Finding the DNA Gap in Arhodomonas Seminole Using DNA Sequencing

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ABSTRACT

Arhodomonas Seminole is an aerobic, halophilic bacterium enriched from salty, crude-oil-impacted soil in Seminole Co, OK. This is a newly found and researched bacteria. Samples of this bacteria were obtained through the instructor. This samples had been pre-ruptured, pre-purified, and pre-concentrated by the instructor so they were ready to be analyzed. These samples were then sequenced using Blastx at the NCBI website. After sequencing was completed, reeds were produced. By overlapping and correctly ordering these reeds, two contigs were formed and ready to be put together. Predicting adjacent contigs based on what is known in related species and other strategies were used. Ultimately, this was achieved by the PCR process. This process was focused solely on one small portion of the DNA. Designed PCR primers were then created and used to complete the PCR process. After PCR the copied DNA was viewed and examined by gel electrophoresis. After this process the final product was finally gathered, which is what will be presented to you here.

INTRODUCTION

Arhodomonas Seminole is a halophilic bacteria that thrives upon crude oil found in oil filed sites. We are examining this bacteria to fill the gap in its' DNA. We know proteins from similar bacteria that could fill the gap and have tested these to see if they will work for Arhodomonas Seminole. We are using DNA sequencing to determine where the gap is and how big it is. Also we need to find a related protein, from a similar bacteria, to fill the gap. Filling small parts of this gap will lead to filling the whole thing and then we will have the entire genome sequenced for Arhodomonas Seminole.

MATERIALS AND METHODS

dH2O (provided) 70 ü   Contig671_Contig465 (Arhodomonas seminole DNA)

10X Taq buffer 10   Fused Contig sequence   50 ng of DNA

10X dNTP's (all 4) 10   Related Protein Sequence   0.6 µM of each primer

Arh. sp. Seminole   PCR sequence   200 µM of PCR nucleotide mix

gDNA (58 ng/ml) 5   Thermocycler   1.75 mM MgCl2

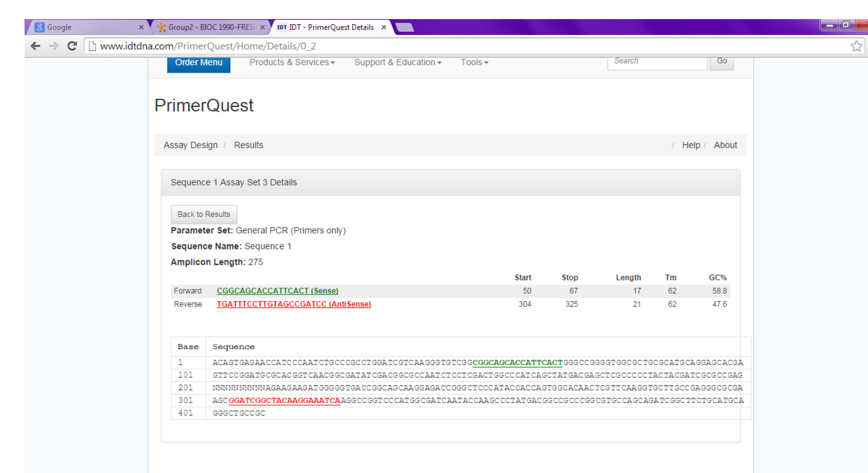
F-primer 2   Gel electrophoresis Chamber 2.5 units Taq polymerase in Buffer A

R-primer 2   Running Buffer   Running Gel

Taq polymerase 1   The 100 µl PCR mix

1) Ran Contig671_Contig465 DNA sequence through BlastX with a related protein sequence
2) Recorded results of both blasts
3) Found the length of the gap in our DNA strand
4) A Fused Contig sequence was then used 200 characters to the left of the N's in the sequence and 200 characters to the right of the N's
5) The Fused Contig Sequence was then ran through BlastX and resulted in to many primers
6) The Forward Primer CGGCAGCACCATTCACT was chosen as well as the Reverse Primer TGATTTCCTTGTAGCCGATCC
7) 2 microliters of each primer was used in the PCR we prepared using dH2O (provided) 70 ü, 10 microliters of10X Taq buffer, 10 microliters of 10X dNTP's (all 4), Arh. Seminole, 5 microliters of gDNA(58ng/ml), 1 microliter of Taq polymerase. All substances were placed in a centrifuge tube.
8) We then placed a small mixture (roughly 5 microliters) of our PCR and dye into a DNA Running Gel.
9) We then used BlastX on the Fused Contig sequence with reference to the related protein to find what protein should fill in the gap; which was 2 keto-D-glucanate-dehydrogenase
10) Through the gel we had a working PCR product that gave us the sequence GTTGCCATCTCTTTCCTAGCTGATTGGCTGCCGAGAAGAAGTTGGGGTTGACCGGCAGCTTGG AGACCGGGCTCCCATAC CACCAGTGGCACATCTCGTTCAATGTGCTTGCCGAGGGCGCGAAGCGGATCTGCTACAAGGAA ATCAA
11) We then ran this our protein sequences through Clustal to align them and form a contig.

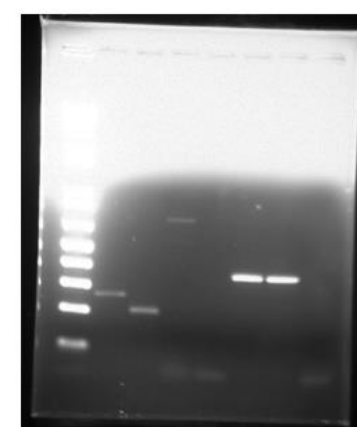
RESULTS



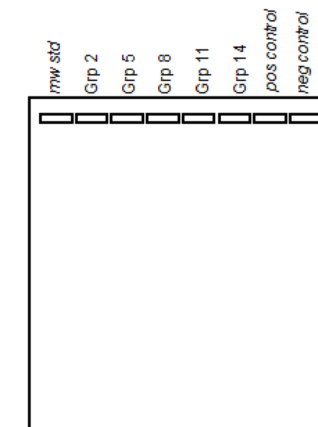
Through the NCBI website we were able to blast a portion of our fused full length contig 200 amino acids before the unknown gap and 200 amino acids after the unknown gap against the related protein. The results we received from the blast were the forward and reverse primers we used in our PCR reactions. Primers are essential to the ingredients needed for the PCR reaction to be successful. Primers act as the zipper to unwind the double helix of the DNA to replicate only the portion of DNA we are trying to manipulate and observe more closely.

The primers were only one of the many ingredients needed to complete our PCR reaction. Among the others are water that acted as the solution for the reaction to take place, a buffer solution that provides the optimal pH in which the reaction needs to take place, several of all four amino acids to help zip up the DNA in only the specific sections we are observing at the ends of the primers, we also added the actual DNA of Arhodomonas Seminole as the backbone of the reaction, and finally Tag polymerase that act like the glue that helps the primers zip along the specific segments of DNA.

Reagent	Volume (□l)	Tube Color	Added
dH_2O (provided)	70		□
10X *Taq* buffer	10		
10X dNTP's (all 4)	10		
Arh. sp. Seminole gDNA (58 ng/□l)	5		
F-primer	2		
R-primer	2		
Taq polymerase	1		
Total	100	-	-



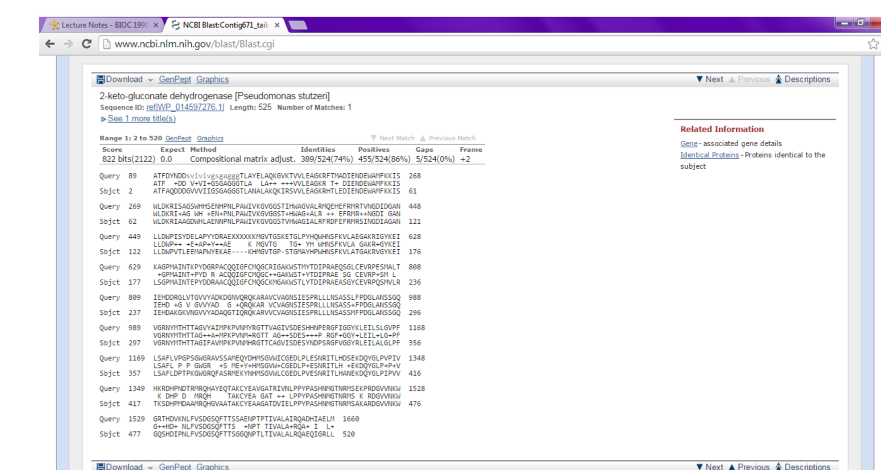
Gel 1.2

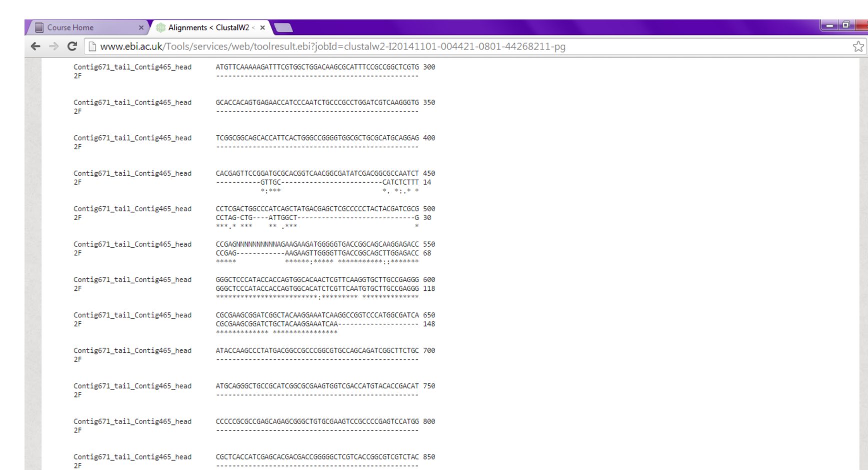


Gel 1.2

As this model shows gel electrolysis which we conducted after performing our PCR experiments, the more vivid the gel sample the better the PCR reaction matched with the forward and reverse primers we found and the mixtures we made to perform the reaction. There is a molecular standard on the far right to be compared in terms of the number of amino acids. The two columns on the far left one is not shown this is the negative control and the second farthest to the left column is the positive control shown very vividly.

One of the most important blast exercises our class performed was one in which we blasted the fused contig and received the related protein 2 keto-gluconate dehydrogenase. This protein allows us to observe a particular section of Arhodomonas Seminole's DNA and according to the blast the closest protein to this particulate sequence is the protein 2 keto-gluconate dehydrogenase. This can allow us to make inferences about what is the function of this section of DNA and what could be the overall function of the bacterium if scientists were able to fill in more of the over 700 missing gaps in the genomic sequence.





This website entitled Clustal added a more detailed view of the gap in the genomic sequence when we compared the fused contig against the forward primer produced from the PCR reaction. By analyzing the amino acids that come before and after the gap scientist can better able determine the functions of the bacterium through related proteins and the cause of the gaps.

DISCUSSION

Arhodomonas Seminole is a bacterium found initially in Seminole County whose function is unclear due to the incomplete genomic sequence. It was the task of Biochemistry 1990 to continue the work of our professor Dr. Canaan in her quest to fill in these missing DNA fragments. Through processes such as PCR and gel electrolysis, four out of the fourteen groups were able to receive forward and reverse primers from their work, or simply one of the two primers. From our mentors going over the remaining groups work three more groups' revealed primers from the PCR and electrolysis experiments. Although Dr. Canaan believes there may be over 700 gaps in Arhodomonas Seminole's DNA sequence, the fourteen groups that participated in this study did not close the case but helped to further her work. Simply because primers were found does not mean they fill the gap but through this we were able to conduct more blast exercises from the NCBI website to find the proteins that most closely relate to this gap. By finding proteins that relate to DNA sequences in Arhodomonas Seminole we can better identify its end function which is the overall goal of this project. Our group identified the protein 2 Keto D Gluconate dehydrogenase another common name gluconate 2-dehydrogengenase and it acts on the CH-OH group of donors. Facts like these can help us identify, can this bacterium be helpful to humans in some way? Is it potentially harmful? Arhodomonas Seminole was found near oil drilling sites and the initial function was thought to be this bacterium could eat oil remnants out of the soil, a toxin that is not properly removed any other way. If when the genomic sequencing is done and it proves this could be true of Arhodomonas Seminole, scientists are then presented with what kinds of soil can it thrive in and what levels will prove too toxic? The work is just never finished.

REFERENCES

•1."Basic Local Alignment Search Tool." Blastx: Search Protein Databases Using a Translated Nucleotide Query. N.p., n.d. Web. 11 Nov. 2014.

•2."American Society for MicrobiologyApplied and Environmental Microbiology." Isolation of a Novel Arhodomonas Sp. Strain Seminole and Its Genetic Potential to Degrade Aromatic Compounds at High Salinity. N.p., n.d. Web. 11 Nov. 2014.