Sequence Determination and Analysis of Parsley GAPDH and Comparison to Arabidoposis GAPDH

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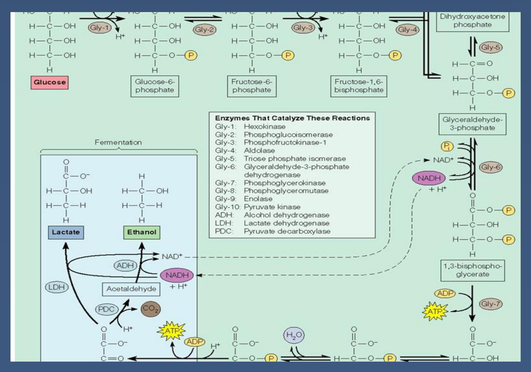
Introduction

Comparing gene sequences can be a very useful tool in discovering the evolutionary similarities and differences across species. We isolated genomic DNA from Parsley and compared the band sizes to those of Arabidopsis.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key enzyme in glycolysis and gluconeogenesis. GAPDH converts glyceraldehyde -3-phosphate into 1,3-bisphosphateglycerate in glycolysis and the reverse reaction in anabolic reaction. (Kubo, 2011) (Figure 1)



(Figure 1: GAPDH reversibly catalyses the 6th step of glycolysis)

Parsley and Arabidopsis

Petroselinum crispum common name Parsley is grass like plant with flat divided leaves. Anyone who eats in a restaurant can easily identify it. It is a good natural source of carotene. It provides an attractive garnish for spicy dishes and it is often used when preparing meat and fish. Parley is found just about everywhere but it is widely cultivated in California, Germany, and France. (Figure 2)

Figure 2: This is a picture of parsley

Arabidopsis thaliana common name thale cress is a small flowering plant that is widely used as a model organism in genetic research because it has one of the smallest genomes in the plant kingdom: 115,409,949 base pairs. Most of its DNA encodes its 25498 genes, and extensive genetic and physical maps of all its 5 chromosomes are available. (Figure 3)

Figure 3: This is a picture of *Arabidopsis*

Objectives

Our primary aim was to develop skills in biotechnology using biotechnological techniques to isolate genomic DNA from Parsley and to compare their band sizes to the already known band sizes of Arabidopsis. Also the detection of differential sequence of the gene can provide great leads in addition of it long established metabolic functions and its implication towards non metabolic process

Materials and Method

DNA Extraction

We used a mechanical and chemical method to extract DNA from Parsley. We ground the young leaves to break open the cells and we added lysis solution to break apart the cellular membrane. Lysis buffer contain a restriction enzyme which cut the target DNA into fragments. We followed the steps as described in the Nucleic acid extract kid.

PCR Reaction

PCR -Polymerase chain reaction -is a scientific technique use to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions copies of a particular DNA sequence. We ran the initial PCR using degenerate primers to amply our target DNA fragments.

Nested PCR

Nested polymerase chain reaction is a modification of polymerase chain reaction intended to reduce contamination due to the amplification of unexpected primer binding sites. We ran nested PCR using nested primers as described in BIO RAD DNA purification kit

PCR Purification

We purified to remove the excess primers, nucleotides, and DNA polymerase . (Figure 4)

Blunting

This restriction enzymes used during extraction produced staggered cuts in our target fragments with protruding ends. Since a different restriction enzymes was used to cut the Plasmid vector, our target DNA fragments 3'- and 5'-protruding ends needed to be blunt in order to be properly attached to the Plasmid vector during ligation.

(Figure 5: Blunting reaction)

Ligation and Transformation

Ligation into Pjet 1.2

We incorporated our DNA fragments into plasmids following the steps as described in the ligation and Transformation instruction manual. Next we incorporated the plasmid into E-coli cells a process called transformation by heat shock. These made the cells more competent with more easily altered cell walls to allow DNA to easily pass through. We placed the E -Coli containing the plasmid vectors in a culture medium where they multiply exponentially copying the DNA vector placed inside them - a process called cloning.

Next we Mini prepped the E-coli cells following the procedure is use to extract our target plasmid DNA from bacteria cells. We did restriction digestion with BgII in order to cut out target fragments and let it stayed overnight

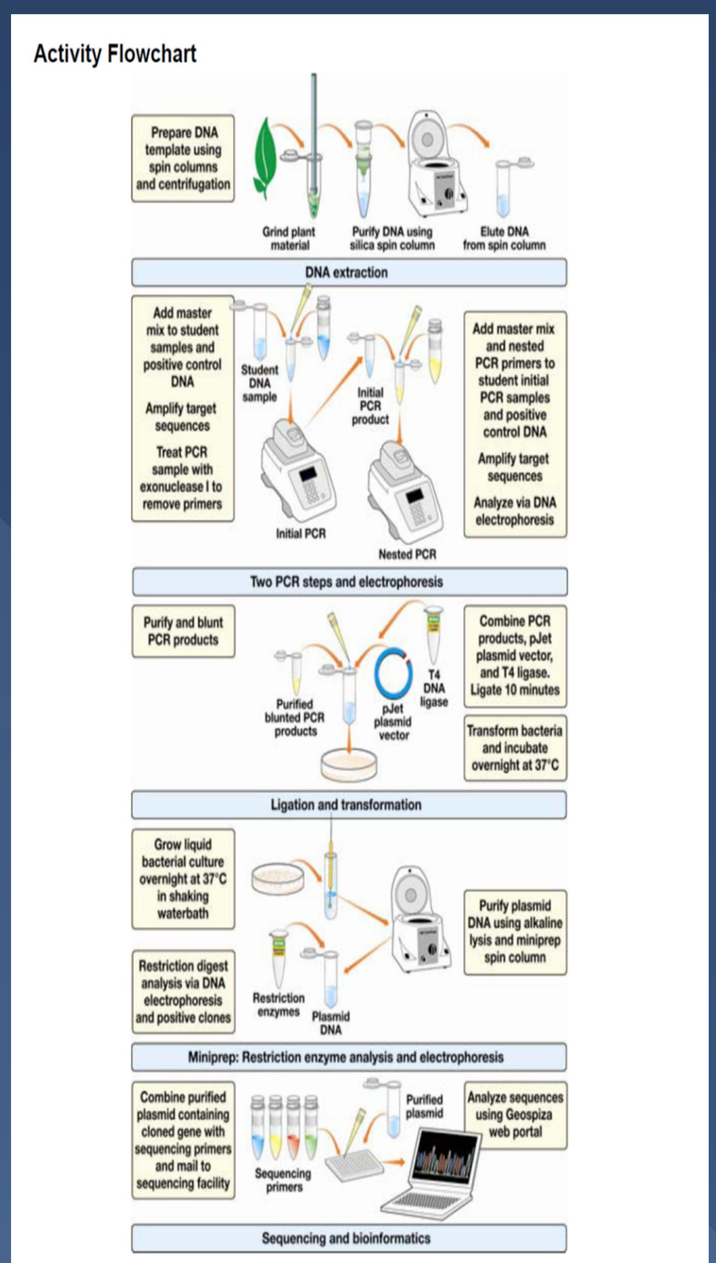


Figure 4: The procedure we used roughly follows the outline of this figure.

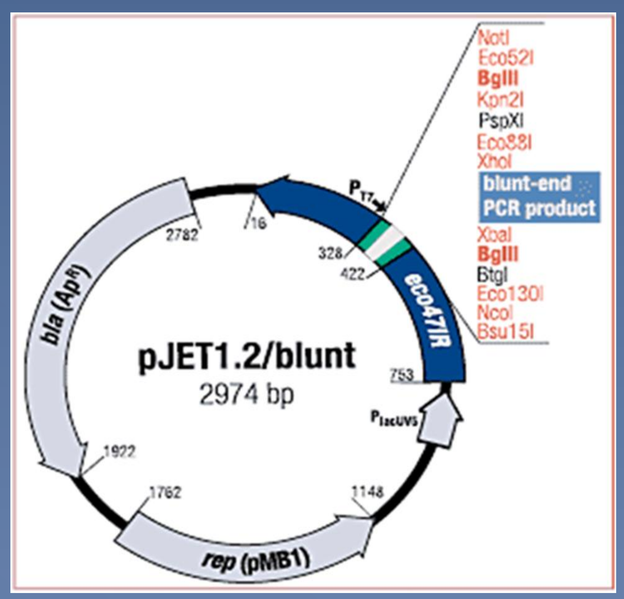
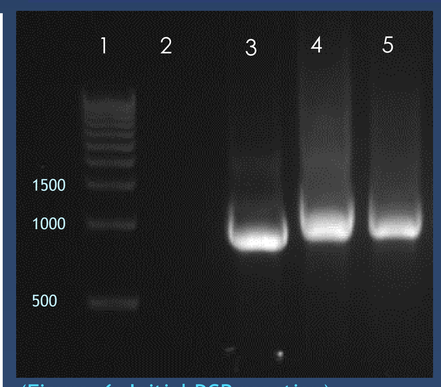


Figure 5: Blunting reaction

Result



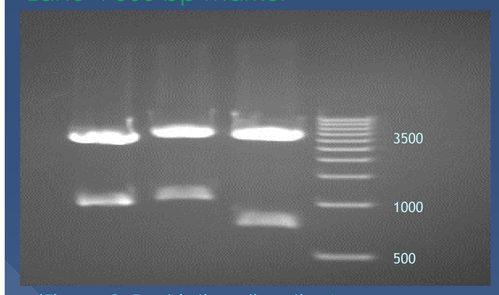
(Figure 6: Initial PCR reaction)

Lane I - 1.5 bp maker, Lane 2- pGap control for PCR

Lane 3- Arabidopsis, Lane -4 Sterile H2O, Lane 5 - Parsley

(Figure 7: Nested PCR purification)

Lane 1-1uL Parsley DNA, Lane 2 – 2uL Parsley DNA, Lane 3-3uL Parsley DNA, Lane 4 500 bp marker



(Figure 8: Restriction digestion)

Lane 1 – Parsley colony, lane 2 – Parsley colony, lane 3 – Parsley colony, lane 4 500 bp maker

We used electrophoresis to separate the DNA fragments into bands of different sizes. Since the fragments are one nucleotide different in size the shorter DNA bands moved faster compared to longer bands. We used DNA makers of known sizes bands traveling once electrophoresis was completed. We compared the band sizes of Parsley to the known band sizes of *arabidopsis* and the band sizes were very similar

After the initial PCR we completed the initial PCR , we ran electrophoresis to confirm that DNA was extracted from Parsley and also to compared the band sizes as shown in figure 6 above. Figure 7: shows the result after we completed nested PCR purification.

Figure 8: shows that we were able to extract our target DNA from the bacteria after restriction digestion

Conclusion

We extracted, amplified , and cloned genomic DNA from Parsley. After analyzing and comparing the band size of Parsley to those of *Arabidopsis*, we realized that they were very similar just as we had predicted. We concluded that GAPDH genes are conserve between *Petroselinum crispum* and *Arabidopsis*.

We are now waiting for sequencing of the Parsley DNA to be completed so that we can further analyze and compare their amino acid regions to those of *Arabidopsis*. We will also indentify their introns and exons

References

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