Sequencing the GAPDH Gene of Rosmarinus officinalis

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Abstract

The enzyme glyceraldehyde-3-phosphaste dehydrogenase (GAPDH) is an important enzyme found in plants that is crucial to glycolysis and is a housekeeping gene. Since it is found in abundance in plant cells, we extracted DNA from the rosemary plant, *Rosmarinus officinalis.* After DNA extraction, we amplified the GAPDH gene through PCR and verified the presence of the gene through gel electrophoresis. *Escherichia coli* (*E.coli)* bacteria was then transformed with the plasmid so that we could extract the plasmid containing the gene and analyze it via restriction enzymes and electrophoresis. After the plasmids were sequenced, we found that we had successfully cloned the portion of the GAPDH gene from rosemary into our plasmid. We are currently considering depositing the obtained sequence into the GenBank. This information could be useful to other scientists who are working with the GAPDH gene or *Rosmarinus officinalis.*

Introduction

As mentioned before, GAPDH plays a crucial role in glycolosis. Glycolosis is the breakdown of glucose into two pyruvate molecules and two ATP. GAPDH catalyzes the sixth step of glycolysis. Since GAPDH is constitutively expressed, much is known about the structure and function of the enzyme. GAPDH has been sequenced in various plants and the GAPDH sequence from *Rosmarinus officinalis* will expand the NCBI database.

Results

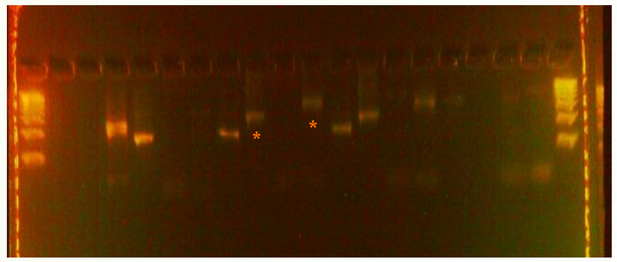

Fig. 1: Gel electrophoresis product of initial PCR and nested PCR. Rosemary samples are indicated as "*".

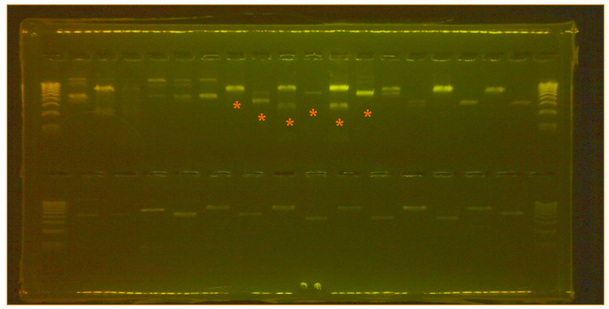

Fig. 2: Gel electrophoresis product of plasmid vector digested by BglII or undigested. Rosemary samples are indicated as "*".

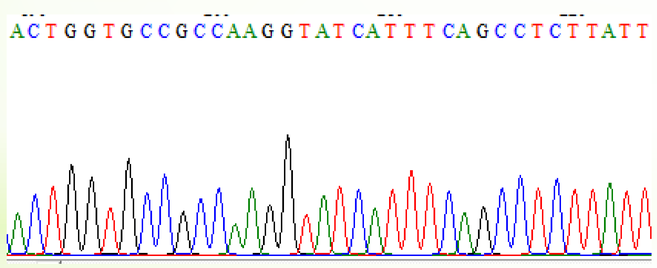

Fig. 3: Sample of sequence chromatogram of *Rosmarinus officinalis*.

TGGTGTCTTCACTGACAAAGACAAGGCTGCTGCTCACTTGAAGGA
GGGTGGTGCTAAAAAGGTTGTCATCTCTGCCCCAAGCAAAGATGCG
CCCATGTTCGTTGTTGGTGTCAACGAGCACGAGTACAAGTCTGACC
TTGACATTGTTTCCAACGCTAGTTGCACCACTAACTGCCTTGCTCCT
CTTGCCAAGGTAGGTTATTAATGACAGGTTTGGCATTGTTGAGGGA
CTCATGACCACTGTCCACTCTATCACTGGTACCTACTCAGAAGACAG
TTGATGGTCCATCAATGAAGGACTGGAGAGGTGGAAGGGCTGCTT
CCTTCAACGTTATTCCTAGCAGCACTGGTGCCGCCAAGGAGGCTGT
TGGGAAAGTGTTGCCATCCCTCAATGGAAAATTGACCGGAATGTCT
TTCCGTGTTCCAACCGTTGATGTCTCAGTTGTGATCTCACCGTTAGA
CTTGAGAAAGCTGCAACATACGACGAAATCAAGAAGGCCATCAAG
TGAGAACCATCAAGTCAGGGAGGAATCTGAAGGCAAAATGAAGG
GAATTTTGGGATACACTGAGGATGATGTTGTGTCTACCGACTTTGTT
GGTGACAACAGGTAGGTCAAGCATCTTTGATGCCAAGGCTG

Fig. 4: Final exon-only sequence for *Rosmarinus officinalis.*

The first round of PCR performed indicated that we had successfully amplified the target sequence around the GAPDH gene by using degenerative primers (Fig. 1). The initial PCR fragments can be identified by their higher position in the gel. The nested PCR fragments can be seen slightly below the initial fragments. The nested PCR fragments are slightly smaller than the initial fragments because the nested primers used were more specific to the gene within the initial fragments. After inserting the gene into the plasmid vector, the restriction enzyme BglII was used to determine the success of ligation. The presence of 2 bands represent the gene and the plasmid vector (Fig. 2). After the samples were sent for sequencing, we received a sequencing chromatogram (Fig. 3). From the chromatograms, we found 6 sequences that were clear enough to be used to help construct the contig sequence. Of the 6 sequences, only 5 were used due to their similarity to each other. Since all of the fragments used were from one bacterial colony, our depth of coverage was one. After using the BLAST system on NCBI, it was found that the nucleotide sequence was similar to that of *Arabadopsis thaliana;* however, the protein sequence was similar to *Sesasum indicum.* Using the BLAST system allowed us to create a final exon-only sequence for the GAPDH gene in *Rosmarinus officinalis* (Fig.4).

Materials and Methods

In order to extract DNA from rosemary leaves, we first cut the leaves into small pieces. The pieces were then placed into a micropestle and ground into lysis buffer. The DNA was then purified using a spin column. Once purified, the DNA was eluted from the column. After the DNA was extracted, the desired sequence was amplified using PCR. We then did a round of nested PCR to further amplify the desired sequence. The products were analyzed using DNA gel electrophoresis. The target sequence was then ligated into a plasmid vector that was transformed into *E. coli*. The transformed bacteria was then grown on LB amp plates. Any successful colonies had successfully been transformed. Restriction enzymes were used to verify that the bacteria had been transformed with the plasmid vector and were not spontaneous mutants. The plasmid DNA was then purified by separating it from the bacterial DNA. The successful samples were then placed in a 96-well plate and sent to be sequenced. Once the sequences were returned, we analyzed the sequences using BioEdit (Hall 1999), CAP3 (Huang and Madan 1999), and NCBI. On NCBI, we utilized the nucleotide BLAST to determine the mRNA sequence. Introns were identified and the exons were used to create a protein sequence.

Discussion

The sequencing of the GAPDH was successful because the nucleotide and protein BLAST sequences showed similar plant sequences. Since the sequence was similar to other GAPDH genes, we were able to obtain an exon-only sequence for *Rosmarinus officianlis.* The sequence is important because it helps better understand the genes involved in glycolysis. The sequenced gene is also able to aid evolutionary biologists in studying the genetic diversities between GAPDH genes in plants. The sequence will be added to the NCBI database and potentially could be useful for scientists researching the plant in future studies.

References

Huang, X., & Madan, A. (1999). CAP3: A DNA sequence assembly program. Genome research, 9(9), 868-877.
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 .