Analysis of Glyceraldehyde 3-Phosphate Dehydrogenase gene from Arugula and Sage

Lauren Lee, Steven Bozell and Dr. Muatasem Ubeidat

Southwestern Oklahoma State University, Department of Biological Sciences, Weatherford, Oklahoma

GAPDH and Arugula and Sage

In glycolysis, GAPDH enzyme produces energy and adds a phosphate group to the glyceraldehyde 3-phosphate. Glycolysis is very important as a first step in energy production in the cell. GAPDH is found in plant cells making it a perfect link between the ancestor of all plants and today's modern plants. Our project involves the isolation and sequencing of the gene obtained from Eruca vesicaria sativa (arugula) and in Salvia officinalis (sage). Knowing the sequences and doing comparative analysis on these sequences of such important gene will help us find out the evolutionary significance of this gene.

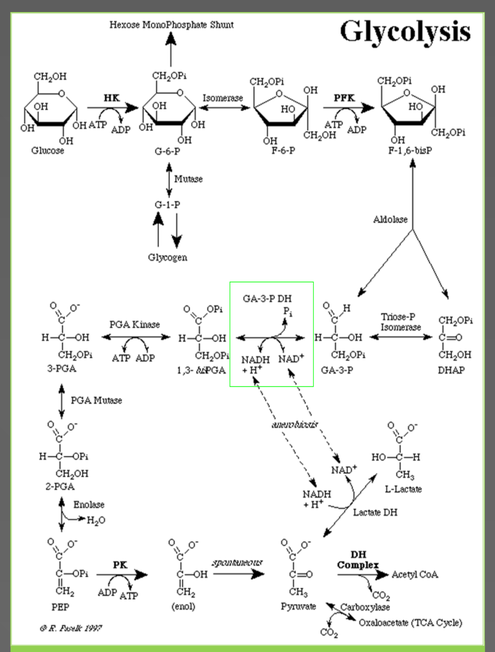

Figure 1: The sixth step in glycolysis using the enzyme GAPDH.

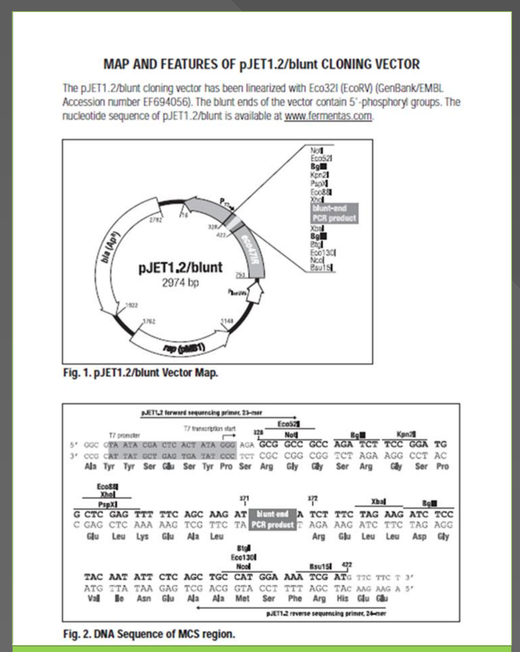

Figure 2: This shows the Vector Map of pJET1.2 which we used with our blunted PCR.

Introduction to Arugula and Sage

Eruca vesicaria sativa, or Arugula, part of the Brassicaceae family or the mustard family is a dicot plant. *Salvia officinalis*, or Sage, part of the Lamiaceae family or the mint family is also dicot plant. Glyceraldehyde 3-phosphate dehydrogenase, is a vital metabolic enzyme that catalyzes the sixth step in glycolysis. GAPDH therefore appears to be a suitable "housekeeping gene" in addition to 25S rRNA as a reference for measuring the relative expression of other genes in sugarcane(Iskandar et al). Because GAPDH is in all plants, it made it a very good gene to study for evolutionary purposes. Because of the known success in research on GAPDH, learning all of the lab techniques necessary for working in a biochemistry and molecular biology based lab was made easier.

Results and Discussion

We lysed the cells, using a lysis buffer, to isolate genomic DNA from Arugula and Sage. Then, using a microcentrifuge to spin down the remaining cell debris, we collected the supernatant and ran an initial PCR, using the program GADPH1, containing degenerate primers, to amplify the gene coding for the enzyme, GAPDH. After the first run of PCR, and checking the amount of DNA in a 1% agarose gel(Fig. 4), we did a second round of PCR, using the program GADPH2, with nested primers. The nested primers were used so that we were sure the only thing being amplified was the target section of DNA. The next step was to purify the PCR product to get rid of all the excess using a BIO RAD DNA purification kit. We then checked our remaining product for DNA with a 1% agarose gel(Fig. 5). After purifying the DNA, we did blunting and ligation into pJET1.2(Fig. 2.), a cloning vector, to prepare for transformation into *E. coli* cells. We transformed the cells by heat shock. After the cells had been transformed we grew cultures from the colonies we grew with the isolated DNA from Arugula and Sage. We then did a Mini/plasmid prep using BIO RAD purification kits as described. We then did restriction digestion with the Bgl II enzyme overnight and tested the final DNA with a 1% agarose gel(Fig. 6.).

Objective

Our objective was to compare genomics with Arugula and Sage. We focused on the GAPDH gene and amplifying it, looking for either the similarities or differences between the band sizes of the DNA isolated from Arugula and Sage. We also gained knowledge and skills in biotechnology and biochemistry.

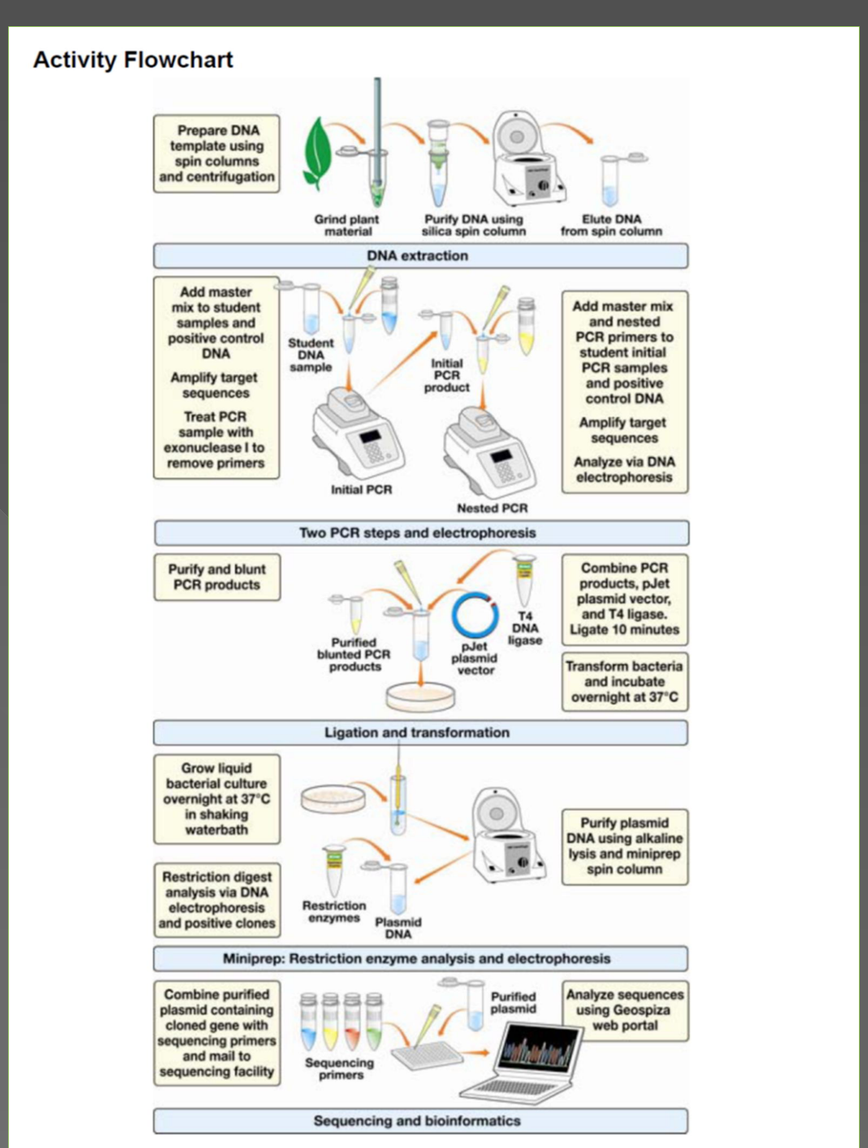

Figure 3: Materials and Methods. This flow chart shows a step by step explanation of how and what we did. In Restriction Digestion we specifically used Bgl II restriction enzyme.

Conclusion

We isolated genomic DNA in order to amplify GAPDH. The results show that Arugula and Sage have different band sizes which points towards the differences in evolution of GAPDH. To further support our indicated results, we are going to sequence our DNA so we can visually see how different they are, based not just on band sizes.

References

Iskandar, H., Simpson, R., Casu, R., Bonnett, G., Maclean, D., and Manners, J.(December 2004). Comparison of reference genes for quantitative real-time polymerase chain reaction analysis of gene expression in sugarcane. PLANT MOLECULAR BIOLOGY Reporter 22(4): 325-337

Results

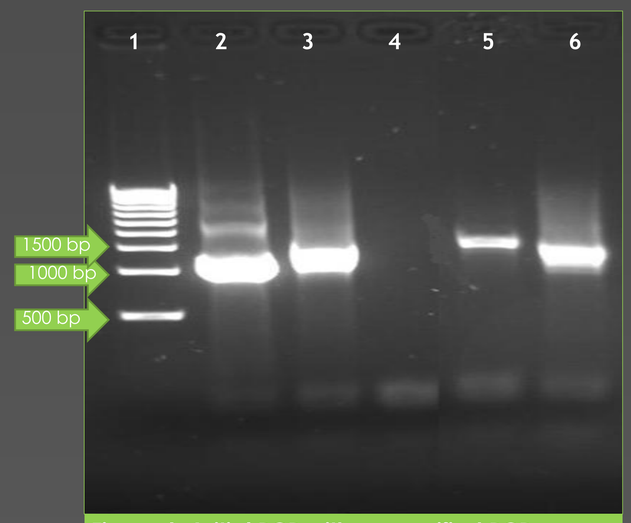

Figure 4: Initial PCR with unspecified PCR primers. Lane 1: 500 bp marker DNA. Lane 2: pGAP control for PCR. Lane 3: Arabidopsis gDNA. Lane 4: Sterile Water. Lane 5: Sage gDNA. Lane 6: Arugula gDNA.

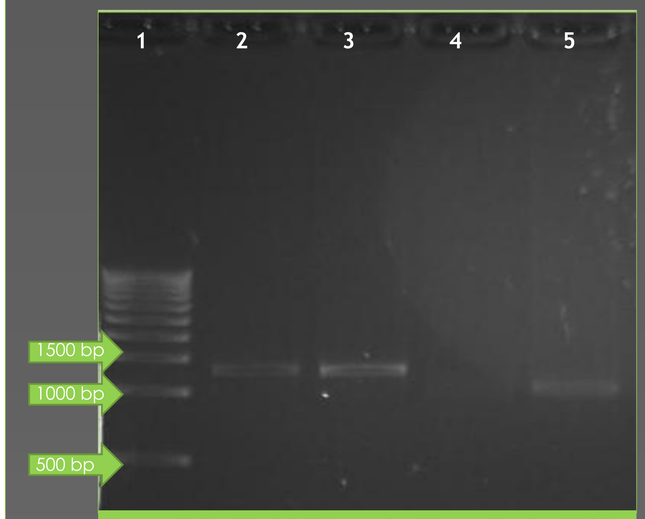

Figure 5: Purified DNA product from Nested PCR. Lane 1: 500 bp Marker DNA. Lane 2: 1 µL Sage DNA. Lane 3: 2 µL Sage DNA. Lane 4: 1 µL Arugula DNA. Lane 5: 2 µL Arugula DNA.

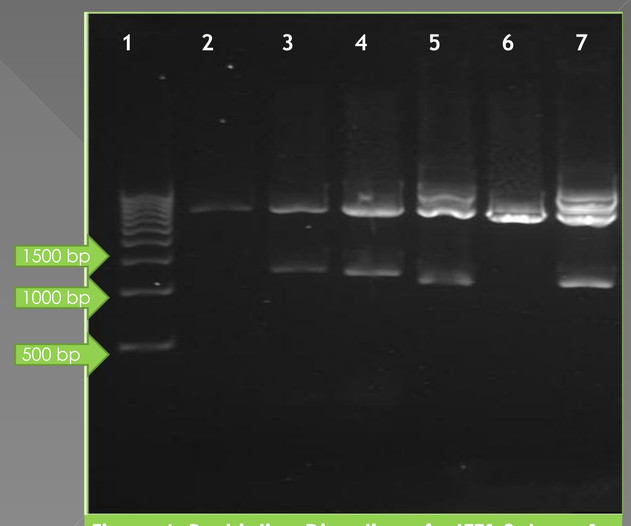

Figure 6: Restriction Digestion of pJET1.2. Lane 1: 500 bp Marker DNA. Lane 2: Sage colony 1. Lane 3: Sage colony 2. Lane 4: Sage colony 3. Lane 5: Arugula colony 1. Lane 6: Arugula colony 2. Lane 7: Arugula colony 3.

Research Opportunities for Biology, Chemistry, and Health Sciences Undergraduate students are available Muatasem Ubeidat's Lab.

If you want more information, come by Dr. Ubeidat's office (SCI 213), call 580-774-3298 or email: muatasem.ubeidat@swosu.edu