ABSTRACT

Arhodomonas Seminole is an aerobic, halophilic bacterium enriched from salty, crude oil impacted soil and 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 bacterium Arhodomonas 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.

INTRODUCTION

Arhodomonas 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 bacterium Arhodomonas 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.”

MATERIALS AND METHODS

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 73, in 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. Our discovery of this protein aligning our upstream and downstream contigs confirmed our belief that our contigs were in fact adjacent to one another.

- The amplification was performed with the program which consists of an initial denaturation at 94°C for 1 min another 10 min at 72°C for the final extension.

DISCUSSION

The results of this experiment have shown that when we used gel electrophoresis to run our contigs it gave us a positive result. Our group got the expected results along with Groups 19 and 17. 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, polymerase 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, Spence, W J, Rhydo, 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(Thioalkalivibrio, Thioalkalivibrio is a "moderately salt-tolerant and obligately alkaliphilic, chemolithoautotrophic sulfur-oxidizing bacterium" (Rychlik, W, Spence, W J, Rhydo, 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.

REFERENCES


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.