A certain aspect of the geochemistry project with Dr. Jim Evans was to analyze potential life forms residing inside the mud of the gryphons and mud pots around the Salton Sea. The gryphons and mud pots create a unique environment – salt appears to be precipitating in or around these formations, the smell of sulfur lingers in the air, the mud is nearly black in some areas which encourages the idea that there could be hydrocarbons present, and lastly, the mud pots are approximately 30º C and the gryphons at 60º C. This sort of environment is borderline suitable for extremophiles, which generally fall under the Archaea branch of the tree of life. However, some bacteria are capable of living in such conditions.

Mud samples (solid and liquid) were collected from the Davis-Schrimpf field in sterile 50 mL tubes, and brought to Dr. Don Robert's Fungus Lab at Utah State University, where a few of the samples were plated on a variety of agar plates. Soon after, growth was found on a few of the plates, of what appeared to be mucoid, yellow-white translucent colonies. Soon after, we took all of the samples (except for the solid one), made a set of serial dilutions, and plated them onto PDAY agar plates, which consists of 4 grams of potato starch, 20 grams of Dextrose, 15 grams of agar and 1 gram of Bacto Yeast Extract per liter. We chose PDAY since this was the medium that displayed growth while the other media did not.

From these plates, we found many of them had growth, and were taken to Dr. Anne Anderson's Microbiology lab at Utah State University for further analysis. We performed single colony isolations to try and isolate single, genetically identical colonies from the plates so we could perform 16s RNA sequencing for identification. After leaving these isolates to grow for approximately 2.5 weeks, we found that there were multiple organisms living in the mud, and not just the dominant mucoid white-yellow colony. Colonies found were small, yellowish, round, hard; reddish/brown discoloured mucoid colonies; and a small, mucoid film colony in the background of the plates. We created patch plates of these to try and further isolate these new colonies, and to see if these colonies found on the original plates are composed of the same organism. From this another set of patch plates were created.

Within this 2.5 week period, my co-worker had obtained 12 new samples from the Davis-Schrimpf field for further microbial analysis. 3 replicate samples were taken at 4 sites – 2 gryphons and 2 mud pots. Upon obtaining these samples a set of serial dilutions were created, and plated out onto PDAY agar plates, and left to grow from Friday to Monday. On the Monday, the growth of the plates were characterized. There were many different types of colonies, such as the dominant yellow/white mucoid colony, a white/green fuzzy fungus, a white mucoid colony and some colonies with a pink-red center or pink outline. From these, patch plates were made to prepare them for 16s RNA sequencing.

From both of the sets of patch plates, cells were gathered and put into separate mixes for a Polymerase Chain Reaction (PCR) to attempt to amplify their DNA in order to sequence and identify the organisms. We used two sets of primers – one of Archaea and one for Bacteria. The results of the PCR was run through a gel electrophoresis process to see if anything amplified.

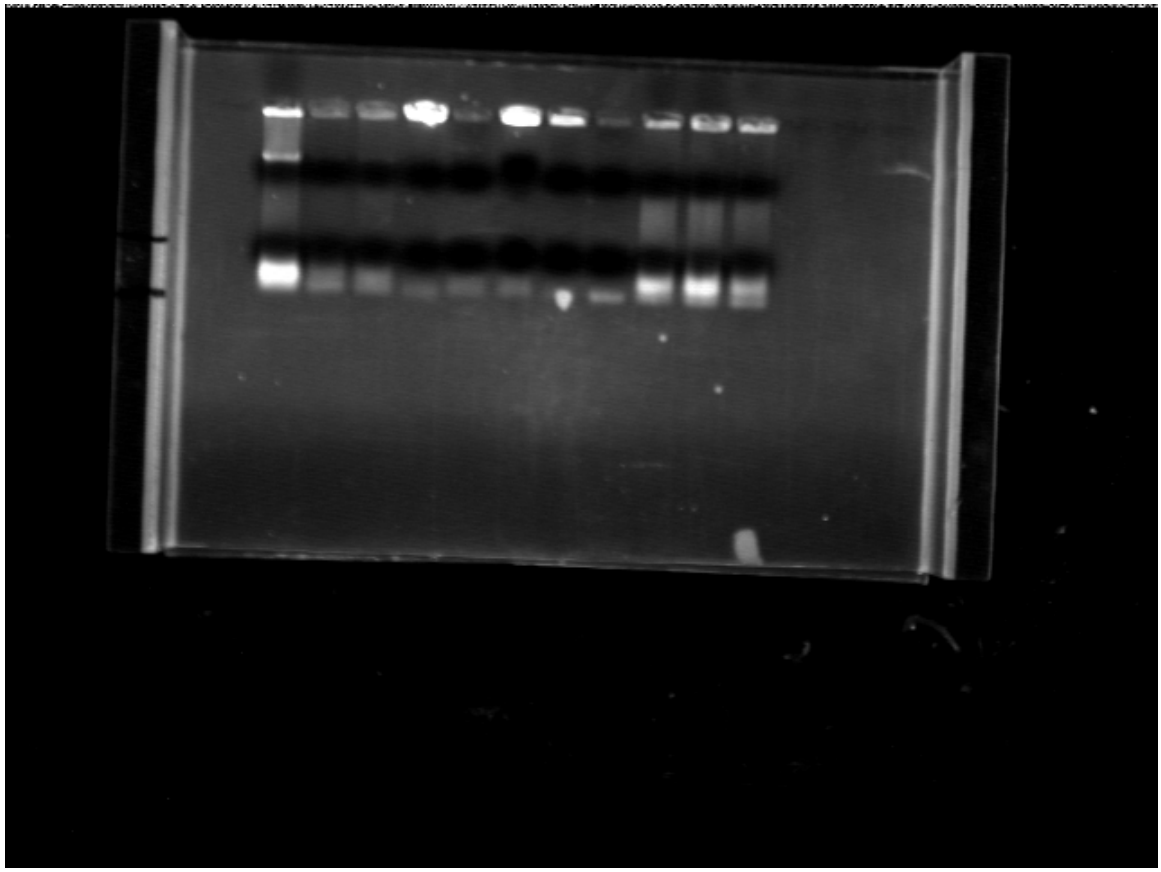

Figure 1. Bacteria Primer results of the PCR

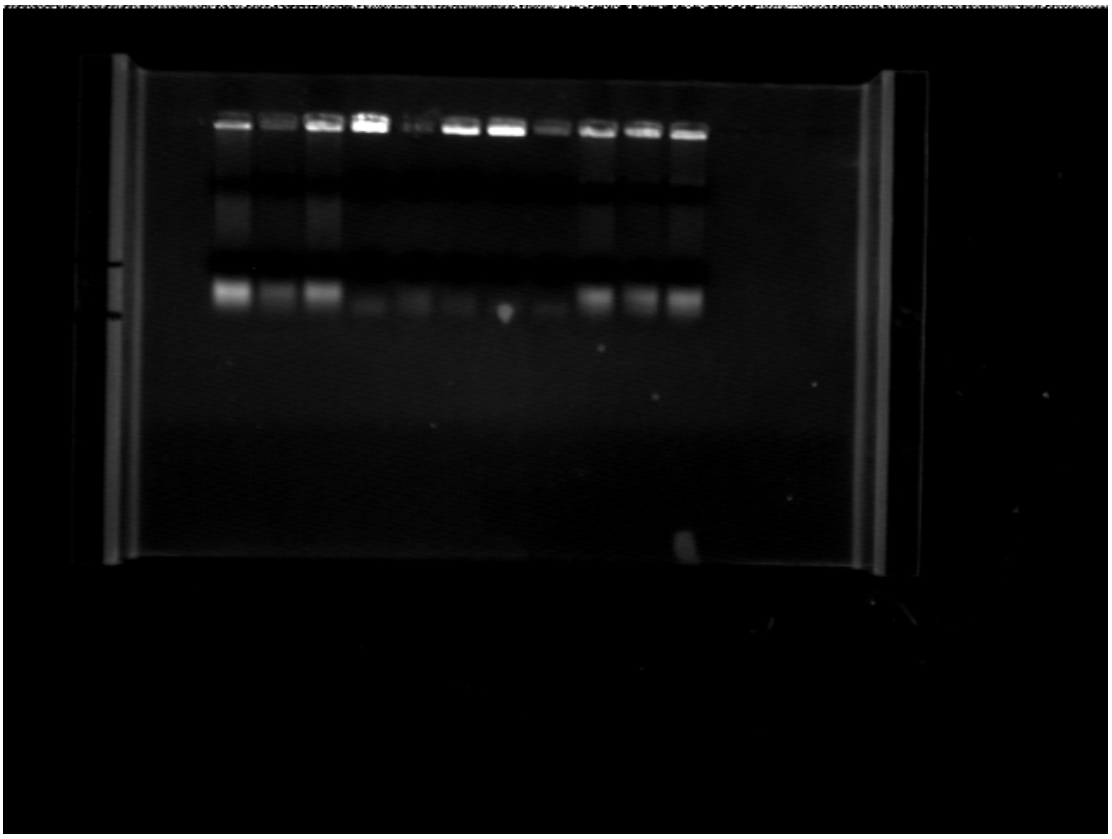

Figure 2. Archaea Primer results of PCR

Figure 1 displays the gel electrophoresis results of the PCR with the Bacteria primers. The first lane is a sample Dr. Anderson had prepared for herself, which conveniently happens to be that of a known bacterium. At the top of lane 1 (most left-hand lane), a distinctive band can be seen, which shows that the DNA was amplified of that bacterium. It also serves as a control sample, showing that the reaction does work. However, in the other lanes, all being the mud samples, nothing appeared except for what is known as "primer dimer." Figure 2 shows the results of the PCR reaction with the Archaea primers, and once again, nothing appears. The control serves well, showing that once again, these primers do not work for a bacterium.

Therefore, the PCR reaction needs to be re-done or perhaps a different approach needs to be taken on how to obtain the RNA/DNA of the organisms. Also, it is interesting to note that there are few culturable colonies – perhaps we didn't explore all the possibilities of medium to grow bacteria on, or there truly aren't many. There are many, many aspects yet to be explored, experiments to be done, however, one thing is we are fairly certain that there is life in the mud at the Davis-Schrimpf field.