Arhodomonas: A Genomic Puzzle

GRP #24
Section #002 and 702

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ABSTRACT

The purpose of our research project was to identify possible gaps in the Arhodomonas Sp. Seminole bacterium's DNA. The Bacteria Arhodomonas sp. Seminole lives and thrives within oil rich soils in the southern United States. It is able to metabolize the harmful chemicals that are produced within the area. In this experiment, we examined the DNA string of the Bacteria Arhodomonas sp. Seminole, our groups were presented with the task of "rebuilding" the DNA string using some methods that we learned in lecture. We were given two contigs and used PCR primers to generate a draft of the gene gap. The DNA sequences we were given were placed into BLASTx and information was gained about the two (Head and Tail) determining whether these two sequences could possibly sit beside each other. With our information, we were given a related protein sequence to determine whether these two contigs contain a protein thus proving that they connect while giving information to what is missing. After this, the groups were taught and helped through the process of PCR in order to create more copies of our contig, then used a technique called Electrophoresis in order to determine similarities/differences between our contigs and the control group. Our groups went through the steps and use the methods presented to us and we were able to determine that our two contigs produce a protein within the Bacteria Arhodomonas sp. Seminole. We obtained results from the forward primer but results were inconclusive from the reverse primer. The results confirmed that the two contigs do line up but we were not able to identify the DNA segment due to limited information.

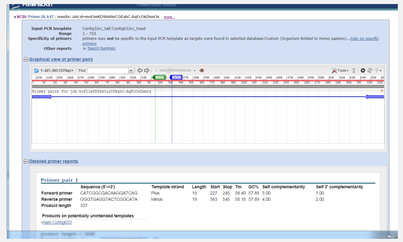
INTRODUCTION

The project that we have done dealing with the bacteria Arhodomonas sp. Seminole, which is an aerobic, halophilic bacterium enriched from salty, crude–oil-impacted soil from Seminole County, Oklahoma, has led us to try to sequence its gaps. We have done this by using what we know about sequencing, which is that the "reads" are produced by sequencing and that the "reads" are in the order that they are in based on overlaps. We have also learned that the assembled "reads" is what is used to create the contigs and the contigs are what we look at to find genes and regions of interest. We wanted our Genomic sequence of Arhodomonas sp. Seminole to be a complete genome but instead in our group we found out that we had two of the 750 contigs and we completed our gap by changing it into a protein sequence and compared it to the related protein sequence that we had. We compared them by copying them both on to the website blastx which let us now how well they matched up.

RESULTS

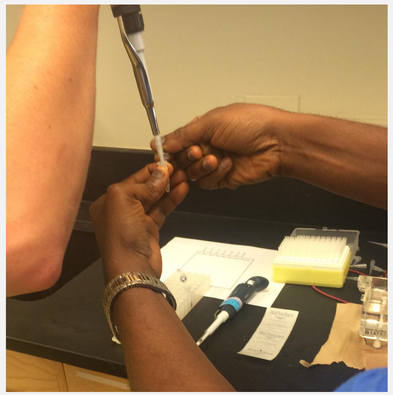
We created forward and reverse primers for PCR. The 100 µl PCR mix contained about 50 ng of DNA, 0.6 µM of each primer, 200 µM of PCR nucleotide mix, 1.75 mM MgCl2, and 2.5 units Taq polymerase in Buffer A.

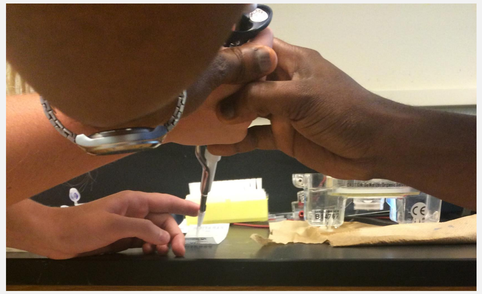
The amplication program began with 30 seconds of 94°C temperature for denaturation, and continued with 30 cycles of this denaturation before annealing for 30 seconds at 51°C and extension of 72°C for one minute and a final extension at the same temperature for 10 minutes



We ran the PCR product through electrophoresis gel

Our experiment yielded a forward primer but not a reverse. This confirms the two DNA segments align but we can not identify the missing segment.





MATERIALS AND METHODS

Materials:
Micropipette
Electrophoresis Gel

We used a micropipette to create the primers for PCR. We amplified the PCR product, added dye and placed the DNA in the electrophoresis gel.

DISCUSSION

Our group worked through this project as Dr. Cannan instructed and we came up with a set of Data and Observations that prove helpful to the puzzle that is the Genome of Arhodomonas sp Seminole. Our group produced a PCR product and discovered that our contigs match and combined produce a helpful protein. The importance of our findings is that it places another piece of the genomic puzzle into place, that will conclusively result in the full sequence of this bacteria being discovered at the end of Dr. Cannan's research. Our group's involvement and research with these helpful bacteria is important solely for the sake of sequencing a new organisms genome. Dr. Cannan primarily set many of the findings and observations made during this project up, so the results of each step of our process shows her expertise in the methods of research used. The only future research needed for this project to reach completion is only repeating the steps used many times finally reaching a complete unbroken Genome for Arhodomonas sp Seminole.

REFERENCES

Dr. Patrica Canaan, "Assembly and Gaps," Lecture Notes, 2014.