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Introduction: The goal of the B.A.S.A.L.T. project is to determine the upper bounds of the biomass that could have been supported on Mars and investigate how those upper bounds inform future requirements to detect extinct life on Mars. Fieldwork is conducted on the Big Island of Hawaii at the latest lava flow in Pahoa and an older flow in the Hawai'i Volcanoes National Park's Ka'u Desert. These two flows serve as analogs to present day and Noachian era Mars respectively. At the two sites, I collect samples of basalt and analyze them in the lab for bacteria and archaea. Once the yield of genetic material is determined, a segment of that DNA is amplified and then prepared for sequencing. This undergraduate research, funded by the Hawai'i Space Grant Consortium, is preliminary work for a larger NASA funded inquiry.

My methods for retrieving the samples from the flows went as follows: All samples were collected with a rock hammer that was sterilized with a 70% bleach solution before and after each use. Bleach was used instead of ethanol because bleach will completely destroy any genetic material residue [1]. Sterile gloves were used to handle the samples and place them into sterile ziplock bags. Each sample was then taken to the lab and crushed with a corresponding mortar and pestle under a specially enclosed hood. The crushed samples were then placed in 50mL sterile tubes and put in a -20°C freezer. Before using the mortar and pestles, rather than autoclaving them, they were baked in an oven at 230°C to ensure no contaminates were there. 230°C is the temperature at which you can achieve DNA pyrolysis, which is when DNA will dissipate [2].

The method of DNA extraction that is being used is called solid phase binding which is when the sample is run through a silica membrane that captures the DNA allowing you to remove impurities [3]. Using a nanodrop, an analytical instrument used to determine the average concentrations of nucleic acids, I am able to determine how successful I was at removing impurities and extracting a large amount of genetic material (see Figure 1).

The section of DNA that is being amplified is the 16S ribosomal RNA (rRNA) fragment which is often used in phylogenetic studies to differentiate microbial organisms from one another. This particular fragment has highly conserved primer binding sites and sequences containing variable regions that are species-specific signatures [4]. After amplifying the 16S rRNA section of the DNA using PCR, Agarose Gel Electrophoresis is used to separate the fragments and analyze them based on their size (see Figure 2).

No conclusions can currently be made about the results of this work because more data needs to be gathered and analyzed.

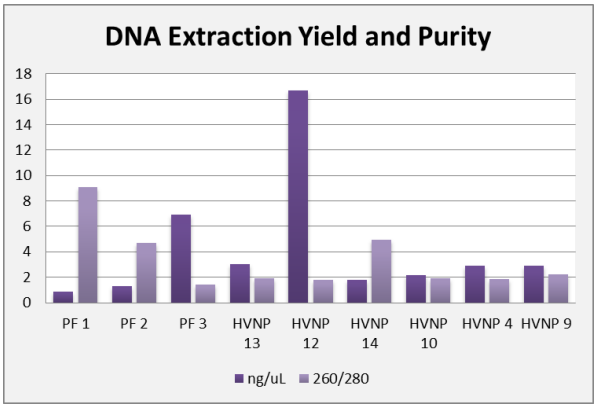

Figure 1. This graph shows the results of my initial DNA extractions and the amount of genetic material measured by the nanodrop instrument in nanograms per microleter. It also shows the 260/280nm ratio where ~1.8 is considered "pure" for DNA.

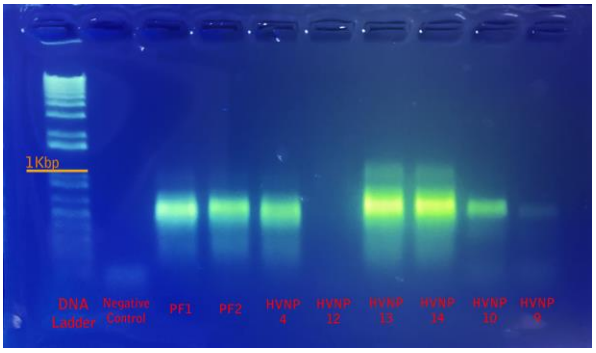

Figure 2. This figure shows the gel electrophoresis results of my PCR products. The bands shown are around 600bp which is the expected size for my 16S rRNA amplification.

References:

[1] Liquid Bleach; MSDS No. VAR9K [Online] (2012). [2] Mainord K. (1994) *The Magazine of Critical Cleaning Technology*, 37. [3] MoBio Laboratories Inc. (2012) *PowerSoil SNA Isolation Kit Protocol*. [4] Woese C.R. et al. (1990) *Proceedings of the National Academy of Sciences 87*, 4576-4579.