Inquiry-Driven Bioinformatics Laboratory Research Module: Metagenomic Study of Student Oral Microbes

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American Society for Microbiology Curriculum Guidelines highlight the importance of enabling students to think critically and learn by doing research. Moreover, information in biology, especially genetics and biotechnology, increases too rapidly for instructors to teach everything. To increase students’ interest and comprehension of important core genetic concepts and to encourage students to practice scientific investigation, we designed a research module for upper-level biology/genetics students to examine oral bacteria. Students extracted their own oral microbial DNA and amplified and analyzed with general genus- and species-specific 16S rRNA PCR primers. The microbial DNA samples were also amplified with conserved bacteria 16S rRNA primers and the amplicons TOPO cloned (topoisomerase-based cloning) and Sanger sequenced. Lastly, the metagenomic microbial DNA samples were also sequenced by Illumina next-generation sequencing and analyzed with bioinformatics tools. We have implemented the module in three iterations of an undergraduate class at a small, liberal arts college. The project culminates in a poster presentation that the students on average performed in a high B range. Pre- and postsurvey analysis of student learning gains revealed significant student learning ($P < 0.05$ one-tailed, paired Wilcoxon signed ranked test, $n = 23$). Next, we surveyed student perceptions of the activity by self-assessment. Significantly more than the medians, the students enjoyed the inquiry-driven module and considered it more effective in teaching about PCR and other molecular genetics concepts than the traditional prescribed laboratory exercises. We conclude that this microbe laboratory module induces research interest and is useful in teaching important genetics concepts.

KEYWORDS laboratory exercises, genetics research module, metagenomic study, student oral microbes, genetic databases, bioinformatics, PCR, hands-on learning, inquiry driven, 16S rRNA gene primers and sequencing

INTRODUCTION

Research in biology in general, genetics in particular, is moving at such a rapid pace that the goal to teach every aspect of genetics in a course is unrealistic. Nonetheless, it is important to make the discovery of scientific knowledge in a field such as genetics accessible and relevant to students. The challenges and proposed changes in science education which are supported by many, have been perhaps best articulated and represented by the Vision and Change program launched by the National Science Foundation and the American Association for the Advancement of Science originally in 2006 and revised in 2015 (https://live-visionandchange.pantheonsite.io/wp-content/uploads/2015/07/VISchange2015_webFin.pdf). In addition to teaching the fundamental principles, any science course should aim to cultivate students’ interests in science and to enable them to become independent and critical thinkers who can carry out their own investigations in any subject. Significant community support and changes in undergraduate curriculum have been reported (http://visionandchange.org/about-vc-unpacking-a-movement-2018/) and encouraged (1). Nonetheless, incorporating research in undergraduate science courses have serious challenges (2). Research units forced poorly into courses (just to show courses have research components) implemented by overworked faculty without sufficient institutional support may not lead to positive gains in student learning (3–7).

Another challenge that scientific education faces is the specialization of subject areas. With the advancement of each field, the required content areas to be covered in each subject area such as genetics, computer technology, and
mathematics increase with time. The integration of these areas is only done at the research level, which often does not benefit the general undergraduate students. Therefore, courses that can bring together different fields of expertise are needed to reflect the true state and necessity of scientific research (8–10). Exposure to and training in quantitative analyses in biological experiments are especially important with the increase in large-data research such as population studies and the vast information in genomics and metagenomics (10–12).

Thus, college educators should be able to access genuine, practical research modules that generate and analyze substantial qualitative and quantitative data and integrate computational and mathematical tools. Ideally, the modules should also be adjustable to flexible time or financial restraints of investigation. Here, a metagenomics research module is presented that integrates genetics, microbiology, and bioinformatics. By implementing a very personal, but safe, student investigation of their oral microbes, students can learn by “doing” science, not by just memorizing scientific facts. Some of the approaches outlined here are fundamentally similar to those in the laboratory manual published by Sanders and Miller in 2010 (13), but here students’ oral microbes are studied instead of soil microbes and a particular statistics platform that leverages the R programming language is demonstrated. Other studies have shown effective student learning and manageability investigating oral microbes in laboratory courses (14–17). Our module is unique in that the module can be used to selectively study the effect of one tooth-brushing event on the microbial community structure, the difference between Sanger sequencing and next-generation sequencing (NGS), the analysis of the results by an R code, or any of these components because of budgetary or time concern.

We chose to study microbes because recent studies have shown important relationships between microbes found on different body surfaces and our health (18, 19). It has been suggested that more than 600 species of bacteria inhabit the human oral cavity (20) and salivary diagnostic tools are being developed to assess hormones and microbes (21, 22). Nonetheless, many of these bacteria are not easy to culture. PCR amplification of DNA extracted from investigators’ own saliva samples can circumvent the challenges of microbial cultures (23). The ease of access allows undergraduate students to investigate relatively safely. Possible relationships between the microbial community composition and hosts’ disease states, diet, gender, ethnicity, and brushing habits can be assessed with different complementary approaches. Working with students’ own samples increase student engagement and agency (14).

(This study was presented as part of the Microbrew session at the Annual ASM Conference for Undergraduate Educators in 2014.)

Intended audience and prerequisite student knowledge

This research module is appropriate for upper-level college courses in genetics, molecular biology, or microbiology and may be expanded for graduate-level coursework. Students should have taken general biology and have knowledge especially in DNA replication, PCR, DNA sequencing, and basic statistics.

Instructors need to be familiar with some genetics databases such as GenBank (https://www.ncbi.nlm.nih.gov/genbank/), RDP (http://rdp.cme.msu.edu/), and others reviewed, for instance, by I. B. Zhulin in 2015 (24). Training workshops like the Bioinformatics Institute offered by ASM and Joint Genome Institute (https://mgm.jgi.doe.gov/) are helpful. Familiarity with a multivariate statistical analysis tool is necessary if the analyses of metagenomic sequencing data are desired. There are free computational packages in R that can be used to analyze, graph, and display metagenomics sequencing data.

Learning time

This hands-on research module can be adapted to a few laboratory sessions (2 to 3 h each) for one branch of the research module (Fig. 1A) or to the entire semester to cover all three approaches of the module. Figure 1B shows a sample laboratory schedule for incorporating this research module as a semester theme.

Learning objectives

Upon completion of this research module, students should be able to (i) prepare and measure solution and reaction mixes with micropipettes to carry out metagenomic PCR, DNA extraction, DNA digestion, gel electrophoresis, bacterial transformation, and/or bacterial vector cloning; (ii) describe PCR, cloning, sequencing, and DNA fingerprinting; (iii) analyze sequencing results, including correctly marking the PCR primer sequences, submitting the insert sequences to genetic search engines, and interpreting BLAST results; (iv) use simple computational tools such as R or Excel to perform analyses of the metagenomics data; (v) clearly communicate experimental results and the advantages and limitations of metagenomic approaches; and (vi) self-report an interest in learning more genetics.

PROCEDURE

Materials

The materials needed for various procedures were as follows: saliva collection and DNA stabilization, Zymo DNA/RNA Shield Saliva Collection kit 9 (details in Appendix S1 in the supplemental material); microbial genomic DNA extraction, Zymo Research Soil DNA extraction kit (details in Appendix S1); and gel electrophoresis, 1.5% agarose gels made with 1× TAE buffer, gel electrophoresis chambers, 1× TAE buffer.

PCR primers were ordered from Hiram Genomics or Invitrogen: SYBR green I (10,000× concentrate in dimethyl sulfoxide from Thermo Fisher [3 μl] in 1.5 ml of 6× gel loading buffer (B7021S from New England Biolabs [NEB]); ethidium bromide staining solution (10 ng/ml...
FIG 1. (A) Oral microbe module outline. (B) Sample semester laboratory schedule (assuming one 3-hour laboratory session per week).

Superscripts a and b indicate possible alternative finish points of the project due to pedagogical preference, time or monetary constraints.

* For once in the semester, students need to come in for an hour 1 to 2 days after the scheduled, weekly laboratory time. Times for R coding training are suggested in a blue font.
from Sigma diluted to 0.5 μg per ml for gel staining), 100-bp DNA Ladder (N3231S from NEB); TOPO cloning, Invitrogen TOPO cloning kit (K457501); oral microbe module student manual, see Appendix S1; faculty support material, see Appendix S2.

For the outlined tentative genetics laboratory exercises (Fig. 1A [flowchart] and Fig. 1B [laboratory schedule]), the following procedures were used.

(i) Oral microbiota sampling (DNA extraction)

An application (see Appendix S2 in the supplemental material) to approve the experimental protocol involving human subjects was submitted to and approved by our school’s Institutional Review Board. Students, who signed the informed consent form (see Appendix S1), collected their own salivary samples, and labeled the samples with number identifiers linked to their survey answers, so the subjects’ names remain anonymous. Metagenomic microbial DNA samples were extracted with the ZR soil microbial DNA extraction kit (see Appendix S1).

(ii) PCR analyses

Some of the extracted DNA samples were amplified with published 16S rRNA primers that can differentiate the different representative taxonomic groups across different groups of bacteria. The presence and relative proportions of PCR products were compared before and after brushing and between students.

(iii) Sequencing

The total metagenomic DNA samples were amplified by PCR with degenerate primers that were designed to amplify the 16S rRNA gene conserved in bacteria (S15F and 909R) (25). The PCR products were cloned into the pCR4-TOPO vector and used to chemically transform TOP10 Escherichia coli (Invitrogen TOPO TA cloning kit). The successful recombinants were selected by the blue/white selection using X-Gal LB/kanamycin plates (13). Ten white colonies per student were selected to be grown in liquid LB/kanamycin cultures. The DNA of each liquid culture was extracted and analyzed by EcoRI restriction to be grown in liquid LB/kanamycin cultures. The DNA of each liquid culture was extracted and analyzed by EcoRI restriction digestion to ensure the presence of an appropriately sized insert (395 bp). The extracted DNA samples from successful recombinant bacterial clones were sent out to be sequenced with the traditional Sanger sequencing at Clemson University.

A sample guideline to students’ sequence analysis is included as Appendix S1 in the supplemental material. Some of the metagenomic oral DNA was amplified to be sequenced by CUGI. For Clemson’s Illumina sequencing, the bacterial primer pairs were tagged with Illumina sequencing adapters and Nextera indexes before the PCRs. The amplicon libraries were then sequenced. The primers for the 16S rRNA gene of Archaea (Arch 349F and 806R) (26) and for 18S (ITS3_KYO2 and ITS4) (27) in fungi were also used.

The sequences, from both Sanger sequencing and NGS, can then be analyzed with bioinformatics tools to identify the species present. The latter is more unbiased since the primers for particular species do not have to be selected before sampling.

(iv) Analysis of metagenomic sequences

Analysis of metagenomic sequences can be done with different kinds of software, including Excel or SPSS. Here, the free computational software R (https://www.r-project.org) was used because it is free and is a powerful statistical tool for students to learn and even use in the future. Students can download the software to their own computers and can analyze the sequences anytime. There are many resources available for learning R. For instance, R for Data Science (28) (http://r4ds.had.co.nz/) is available online for free. There are also free introductory, interactive online courses such as “Introduction to R” and others offered by DataCamp (https://www.datacamp.com/groups/education). To date, teaching with the online, interactive DataCamp courses is free in the classroom.

Safety issues

Most oral microbes in healthy individuals are characterized under BSL-1 (https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-Pdf.pdf). Due to concerns regarding SARS-CoV-2, extra protections are suggested (details in Appendix S1). Essentially, students should handle only their own samples. Any procedure that may produce aerosols should be conducted under a biological safety hood with personal protective equipment (https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-Pdf.pdf, https://asm.org/ASM/media/Education/Biosafety-Guidelines-Appendix.pdf). Students should be instructed on safe collection of saliva at home with the Zymo DNA/RNA shield saliva collection kit (https://www.zymoresearch.com/products/dna-rna-shield-saliva-collection-kit) that inactivates pathogens in collected samples.

The laboratory benches should be disinfected before and after each laboratory session to minimize contamination to or from the environment. All bacterial media need to be autoclaved before disposal. Students should be instructed in the proper handling of the electrophoresis gels with ethidium bromide or other dyes which are potential mutagens. The staining solution should be reused and discarded as biohazardous chemical waste. Protective barriers to UV light should be used when viewing the gels with UV transilluminators.

DISCUSSION

Field testing

This activity was initially conceived and implemented for undergraduate students in a genetics course at Trinity
International University, a small liberal arts college located in the midwestern United States. Our programs in the sciences are primarily undergraduate. Some of the authors have previously attended the bioinformatics workshops hosted by the American Society for Microbiology and the Joint Genome Institute.

We tested this research module in the 2013 genetics class (11 students with diverse ethnic background, including Americans of descent from Asia, Africa, the Middle East, and Europe) among other genetic experiments, and the class only had time to analyze the Sanger sequences from the TOPO clones. Students overwhelmingly showed enthusiasm and motivation in the new module, and quite a few students would come into the laboratory to spend extra time redoing experiments to produce better results. To facilitate easier faculty/staff preparation for this kind of research module in the laboratory, more gel electrophoresis chambers and multichannel pipettors were purchased.

In 2015, the genetics course was repeated with nine students in the class with similar diverse ethnic backgrounds. The research module was extended to include the NGS and statistical analyses. The goal was to incorporate bioinformatics, quantitative analysis, and research components into the upper-level genetics course. Two students have furthered the class investigation in their independent research projects and presented their preliminary results at the 2014 and 2015 Student Symposia of the Associated Colleges of the Chicago Area (https://acca.cuchicago.edu/). The initial 2013 class data were also presented in the Microbrew session of the ASMCUE in 2014 (29). With the feedback and assessment results received from the students, modifications have been made in this laboratory module. First, the oral, microbial DNA samples were sent out earlier for sequences, so that students can receive the sequences earlier to have more time to prepare for their poster presentation. Second, the module has been expanded to take up a whole semester with more introduction and laboratory meeting times to facilitate better understanding of the research project and to allow more communication between the instructor and students and among the student partners.

Four students enrolled in this elective course in 2019. On the whole, students showed good engagement and performance in the poster preparation. Significant student learning gain was shown by multiple assessment methods detailed below. Two factors might have negatively affected the 2019 course. The DNA samples sent for Sanger sequencing were damaged in the shipping process, so the sequencing results were delayed for 2 weeks. Second, two students were planning to present their research results in an annual Spring student symposium, but they could not because the conference was cancelled due to COVID-19.

SUGGESTION FOR DETERMINE STUDENT LEARNING

Frequent, short discussions and presentations to assess learning objectives 1 to 5

To increase students’ confidence and participation and to promote high-level thinking and classroom equity, students were grouped in pairs at the beginning of the semester and seated together in the laboratory times. During laboratory sessions, students were asked questions, and we frequently utilized quick “think-pair-share” strategies (30, 31) to encourage voluntary responses. We also practiced extended “think-pair-share” to elicit regular reports of sequence analyses and gel interpretation in “laboratory meetings” (32–34) to continuously assess learning objectives 1 to 5. Throughout the semester, student partners were occasionally given laboratory meeting times to analyze and share new results with the partners and then the whole class. The frequent, short oral presentation was helpful to assess student understanding and clear up confusion early.

Poster presentation to assess learning objectives 4 and 5

A poster presentation was scheduled at the end of the semester within the laboratory time. Poster presentations have been shown to increase student learning, engagement, and improve communication skills (35–37). Besides the common project flow, each student was assigned either a particular technique to explain in more detail such as Sanger sequencing, NGS, TOPO cloning, use of dilution curves for PCR sensitivity and linearity in amplification; or a particular type of microbes and a particular factor to assess correlation with the microbes present such as ethnicity, gender, and diet. In the presentation, slides that illustrate different portions of the poster were projected to facilitate the presentation to a large group of audience. The posters are also displayed in the laboratory. We encouraged other science faculty members to offer extra credits for the students in their classes to attend. Typically, quite a few science faculty members and students attended the poster presentations. A sample grading rubric can be found in both student and teacher manuals (see Appendices S1 and S2). Students in general presented well and answered audience questions sufficiently reflecting significant engagement and effective learning gains in objectives 1 to 5: average score = 89.3% (standard deviation = 6.7%, n = 23). In the poster grading, understanding of experimental procedures, results, and student interpretation of the results, proper citations were assessed. A sample poster template and a student poster from the 2019 class are included in Appendix S1.

Pre- and postunit assessments to assess learning objectives 1 to 3

Short pre- and postmodule assessment quizzes (see Appendix S2) were administered to the genetics classes. This assessment consisted of five multiple-choice questions that tested understanding of PCR and metagenomic study utilizing rRNA gene primers.

Summary of pre- and postmodule learning assessment

There is a statistically significant difference between the mean pre- and postmodule assessment scores with a P value
smaller than 0.05 (0.0001 for one-sided Wilcoxon signed-rank test with \( n = 23 \)) (Table 1) (38). The means and standard deviations for the pretest scores were 2.35 and 1.07, respectively, whereas those for posttest scores were 3.65 and 1.1. The statistically significant increase in the students’ average score demonstrates learning gains related to the learning objectives 2 and 3.

Summary of student perception self-assessment to assess learning objective 6

The student learning self-perception assessment (Table 2; see also see Appendix S2) was given to the same students to evaluate their perception of the inquiry-based research module. The pilot data indicated that significantly more than the median rankings (Wilcoxon one-sided, one-sample test, \( P < 0.05, n = 23 \)), the students enjoyed the inquiry-based module, considered it effective in teaching about PCR and genetic fingerprinting of organisms using the rRNA gene and in general more effective in teaching molecular genetics concepts than the traditional laboratory exercises. However, the students indicated that the module was not effective in learning about the concept of microevolution. The mediocre evaluation score for question 4 suggested that the term “microevolution” needs to be better defined in the course in the future. Students have also asked for more and earlier information on the poster presentation; thus, a grading rubric has been included in the student manual (see Appendix S1) starting with the 2019 class.

The student answers to the open-ended question 6 regarding feedback and comments on the oral microbial unit can be found in Appendix S2. Every respondent indicated increased interest and engagement in the laboratory unit suggesting the achievement of learning objective 6.

SAMPLE STUDENT DATA

Sample gel pictures of PCR results

Interpretations of figures were performed as follows (Fig. 2 and 3).

For the interpretation of Fig. 2, in this gel electrophoresis, only student 4 showed a visible amplicon around 316 bp both before and after brushing. The *Treponema denticola* amplicon did not show up commonly in the student population in the genetics classes sampled. It is indicated in the causation of periodontitis (39, 40), dental infection (41), and oral cancer (42).

For the interpretation of Fig. 3, in this gel, every student sample showed a visible amplicon of about 705 bp before and after brushing. This pattern was seen for all of the students tested. *Fusobacterium nucleatum* seemed to be quite ubiquitous among the student populations in the genetics classes sampled.

TOPO cloning with 16S bacterial primers and Sanger sequencing

See Appendix S1 for the guidelines to the analysis of the Sanger sequences and our class sample results. BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was introduced. Students were very excited in the analyses of the sequencing results to find out what microbes were in their mouths. We used a

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**Table 1**

| Parameter | Premicrobiota | Postmicrobiota |
|-----------|---------------|----------------|
| Mean      | 2.35          | 3.65           |
| Sample SD | 1.07          | 1.03           |
| Observations | 23           | 23             |
| \( P \) value\* | 0.0001\*      |                |

\* \( P < 0.05 \) (one-sided, rank Wilcoxon).

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**Table 2**

| Question no. | Possible answer range | Sample median | Hypothesized median | \( P \)  |
|--------------|------------------------|---------------|---------------------|---------|
| 1            | 1–5                    | 4.0           | 3                   | \( 1.7 \times 10^{-5} \* \) |
| 2            | 1–5                    | 4.0           | 3                   | \( 1.1 \times 10^{-5} \* \) |
| 3            | 1–5                    | 4.0           | 3                   | \( 7.1 \times 10^{-4} \* \) |
| 4            | 1–5                    | 3.0           | 3                   | 0.67    |
| 5            | 1–4                    | 3.0           | 2.5                 | \( 4.8 \times 10^{-4} \* \) |

\* \( P < 0.05 \).
laboratory session like a conference presentation session for the students to take turns presenting their findings. Each student was assigned to present on several PCR primers used to amplify species- or genus-specific (such as Treponema denticola or Fusobacterium nucleatum) amplicons from the salivary DNA samples. In this way, students needed to do literature searches regarding the prevalence and significance of several bacterial genera or species in the oral cavity.

NGS

The 2013 and 2015 DNA samples were sent to CUGI for Illumina sequencing. The PCR with the tagged fungus-specific primers, ITS3_KYO2 and ITS4, did not yield enough amplicons for sequencing. The sequencing with the tagged archaeal primers, Arch 349F and 806R, yielded very similar operational taxonomic units (OTU) as those from the bacterial primers. At this point, it cannot be concluded whether the lack of archaeal sequences from the sequencing...
was because of this particular pair of primers, the particular Illumina platform at CUGI, the low concentrations of archaea/fungi species in the oral cavities of this sample of students, or a combination of these reasons. CUGI did not accept NGS orders in 2019. Sequencing data for the four 2019 student samples from MR DNA (www.mrdnalab.com) are not included in the present study due to problems discussed under “Field Testing.”

**Statistical analysis of the presence of microbes**

Correlations of students’ eating habits and dental hygiene, cultural and ethnic backgrounds, and family dental histories with specific bacteria in their mouths would require many more subjects than were available in our classes. Nonetheless, it was a good starting point to determine whether the relative abundance of different kinds of bacteria changed before and after brushing. (Because our school did not have a thermocycler that could conduct quantitative PCR, we were only comparing relative proportions of bacteria after PCR, not their absolute quantities.) The analyses provided an opportunity to discuss the importance of statistics in the analysis of large numbers of sequences as well as the complicated nature of microbial community and biofilms.

The R code used for the genetics class’ (in 2015) applies Wilcoxon two-sided tests at the taxonomic Class level between the salivary DNA samples before and after brushing and the analysis results can be found in Appendix S3. Each data column is normalized so that the relative OTU abundance values are summed up to 1. Each OTU relative abundance is compared between the DNA samples before and after brushing. The Wilcoxon tests at the class level showed that the relative abundances of *Coriobacteriia*, *Bacteroidia*, *Bacilli*, *Clastridia*, *Fusobacteria*, *Epsilonproteobacteria*, *Gammaproteobacteria*, *Spirochaetes*, TM7-3 (of phylum *Saccharibacteria*), and *Mollicutes* are significantly different after brushing. Similar analyses can also be done at other taxonomic levels.

**Possible modifications**

The three branches of the research module (Fig. 1A) can be selected to be conducted separately or together depending on pedagogical preferences, class size, and time and monetary concerns. This research module can also be expanded to collaborations with microbiology classes, especially in culture-dependent microbe identification and with statistics classes in assessing the correlation between microbes and other factors collected in the student surveys (such as ethnicity, gender, diet, or toothpaste). Refer to the *ASM Guidelines for Biosafety in the Teaching Laboratory* for safe practices (https://asm.org/Guideline/ASM-Guidelines-for-Biosafety-in-Teaching-Laboratory). With this particular module, a large amount of data was generated each semester. We only had time to have a limited analysis of a few kinds of bacteria/trends in one semester. If anytime remotely taught, “dry” laboratory sessions are demanded, for instance during self-quarantine periods caused by COVID-19, there can be more sequence analyses in silico or in graduate-level courses.

In schools where the laboratory sections have more students and the experiments need to be scaled up, it will be more feasible if there is enough gel electrophoresis equipment, ideally one set per student or at least one per two students. Having more thermocyclers and UV transilluminators allows more parallel progression of experiments. At our institute, we only have one thermocycler compatible with the touchdown PCR programming, and one UV transilluminator that accommodates large gels, so at times there was a line waiting for gel examination and picture taking. Moreover, PCRs were set up and frozen before their turns to be put in the thermocycler outside of the laboratory section. The number of TOPO clones to grow in the liquid media should also be adjusted depending on the setup of the individual laboratory.

In the future, more emphasis could be put on developing students’ higher-order learning, including their ability in forming hypotheses regarding the effect of brushing or other factors on the relative bacterial proportions and recognizing the limitations and ambiguities in sequence analyses and identification of bacteria.

**CONCLUSION**

Due to the small class size at our college and that genetics is an elective that is offered every other year, a controlled comparison between this research-oriented genetics course and a traditional, prescribed laboratory course is not feasible. Nonetheless, the student oral microbe research unit appears to be a practical, flexible laboratory module that increases student engagement and effectively integrate quantitative and bioinformatics analyses and microbial knowledge. Overall, students have shown a positive learning gain and attitude toward this module. In general, students performed professionally in the poster presentation. Guest science faculty and student audience were impressed with the poster presentations and celebrated the culmination of the semester-long research project. A sample student poster is included in Appendix S2. A majority of the student presenters consulted the course instructor and the statistics professor in the poster preparations, showing ownership and active engagement. During the presentation, student presenters were equipped to explain the detailed procedure, summary of the data, and implications. Some presenters pointed out possible trends in the correlations between the brands of toothpaste (and/or cultural differences in diet) to different microbial communities. Utilizing the poster grading rubric, 90% of the students have scored at or above 80%. The iterative assessment tools (pre- and postmodule assessment, student learning self-assessment, and poster presentation) can be used to assess and implement modifications over time.
SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, DOCX file, 3.4 MB.

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There are no conflicts of interest.

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