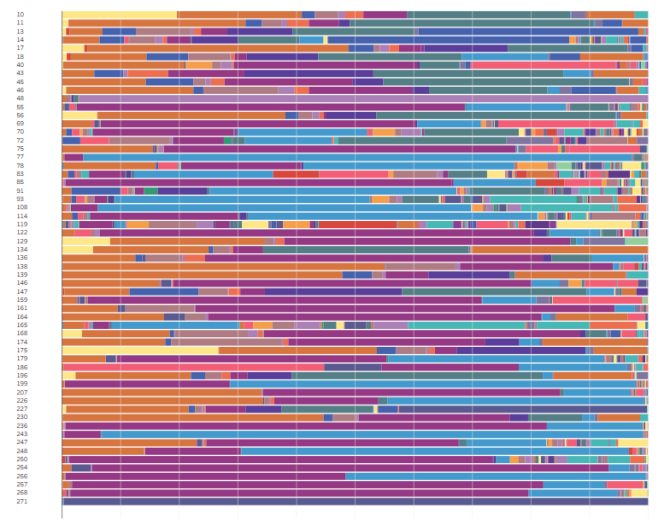
The rationale behind this hypothesis comes from both the immune system of younger people and the exposure to pathogens that differs in age groups.

By organizing the data by age and comparing the number of possibly pathogenic versus non-pathogenic families of bacteria in each age group, the hypothesis can be tested.

Microorganisms (by family) found in both age groups



- 0 to 20 year olds' microbiota



- 40 to 60 year olds' microbiota

Closer look at the families of organisms

TOP SEQUENCES	
k__Bacteria,p__Firmicutes,c__Bacilli,o__Bacillales,f__Staphylococcaceae,g__s__	13,368
k__Bacteria,p__Actinobacteria,c__Actinobacteria,o__Actinomycetales,f__Corynebacteriaceae,g__s__	8,768
k__Bacteria,p__Firmicutes,c__Clostridia,o__Clostridiales,f__Clostridiaceae,g__s__	7,719
k__Bacteria,p__Bacteroidetes,c__Bacteroidia,o__Bacteroidales,f__Porphyromonadaceae,g__s__	4,079
k__Bacteria,p__Firmicutes,c__Bacilli,o__Bacillales,f__Alicyclobacillaceae,g__s__	3,491
k__Bacteria,p__Proteobacteria,c__Gammaproteobacteria,o__Pseudomonadales,f__Pseudomonadaceae,g__s__	2,678
k__Bacteria,p__Proteobacteria,c__Gammaproteobacteria,o__Enterobacteriales,f__Enterobacteriaceae,g__s__	2,646
k__Bacteria,p__Cyanobacteria,c__Chloroplast,o__Streptophyta,f__g__s__	2,590
k__Bacteria,p__Firmicutes,c__Bacilli,o__Lactobacillales,f__Streptococcaceae,g__s__	1,512
k__Bacteria,p__Proteobacteria,c__Alphaproteobacteria,o__Sphingomonadales,f__Sphingomonadaceae,g__s__	1,452

- Top sequences in 0 to 20 year olds

Closer look at the families of organisms

TOP SEQUENCES	
k__Bacteria,p__Actinobacteria,c__Actinobacteria,o__Actinomycetales,f__Corynebacteriaceae,g__s__	17,486
k__Bacteria,p__Firmicutes,c__Bacilli,o__Bacillales,f__Staphylococcaceae,g__s__	9,824
k__Bacteria,p__Firmicutes,c__Clostridia,o__Clostridiales,f__Clostridiaceae,g__s__	7,457
k__Bacteria,p__Bacteroidetes,c__Bacteroidia,o__Bacteroidales,f__Porphyromonadaceae,g__s__	4,432
k__Bacteria,p__Bacteroidetes,c__Bacteroidia,o__Bacteroidales,f__Prevotellaceae,g__s__	1,871
k__Bacteria,p__Proteobacteria,c__Epsilonproteobacteria,o__Campylobacterales,f__Campylobacteraceae,g__s__	1,577
k__Bacteria,p__Actinobacteria,c__Actinobacteria,o__Actinomycetales,f__Actinomycetaceae,g__s__	1,562
k__Bacteria,p__Proteobacteria,c__Gammaproteobacteria,o__Enterobacteriales,f__Enterobacteriaceae,g__s__	1,168
k__Bacteria,p__Firmicutes,c__Bacilli,o__f__g__s__	1,043
k__Bacteria,p__Synergistetes,c__Synergistia,o__Synergistales,f__Dethiosulfovibrionaceae,g__s__	1,029

- Top sequences in 40 to 60 year olds

The top sequences analyzed

0 to 20—top 5 sequences and whether they are possibly pathogenic or innocuous

1. Staphylococcaceae: can be pathogenic
2. Corynebacteriaceae: innocuous
3. Clostridiaceae: can be pathogenic
4. Porphyromonadaceae: innocuous
5. Alicyclobacillaceae: innocuous

Number of harmless sequences: 16,338

Number of possibly pathogenic sequences:
21 087

40 to 60—top 5 sequences and whether they are possibly pathogenic or innocuous

1. Corynebacteriaceae: innocuous
2. Staphylococcaceae: can be pathogenic
3. Clostridiaceae: can be pathogenic
4. Porphyromonadaceae: innocuous
5. Prevotellaceae: can be pathogenic

Number of harmless sequences: 21,918

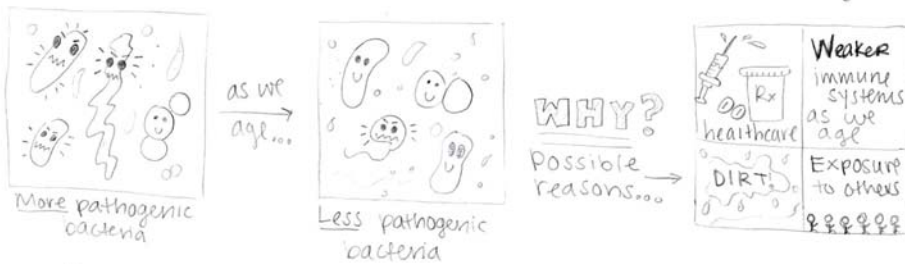
Number of possibly pathogenic sequences:
17 281

Conclusion

According to the perimeters set by the hypothesis, it was **supported** by the data analyzed

Possible limitations

How do human microbial communities change as we age?



How could this information be used?



Model of findings and future research possibilities

References

<http://www.ncbi.nlm.nih.gov/pubmed/15509322>

<http://www.gutmicrobiotaforhealth.com/parkinson-gut-microbiota-7260>

https://books.google.com/books?id=ZSGNbeFw9LcC&pg=PA92&lpg=PA92&dq=Corynebacterium+innocuous&source=bl&ots=wJcSxKGbBq&sig=MYiyvqBsA_BEwiwmTJM5zVhh-Lk&hl=en&sa=X&ved=0ahUKEwj4pP-xaXJAhVDVyYKHU_oB7gQ6AEIKzAC#v=onepage&q=Corynebacterium%20innocuous&f=false

<http://textbookofbacteriology.net/clostridia.html>

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3639412/>

Appendix 6: Example Student Comments about the Activity

Example Student Comments about the Activity

Post-Survey Question:

“In two sentences, which aspect of this activity was the most challenging and/or frustrating?”

Student Respondent - KC#018

“PHINCH was really cool, but there were some parts that weren't as easy to use as others. The website could use a bit of work in just the user [friendliness].”

Student Respondent - NCSU#017

“The analysis of the data was extremely frustrating. Phinch is somewhat difficult to navigate and is very restricted on the way the information is presented.”

Student Respondent - NCSU#032

“Phinch was not the perfect data organizer for me, it was a bit hard to work with.”

Student Respondent - KC#012

“Synthesizing a model on why microbial populations varied [was] at first difficult, since there could be many reasons.”

Student Respondent - NCSU#031

“The most frustrating part was trying to analyze the phylum/species/genus etc of the microbiota. This was because we were not told how in depth we should go, and there was too much information.”

Student Respondent - UMBC#008

“The most challenging part of the activity was coming up with an experimental question to test and interpreting the data into relevant information regarding the role microbes play in human health. For my particular topic of research, I did not connect the relevance of impact of microbiota specifically.”

Appendix 7: Possible Modifications to Activity

Possible Modifications

Alternative Final Projects. Alternatives to the oral presentation or written report are possible. A variety of media forms could be used, including producing simple web pages, written abstracts, or preparation of posters for an in-class mock symposium. Designs that allow concurrent presentations could reduce the amount of time required for presentation of final projects. The UMBC cohort had success with a variation in which students wrote abstracts and presented posters at a campus undergraduate research symposium.

Peer Review. Peer evaluation could be introduced to encourage critical thinking. Students could review the project of a partner using the provided rubric before final submission. If used, we strongly encourage that instructors establish a method of peer review that encourages cooperation and mutual improvement, instead of competition, criticism and/or plagiarism.

Different Data Sets. The present version of this activity incorporates a data set of the human belly button microbiome. However, any properly formatted metagenomic data file can be introduced. The Phinch.org web tool can accept files in BIOM 1.0 format, which can be generated using QIIME software versions <1.8. Additional data sets of bacteria/archaea and fungi collected from residential homes and others are currently available on the Rob Dunn lab webpage at: <http://robdunnlab.com/projects/wild-life-of-our-homes/data-visualization/>. Instructors should be aware that BIOM 2.0 or more recent .biom files may need to be converted to BIOM 1.0 or modified further, depending on how the contents of the file have been organized (see <https://github.com/PitchInteractiveInc/Phinch/wiki/Quick-Start>).

Student Data. In advanced classrooms producing student-generated data sets, this activity could be adapted for visualization and simple comparison of the results. Because it is open-source and free, Phinch.org offers a simple solution to the process of visualizing metagenomics data.

Different Analysis Platforms. Several other web-based analysis platforms are available that offer different types of analyses of metagenomic data sets. OneCodex (<https://www.onecodex.com/>; free for academic users but requiring an account) accepts user data and offers several example data sets of clinical significance that could be used with this activity. The MG-RAST server (<http://metagenomics.anl.gov/>) at Argonne National Laboratory allows users to visualize public metagenomic data sets and easily compare samples using several graphic tools.

Project Continuation within a Curriculum. This activity could be adopted within a curriculum, where students in a course series that starts with introductory biology that extends (with the same students) into upper level microbiology, molecular biology, or ecology courses. This metagenomics lesson could serve as a starting point for lab based studies of cultured microbes or the novel sampling and sequencing of microbes from belly buttons or other habitats of interest.