### ABSTRACT

Our group was interested in determining the gap for the genomic DNA sequence of Arhodomonas sp. Seminole. Our interest in this experiment was to try to reconstruct the DNA of a species that will survive in the harsh environment around oil fields in Seminole county. In order to accomplish our goal, we obtained two contigs of the DNA of the species and constructed a PCR product by using Blastx. This gave us possible primers that fused contigs together to give us a possible gene. Once we had a possible PCR product, we used agarose gel electrophoresis to test the validity of our PCR. Within the agarose gel electrophoresis, we had a control positive and negative. Unfortunately, our PCR failed when the agarose electrophoresis gel gave us a false negative.

#### INTRODUCTION

We are studying the genomic DNA of Arhodomonas sp. Seminole to analyze how the bacterium can live in high-salinity conditions. The bacterium was found near oil fields in Southeast Oklahoma. Because of this, we know that it can survive in these conditions. To isolate the gene, we obtained two contigs (the head of one and the tail of the other) of the species DNA that we believe are connected. When connected we believe that they hold the genomic sequence of the gene that enables the bacterium to survive in this environment.

### **MATERIALS AND METHODS**

8 small test tubes with lids Micropipette with disposable plastics tips 8 small test tubes with lids Blastx Thermo cycler 1 well plate Agarose gel Tracking dye

We took our two contigs from Arhodomonas Seminole to design PCR primers to connect the gap. First, we had a forward primer for the downstream contig and a reverse primer for the upstream contig. Once those were designed, we sent them to the IDT DNA website in which the company made them. Once we received the PCR product, we started the PCR amplification by combining the following reagents: dH20 (provided), 10X Taq buffer, 10X dNTP's(all 4), Arh. Sp. Seminole gDNA(58 ng/ul), F-primer, R-primer, and taq polymerase. After combining our reagents in the reaction tube, we added a tracking dye and inserted it into the agarose gel electrophoresis. This is will determine if the PCR product will connect our two contigs.

# Arhodomonas sp. Seminole and the PCR Product

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RESULTS PrimerQuest Reverse CGACTGAAGCCGGTGAG (AntiSense Assay Design / Result Help / About Assay Set Locations for Sequence 1 This shows the possible primers that could connect our two contigs.

These are the forward and reverse primers that we designed to connect the contigs. These primers had too many bases.



This is a gel image of our false negative result after going through the agarose gel electrophoresis.

Further analysis was conducted to reassess the results that were presented after the electrophoresis. This included inserting our product into a thermo cycler ranging from 30 seconds to 10 minutes and temperatures from 51 to 94 degrees Celsius. The results still produced a false negative.







## DISCUSSION

This experiment was to reconstruct the DNA Arhodomonas sp. Seminole. By doing so we obtained two contigs in order to reconstruct the DNA and identify possible genes. During this experiment, we analyzed the DNA and designed primers to fuse contigs together. Once designed, we use agarose gel electrophoresis. Unfortunately, our results were a false negative. This is caused because our primers were faulty and unable to connect the two contigs.

Our results showed a false negative which means a reagent was poorly made and lead to no production of a band. This was caused by possibly contaminating our PCR product when adding the different reagents. Our hypothesis was centered around whether our contigs would connect properly or not and we said that with our PCR products would fill in the gap. Our prediction would help us complete the genome of Arhodomonas sp. Seminole.

Since our results were inconclusive, our next step in this experiment would be to go back to our designed primers and redesign them with a different set of parameters, such as a smaller base count and have them closely aligned. This would help give us a more accurate analysis and the primers a better chance to connect the contigs. Once the results show that our contigs our connected, we would analyze the DNA to predict a gene if there is one there. Once that gene is present, we can analyze and determine what the gene does for the species as a whole.

Our conclusion is that our hypothesis is disproven; however, this does not disprove the entire experiment as a whole. Our group disproved the hypothesis of our alignment with the PCR products due to how massive the bases were. Other tests and experiments should done with these contigs to further analyze and form a correct hypothesis.

### REFERENCES

• Basic Local Alignment Search Tool. (n.d.). Retrieved November 11, 2014. • Canaan, P., Dalvi, S., Najar, F., Roe, B., Hartson, S., Nicholson, C., & Fathepure, B. (n.d.). Result Filters. Retrieved November 12, 2014.

