**Title: Chemical Approaches to Microbiome Logic**

**Clearly articulate the specific research question and the goals of the project:**

Globally, this project attempts to provide primitives to the research community such as: given microbiome community A (which may come from the microbiomes of many individuals) and microbiome community B, create the communities A intersect B, A-B, and B-A. As a step towards this goal, my project aims to construct a set of DNA probes that will test the presence or absence of certain microbial species in a microbiome community. For example, if a probe of a species from A is present in B, then that species is in the intersection of A and B. In operation, the probe will be able to ligate to specific types of bacterial 16S rRNA and fluoresce after doing so. The 16S rRNA gene sequence can distinguish bacterial species from others. Taking advantage of this fact with a DNA probe would allow for detection of the type and (potentially) concentration of a bacterial species in an environment. Multiple rounds of PCR and linear amplification will be performed on the DNA components to form the final structure of the hairpin loop with an attached fluorophore and quencher (see Figure 1).

## Provide sufficient background to contextualize your question or problem. An educated, non-expert reader should be able to fully understand your topic. Be sure to describe the significance of this research as well: e.g. How is it unique? Why is it important? What will it contribute to the field? Include references:

This DNA probe can be used to compare the microbiomes of two hosts. For example, if Host A is ill, the probe can be used to detect the presence/absence of a specific set of microbial species in both Hosts A and B. Consequent comparison of bacterial species concentration can allow for evaluation of their impacts on their respective host’s health. This has major implications for the medical field, providing a method for determining which bacterial species are responsible for health defects. Further, the set B-A might be a treatment for patients having the same illness as A.

The DNA probe, which will be around 45bp, will have two distinct components (see Figure 1). The first component (yellow region) contains the probe sequence that will be complementary to a bacterial strain’s 16S rRNA variable region. The second component (gray region) contains the hairpin loop with the fluorophore and quencher. When the probe sequence binds to the bacterial rRNA, the complementary ends of the second component will bind together and the hairpin end will open up, separating the quencher from the fluorophore. Fluorescence can then be measured to quantify concentration of the bacterial species in question.

## Describe the methodological approach you will employ to carry out your proposed research:

Polymerase chain reaction (PCR) will be the main method used to create and amplify sections of the DNA probe. After each round of isolation/annealing to create a certain component of the probe, gel electrophoresis will be conducted to analyze the yield of the PCR. After final construction of the DNA probe, experiments can be run to evaluate the functionality of the probe.

## Describe your timeline for completing this research, including the project's start and end dates and estimated number of hours per week dedicated to the work:

This project will be conducted from May 15 to September 1 over the summer term. I will be spending an average of 40 hours per week in the lab. This project may carry over into the fall term.

## How is your project relevant to your academic interests and goals?

I am currently majoring in chemistry on the pre-med track and I tend to wonder how these two fields could be explicitly connected. This project bridges my two interests with its chemistry background and potential medical application. This project has major implications for the medical field, especially if future research can expand on this area. Being able to attribute a certain concentration of bacterial species to a specific illness would be extremely advantageous for physicians trying to determine the best course of treatment.

## Describe your relationship with your project mentor, addressing the following points: How did you identify your project mentor? In what capacity did you work with your mentor in developing your research project? How will you work with your faculty mentor on this project? How often will you meet?

My recitation instructor for Quantum Mechanics, Dubravko Sabo, made an announcement to the class that Professor Ohayon was looking for students to help out in his lab. I met with him shortly after to discuss which project I would be assigned to, and Professor Ohayon connected me with a senior student who was working on this project. Professor Ohayon has been following and guiding me along this project, assisting me if I have any issues with laboratory procedures or questions about the project itself. Professor Ohayon is usually available when I go into the lab to conduct research, and we have been having weekly meetings with Professor Dennis Shasha to discuss updates on the project’s progress.

**Please use the space below to itemize each anticipated expense associated with your project. Indicate specific costs and provide explanations and rationale.**

See Figure 2 for a comprehensive list of materials and reagents needed for this project. All items on that list will be used to carry out laboratory procedures for the project. In addition, we will be ordering DNA strands to construct the probe; this price is estimated to be around a few hundred per order.