Microbiome DNA Sampling

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Abstract

This experiment assesses the relative health for bacteria in two different locations. Health will be assessed based on the relative biodiversity of the bacterial species from these two locations

The two locations compared were the ECO-Lab and the Marian Main Campus. The ECO-Lab is expected to have a higher biodiversity due to its lack of human footprint.

Barcoding technique will be used to develop the experiment. This technique refers to tagging individual samples with the unique DNA sequence known as barcodes to allow its identification. In this case we will be trying to identify bacteria in our two soils samples.

Introduction

The two locations where dirt samples were obtained were the ECO-Lab and the Main Campus area. The ECO-Lab, being a natural place, will hopefully have higher biodiversity. The Main Campus should have less because of chemicals . The goal is to identify and compare how many bacteria can be found in each location.

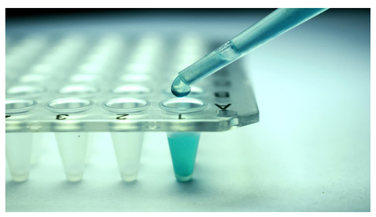

Figure 1. All procedures were completed using various strategies for pipetting. This skill, learned earlier this semester, has been very important for the completion of this project.

Materials and Methods

Samples: Six different samples were gathered

Storage. Each was stored in a fridge to preserve any DNA or other potential findings.

Assistance: Dr. Sara Justice assisted in the procedures

Extraction: DNA Easy and Blood Tissue Kit used to extract initial samples

Gel Protocol and Primers: 16s primers and Barcoding Kit and LongAmp™, see references

Finding 1

The samples from the campus had less DNA than those of the ECO-Lab. Higher concentrations of DNA indicate higher biodiversity in the ECO-Lab. This suggests that early predictions of where more DNA would be found are correct.

Sample 1: 2.18	Sample 4: 1.19
Sample 2: 2.66	Sample 5: 1.37
Sample 3: 3.42	Sample 6: 1.93

Figure 2. The first three samples were from the ECO-Lab, and the last three are from the Main Campus. All units are in ng//uL

Finding 2

The initial extraction protocol did not work. The primers were all seen in the gels, indicating we did not have bacterial DNA. Because the soil was extracted from more forested areas, it is possible that many other fungi or plant DNA were in the sample.

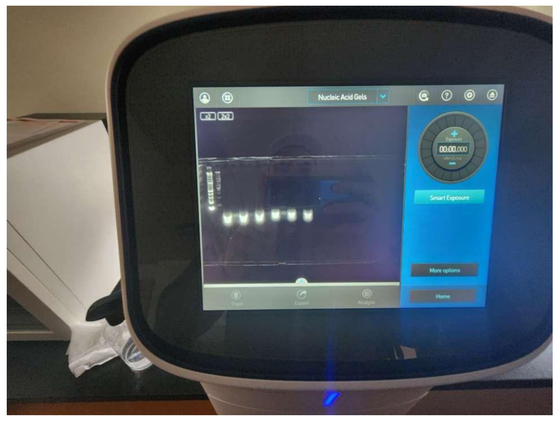

Figure 3. Gel picture : one can see that all samples only have the primers because we did not have bacterial DNA

Results

Campus ECO-Lab

Figure 4. Both of the cites where we got our samples from the Eco-Lab and the Campus

Our results were inconclusive because of time limitations on this project. Our goal of identifying different bacterial species failed. Our 16s primer never yielded tangible results. Thus, we concluded there was other organismal DNA in the soil.

Conclusions

Overall, the procedure did not yield strong results. However, the DNA extracted still showed a strong presence of life in the soil. The question remains, what DNA did we extract? It is not possible to conclude now. Moving on, this experience reminds how science is built not only on success, but our failures as well.

Due to time constraints, we couldn't explore DNA from fungi or plants. Given more time, we'd conduct two additional PCR runs: one using fungi primers and another with plant primers. With the significant amount of DNA already found in our samples, these tests could reveal further insights.

References

Qiagen, "DNeasy Blood & Tissue Kits," 2013.

Kai S, Matsuo Y, Nakagawa S, et al. "Rapid bacterial identification by direct PCR amplification of 16S rRNA genes using the MinION™ nanopore sequencer." FEBS Open Bio, 2019 Jan 29;9(3):548-557.

Elite Orthopaedic, "What's the Deal with Stem Cells," [Figure 1].

Acknowledgments

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