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A Microbial Sampling and Community Reconstruction Activity for Introducing Students to the Burgeoning Field of Metagenomics†

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INTRODUCTION

The identification and characterization of microbial communities within diverse biomes is important in many areas of science, ranging from healthcare to agriculture. Traditional methods of bacterial community analysis would often begin by attempting to culture sample populations on agar; however, we now know that these methods fall short since only a tiny proportion of bacteria in a sample are culturable on standard agar plates (1–3). Metagenomics is a cutting-edge, culture-independent technique that involves the sequencing of all DNA extracted from a given sample, referred to as whole genome sequencing (4). This approach has allowed for the rapid identification and cataloguing of microorganisms in any environment or biome, telling us "who" is there and allowing for an estimation of the relative abundance of known, novel, and unculturable species within a sample (4). The term "metagenomics" is sometimes used to describe community profiling, whereby the members present in a given community are determined by sequencing a specific often universal region of DNA (or "barcode") from an extracted sample, such as the 16S ribosomal RNA (5). These sequences are then compared with a database to determine the identity of the microbes (5). Metagenomics has now become mainstream across many disciplines, including medicine, biotechnology, agriculture, and ecology, with applications ranging from identifying and improving microbial communities in agricultural soils to characterizing bacteria in the human gut that may contribute to various diseases and disorders (3). With the advent of next-generation sequencing and the reduction in sequencing costs, this mainstream scientific approach has broadened our understanding of the role of microbes in our environment and in our everyday life (4).

To introduce students to the field of metagenomics and its fundamental concepts, we propose a hands-on sampling activity that will allow students to conduct a basic metagenomic analysis of a simulated community. The exercise uses a collection of instructor-defined phrases to represent different microbes, with varying proportions of each phrase representing the relative abundance of each microbe in a given community. Students sample sentence fragments on strips of paper (representing portions of DNA) from an envelope (representing the community) and then match the fragments to a reference sheet (representing the database) that links each phrase to a specific microbe. Follow-up questions completed after the activity help to reinforce key concepts addressed in the activity. Students will learn the fundamentals of metagenomics and how the approach is used, and will be introduced to important concepts, such as sampling and accuracy, rarefaction, and the challenges of unclassifiable and undescribed bacterial species. This activity requires minimal preparation time and can be used in a classroom or laboratory setting to introduce metagenomics to junior and senior high school students and early year undergraduates.

PROCEDURE

Materials required for this activity include scissors, hard copies of the template sequences (Appendix 1), envelopes (one for each group), scoring sheets (Appendix 3; one for each student), pens or pencils, and a desk/table space. The class should be divided into smaller groups of two or three people. To set up the activity, the provided communities are printed, each sentence cut out, and each sentence fragmented again along the grey vertical lines. These will serve as the "sequences" that students will sample and compare with the reference "database" sentences. If additional communities are needed, multiple copies of the provided communities can be made. Instructors may also create their own novel communities by either altering community composition or adding/subtracting specific microbes (see Appendix 2 for additional information on set up).

The activity begins with a brief introductory lecture covering the basics of traditional microbial culture-depen-

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dent sampling of microbes and its limitations, followed by a description of metagenomics (definition, work-flow, benefits, and applications; see Appendix 2 for more information). The introduction to the activity should include a brief description of the bacteria they will encounter in the activity, as well as an overview of the sampling and tallying strategy (see Appendix 2 for more details). Each group will be given an envelope of segmented sentences, labeled with a community number, and a copy of the scoring sheet provided that includes step-by-step instructions for students (Appendix 3). Each group (or a representative from each group) will pull out one piece of paper at a time from the envelope and make a tally beside the sentence that matches their fragment. Sampled sequences are not returned back to the envelope after each sampling. It is possible to have fragments that match more than one microbe, as well as fragments that do not match any microbe. Both of these types of fragments should be scored under the "unclassified" category. Additionally, two sequences, those belonging to *Streptococcus mutans* and *Streptococcus pneumoniae*, are almost identical, which highlights the challenges of distinguishing between two very closely related species using a barcoding approach. Students will sample 10 sequences from the envelope and calculate the percent abundance for each bacterium. This process will be repeated three more times, with the percentages being calculated out of 20, 30, and 40 for the successive rounds. Once students have calculated the percentage for the final round, they can be provided with the actual percentages for each bacterium in their community. Students are then provided several follow-up questions to work through individually or in groups (Appendix 3). These questions can be assigned as homework and/or discussed as a class. Answers to the follow-up questions, as well as a completed example score sheet are included in Appendix 4. The pre-activity lecture, activity, and follow-up questions can fit within a 50-minute class period, with approximately 20 to 30 minutes being designated for the activity itself.

Safety issues

None.

CONCLUSIONS

This metagenomics activity will introduce students to the concept of community profiling through an interactive, intuitive exercise. After completing this activity, students will have an understanding of the fundamentals of metagenomics, including the work-flow, sampling, benefits over culture-based methods, and the limitations of microbial community profiling. We have conducted a simplified version of this activity for two groups of 12th grade biology students, usually following a traditional bacterial isolation activity, whereby students collect environmental samples and observe them on agar plates. The community composition recovered by the students for each of their communities were quite similar to the actual composition after only three rounds of "sequencing" (Appendix 5). Students were able to answer the follow-up questions and were able to understand the concept of sampling and its relationship to the accuracy of community reconstruction. The student response was extremely positive, with students commenting that the activity was fun and easy to understand. Overall, our metagenomics activity allows students to begin to conceptualize the multitude of applications and benefits of identifying microbes in their environment through a DNA-based approach.

SUPPLEMENTAL MATERIALS

Appendix 1. Six sample communities
Appendix 2. Notes for the instructor
Appendix 3. Score sheet and follow-up questions
Appendix 4. Example score sheet and follow-up question answer key
Appendix 5. Data obtained by two 12th grade classes for six simple communities

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REFERENCES

1. Martiny AC. 2019. High proportions of bacteria are culturable across major biomes. ISME J 13:2125–2128.
2. Rappé MS, Giovannoni SJ. 2003. The uncultured microbial majority. Ann Rev Microbiol 57:369–394.
3. Jurkowski A, Reid AH, Labov JB. 2007. Metagenomics: a call for bringing a new science into the classroom (while it's still new). CBE Life Sci Educ 6:260–265.
4. Garza DR, Dutilh BE. 2015. From cultured to uncultured genome sequences: metagenomics and modeling microbial ecosystems. Cell Mol Life Sci 72:4287–4308.
5. Hugerth LW, Andersson AF. 2017. Analysing microbial community composition through amplicon sequencing: from sampling to hypothesis testing. Front Microbiol 8:1561.