Sequencing Arhodomonas sp. seminole: An Oil Well's Maid

Group 20
Section 702

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ABSTRACT

In this project, our goal was to sequence the gap between two contigs of Arhodoonas sp. Seiminolen DNA, an bacterium found in the salty, oil saturated environments of well sites and use PCR to determine if the hypothesized protein fit the gap. After sequencing the DNA, we noted the gap between contigs was small and the protein extended over the hypothesized gap. We designed primes for the sequence. PCR was our next step, in which we put our primers to the test. The PCR was run several times, but came back negative under normal biological conditions. Another test suggested a better protein for the gap would be a formate transporter.

INTRODUCTION

In our research, we will study an aerobic, halophilic bacterium enriched from salty, crude-oil-impacted soil in Seminole Co, OK known as Arhodomonas sp. Seminole. The Arh. sp. Seminole needs salt to grow and it grows on many different carbon sources including lactate. When it grows, it can metabolize hazardous acids and other compounds such as benzene, phenol, and toluene. In order to fix this problem, we must first know the complete genomic sequence of the Arh. sp. Seminole. When the bacteria was first sequenced, it was found to have 750 contigs instead of just one contiguous piece of DNA. We must now DNA sequence one single gap of the bacteria to bring two contigs together and determine what belongs in the gap in order to get closer to determining what the contiguous piece of DNA looks like. We will do this by designing PCR primers and conducting a Polymerase Chain Reaction (PCR) to "photocopy" the selected portions of DNA into millions of strands and then we will examine the DNA using DNA Gel Electrophoresis. If the DNA Gel Electrophoresis produces a product, we can determine that the two contigs do in fact come together and we can determine what is in the gap.

MATERIALS AND METHODS

70 microliters of dH20
10 microliters of 10X Taq buffer
10 microliters of 10X dNTP's
5 microliters of Arh. Sp. Seminole gDNA
2 microliters of F-primer
2 microliters of R-primer
1 microliter of Tap polymerase.

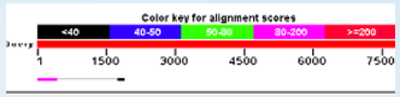
We mixed these together in an eppendorph tube using a micropipette. The PCR then went through the thermo cycler to determine the missing gene.

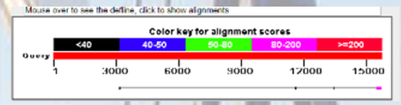
The 100 µl PCR mix contained, ~50 ng of DNA, 0.6 µM of each primer, 200 µM of PCR nucleotide mix (Fisher Bioreagents, Fisher Scientific, Pittsburgh, PA), 1.75 mM MgCl2, 2.5 units Taq polymerase in Buffer A (M1865, Promega Chemicals, Madison, WI).
The amplification was performed with the program which consists of an initial denaturation at 94° C for 30 sec, 30 cycles of denaturation at 94° C for 30 sec, annealing at (51° C, 53° C, 54° C or 55° C) for 30 sec, extension at 72° C for 1 min another 10 min at 72° C for the final extension.

The website www.ncbi.nlm./Blast.cgi were used to sequence the DNA and analyze the hypothesized protein that fit the gap.

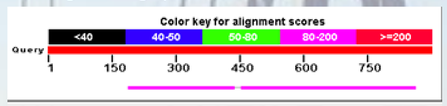
RESULTS

We started out with two contigs that we analyzed on www.ncbi.nlm./Blast.cgi contained 7,780 nucleotides for the first and 15,642 nucleotides for the second. The related protein contained 289 nucleotides. Contig 497 has 51% identity match, at the very beginning. The amino acid alignment begins at 37 and ends at 286, so it fits well. Contig 497 has 51% identity match, at the very beginning. The amino acid alignment begins at 37 and ends at 286, so it fits well. Contig 604 has much less alignment, with many broken parts along but no continuous matches. Part of the contig aligned as much as 75%, but only at a length of 23. This match is toward the tail of the amino acid.





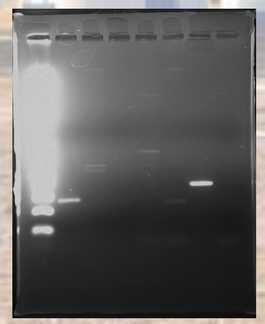
The fused contig we were given was 9 nucleotides long. The gap was small, located in the middle



We then designed primers from the contig sequences given.

Forward Primers: GGAGCTGTTCACGACCAATAA Reverse Primers: AAGCCGCACAAGGAAGATAG

We added our primers and other materials to synthesize the DNA. After this process, we put our PCR product and dye into Agarose gel to run electrophoresis.



Under the conditions we were originally given of 94 degrees for 30 seconds, the PCR failed. While we were unable to match the protein to the DNA, under different conditions, 51, 53, 54, and 55 degrees for 30 seconds, later performed the PCR was able to work.

We ran a final test on www.ncbi.nlm/Blast.cgi to find out more about The hypothesized protein. Our results, listed at the right, show that the protein did not fit as we thought it did.

DISCUSSION

Although the gap between the contigs was small, the hypothesized protein did not fuse them together. Our hypothesis was not proven by this experiment; it could be due to a wealth of factors. The PCR method of sequencing is proven to be valid, but finicky and troublesome. The allotted amount of time given for this project in the classroom proved not optimal for using PCR. The results suggested that more PCR tests could provide better data for our hypothesis. When tested under less common temperature settings, the PCR seemed to work. These different temperatures are not conducive to real biological processes, so it whether the alternate PCR findings are realistic or fictitious is unknown without further research. The final analysis about the protein suggested that a formate-nitrite transporter would fit the gap better than the protein we had. We used www.wikipedia.com to asses the function of the new protein and the types of bacterium it is found in.

REFERENCES

•www.ncbi.nlm./Blast.cgi
•www.wikipedia.com
•Dr. Patricia Canaan, D2L website powerpoints