Sequencing of GAPDH Gene in *Coriandrum sativum* (Cilantro)

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Abstract

Coriandrum sativum (Cilantro) is a common herb used in cooking, spice, and in nutritional cleansing. According to the Global Healing Center, cilantro has good nutritional benefits and is a powerful cleansing agent that can target toxic metals in the body. Cilantro's prominent use in food and nutritional benefits led us to sequence the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. The GAPDH gene was chosen as the gene of interest because it is important in glycolysis and therefore found in most organisms. The purpose of this semester long project was to sequence the GAPDH gene of *Coriandrum sativum*. This was accomplished using laboratory techniques including DNA extraction, initial PCR, nested PCR, gel electrophoresis, purification, bacterial transformation, and sequence analysis. We were able to sequence the portion of the GAPDH gene using the methods listed above.

Introduction

The GAPC gene was studied in *Coriandrum sativum* (cilantro). The GAPC gene was studied because it is a gene that is generally highly conserved in most organisms. The GAPC gene encodes for an essential protein need in glycolysis, glyceraldehyde-3-phosphate dehydrogenase. The enzyme, oxidoreductase catalyzes glyceraldehyde-3-phosphate by oxidizing the aldehyde groups of the triode sugars and inorganic phosphate is added to form 1,3-bisphosphoglycerate (1,3BPG), in order to eventually turn glucose into pyruvate. After glycolysis, pyruvate can enter the Krebs cycle in order to produce energy. The goal for this project was to clone the GAPC gene of *Coriandrum sarivum*.

Figure 5: Leaves of Cilantro, *Cariandrum sativum*.
Source: http://www.foodpoisonjournal.com/foodborne-illness-outbreaks/cilantro-from-mexico-sickens-hundreds-in-us-with-cyclospora/#.WO-7vvnyvIU

Materials & Methods

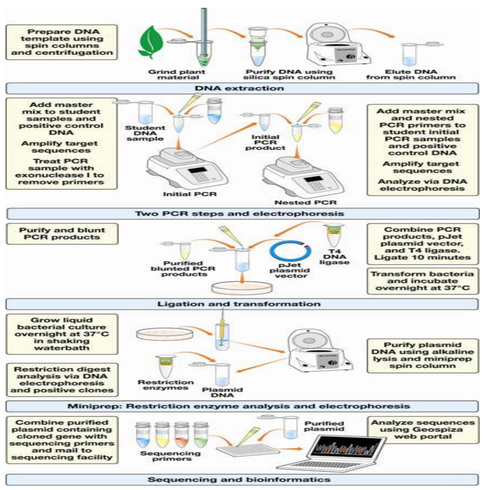

Figure 6: Procedures for DNA extraction, both PCR steps and electrophoresis, ligation and transformation, miniprep: restriction enzyme analysis and electrophoresis, and sequencing of bioinformatics.
Source: BioEdit: Hall, T. A. 1999 . BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT . Nucleic Acids Symposium Series 41 : 95 – 98. CAP3: Huang, X., & Madan, A. (1999). CAP3: A DNA sequence assembly program. Genome research, 9(9), 868-877

Degenerate and nested polymerase chain reaction (PCR) techniques were used in order to amplify the GAPC gene for *Coriandrum sativum*. Nested PCR is required due to the possibility that degenerate primers produced a non-target amplification of a region, which then could be seen on the gel using electrophoresis. After performing nested PCR, and electrophoresis; the products were cloned, sequenced, and analyzed in order to determine the presence of the GAPC gene.

Results

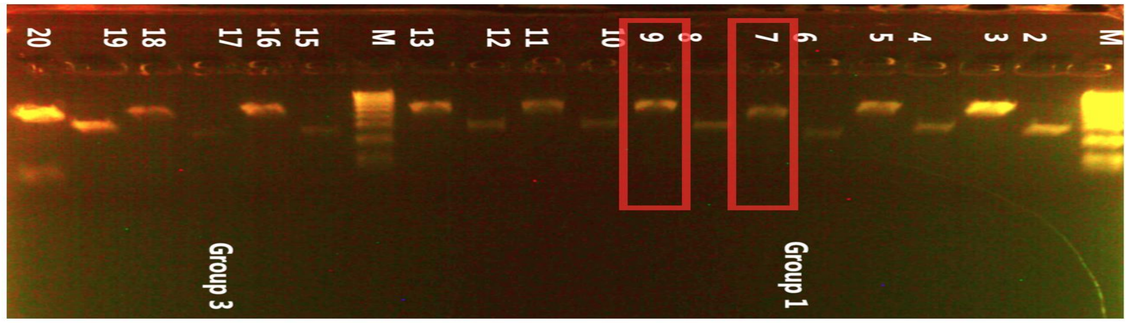

Figure 1: Initial and purified PCR product. Nested PCR increased the specificity of DNA amplification. Lanes 9 (nested) and 7 (initial) show cilantro PCR product. GAPC gene was isolated from the initial PCR (lane 7) and purified for insertion into pJet 1.2 vector.

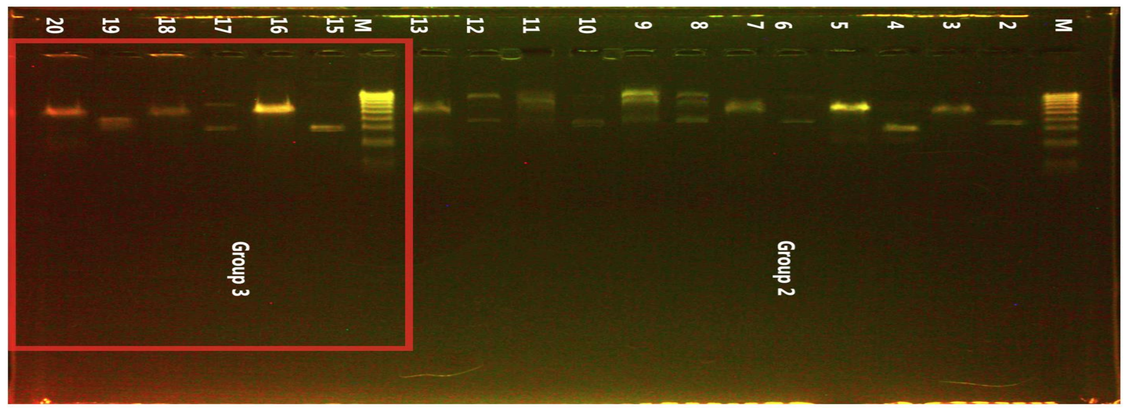

Figure 2: Verification of Plasmid Miniprep. Restriction enzyme digestion was used to verify that the correct insert was taken. Comparison of digested (lanes 16, 18, and 20) and undigested (lanes 15, 17, and 19) showed bands at ~1000 bp (insert) and 3000 bp (plasmid).

Figure 3: *E. Coli* bacteria plates showing growth of bacteria producing cilantro DNA.

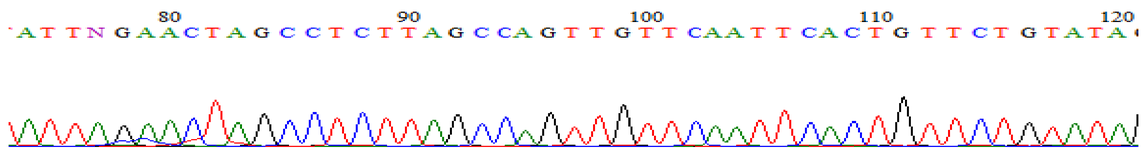

Figure 4: Chromatogram results of cilantro. Each peak and color represents a signal from an individual nucleotide base identified above the peak.

We sent and received a total of sixteen cilantro sequences using forward and reverse primers (JF, JR, GF, GR) from the Eurofins sequencing company. Upon editing we found that five of the sequences were adequate for further examination. Then we eliminated one due to colony origination, providing us with a depth of coverage of one since all the sequences used were from the same colony. With the final four we created a contig sequence and analyzed that with the bioinformatics program, NCBI. Using the information gathered, we cut the exons from the sequences and performed another round of bioinformatics.

Blast Results

DNA Sequence	*Brassica napus* cultivar ZS11 chromosome A5, *Brassica napus* assembly_v1.0, whole genome shotgun sequence
mRNA	*Daucus carota subsp. sativus* glyceraldehyde-3-phosphate dehydrogenase
mRNA (Exons only)	*Camelina sativa* glyceraldehyde-3-phosphate dehydrogenase GAPC2
Protein Sequence	Glyceraldehyde 3-phosphate dehydrogenase, catalytic domain-containing protein

Table 1: Blast results indicated that our predicted GAPC gene is *Daucus carota* subsp. sativus glyceraldehyde-3-phosphate dehydrogenase, cytosolic.

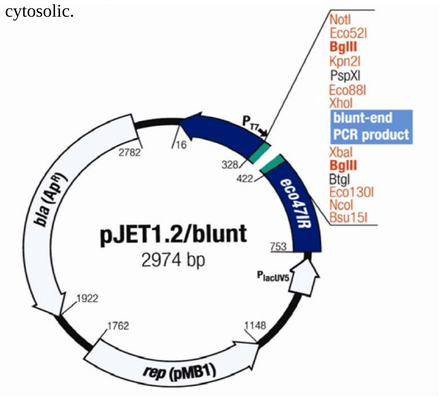

Figure 8: Amplified DNA inserted into the plasmid vector. Successful results were analyzed via gel electrophoresis.

Conclusions and Discussion

The insertion of the GAPC gene from cilantro into the pJET plasmid was successful and E. coli successfully expressed the cilantro DNA. The use of easily accessible bioinformatics tools allowed for sequencing and verification of the portion of the GAPDH gene.

We were able to obtain a short portion of the GAPC-2 gene that was 128 bases in length. The small size of this gene portion is due to the low quality of sequences we obtained. As a result we were not able to increase the depth of coverage. Only 4 sequence fragments were used to obtain the contig sequence. Nevertheless the portion of the GAPC-2 gene we obtained is a unique sequence which had not yet been deposited to the GenBank. Further studies can focus on increasing the depth of coverage which will allow researchers to actual be able to deposit this unique sequence into GenBank and use it in different genetic studies.

Acknowledgments

Presented at Grand View Scholarship Symposium Day 2017.
Grand View University, Department of Biology for supporting funds.
Jennifer Donnelly, lab coordinator at Grand View University for setting up the lab.