PCR and DNA Sequencing of DNA Gap in Arhodomonas sp. Seminole

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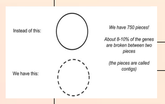
Group 13
Section 001

ABSTRACT

Arhodomonas Seminole is an aerobic, halophilic bacterium enriched from salty, crude-oil-impacted soil and has been discovered in Seminole Co, Oklahoma. About 8-10% of the genes within the genetic sequence of Arhotomonas Seminole are broken between two pieces, totaling about 750 separate contigs. Our goal was to generate DNA sequence data for a gap in a particular region in order to bring 2 larger pieces together. We needed to predict adjacent contigs based on what is known in a related species, and we were going to prove this using processes such as PCR and DNA sequencing. In order to complete the PCR reaction, we required forward and reverse primers. We obtained these by using fused contig sequences that we inputted into a blastX website, which then outputted the primer sequences. Once we had these primers we were able to complete our PCR reaction and proceed to a process called Agarose gel electrophoresis. This process allowed us to visualize our results and determine the DNA fingerprint, which was revealed in the pattern of the PCR product size. This allowed us to determine that the protein that connected our upstream and downstream contigs was Thioalkalivibrio sp. ALJ17, which confirmed that the two contigs were in fact adjacent.

INTRODUCTION

Arohdomonas Seminole, halophilic bacterium enriched from salty, crude oil impacted soil was found in Seminole County, Oklahoma. It requires salt and grows on a wide variety of carbon sources (Canaan, 2014). After extracting the DNA from the genome of the bacteria Arohodomonas Seminole, it was left in 750 pieces. About 8-10% of the genes are broken between two pieces, and these broken pieces are known as contigs. We needed to map the genome using PCR, Polymerase Chain Reaction, to identify the gaps in the genetic information. Our mission was to determine what was missing in the DNA, generate a novel sequence of the gap, and put the two together. Ultimately, planning to "fill in the blanks." We hypothesize that the end of Contig 637 is the head end that matches to the tail of Contig 375. After PCR, we will examine the copied DNA by gel electrophoresis to see if we had a successful product.



MATERIALS AND METHODS

Materials:

Micropipette
DNA
PrimerQuest
BlastX

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 53˚ C for 30 sec, extension at 72˚ C for 1 min another 10 min at 72˚ C for the final extension.

Methods:

Analyze the positives and negatives of our experimental design with the contigs that we received from Dr. Canaan. We used the BlastX to run our contigs, Contig 637 and Contig 375, to test if there was an alignment or match. We found that there was indeed an alignment in our contigs, so we ran them through Primer Quest to find their forward and reverse primers. These primers were then combined with template DNA, DNA nucleotide building blocks, and a buffer. After combining these components in a small test tube, the tube was placed in a thermocycler to synthesize the DNA copies. Then we placed our copied DNA into electrophoresis gel to run a PCR test on the contigs. A picture of the gel results can be found under the "Results" portion of this poster. These results from the PCR were then used through BlastX to find the protein Thioalkalivibrio.

RESULTS

The results from the PCR did in fact produce a visible product. It is shown in the image below, circled in red. Through these results, using blastx, we determined that the missing gap in the contigs was concurrent with the DNA sequencing for the protein Thioalkalivibrio sp. ALJ17.

Genome sequencing of haloalkaliphilic and chemolithoautotrophic sulfur-oxidizing bacteria from the genus Thioalkalivibrio. These bacteria, that are isolated from soda lakes, can thrive at pH 10 and salt concentrations up to 4 M sodium and 3 M potassium. The haloalkaliphilic SOB uses reduced sulfur compounds as an energy source and CO_2 as a carbon source, because of this they play an important role in both the sulfur and carbon cycles. They are used in remediation of sulfur pollution, such as toxic H2S, from wastewater, spent caustics, flue gas, natural gas, and biogas (National Center for Biotechnology Information, 2014).

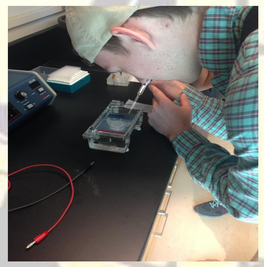


Figure 1: Loading our gels using a micropipette.

Figure 2: Gel electrophoresis image of our PCR product, circled in red.

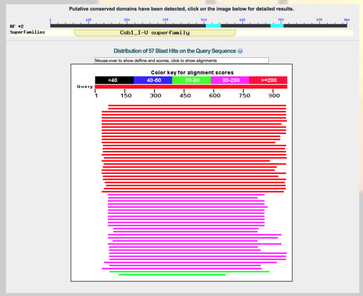


Figure 3: Results from the BlastX of our PCR reaction products

DISCUSSION

The results of this experiment have shown that when we used gel electropherisis to run our contigs it gave us a positive result. Our group got the expected results along with Groups 5 and 19. We found that there was an alignment in our contigs so we ran them through Primer Quest to find their forward and reverse primers. Our outcome was used to run a PCR test on the contigs. A PCR test was ran on our primers. PCR is like DNA "photocopying" where we amplify trace amounts of a DNA piece into millions of copies in order to study it. We used distilled water, 10X taq buffer, polymerse enzymes, and electrophoresis gel. The "purity and yield of the reaction products depend on several parameters, one of which is the annealing temperature (Ta). At both sub- and super-optimal Ta values, non-specific products may be formed, and the yield of products is reduced. Optimizing the Ta is especially critical when long products are synthesized or when total genomic DNA is the substrate for PCR" (Rychlik, W, Spencer W J, Rhoads, R E. 1). We found that after the PCR and genomic sequencing, the CRISPR-associated protein with the highest identity Thioalkalivibrio sp. ALJ17. Similar to Ahrodomonas, Thioalkalivibrio is "a moderately salt-tolerant and obligately alkaliphilic, chemolithoautotrophic sulfur-oxidizing bacterium" (Rychlik, W, Spencer W J, Rhoads, R E 1). Our discovery of this protein aligning our upstream and downstream contigs confirmed our belief that our contigs were in fact adjacent to one another.

Groups 5, 13 and 19:

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 53°C for 30 sec, extension at 72°C for 1 min another 10 min at 72°C for the final extension.

Details to our PCR procedure, including ingredients and temperatures

Results from the BlastX our PCR reaction products

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