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Cloning and Sequencing the GAPC Gene of Delospermia cooperi

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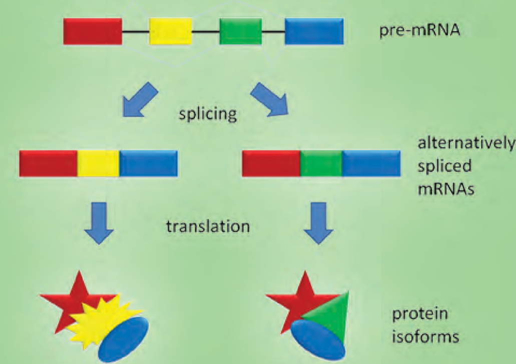
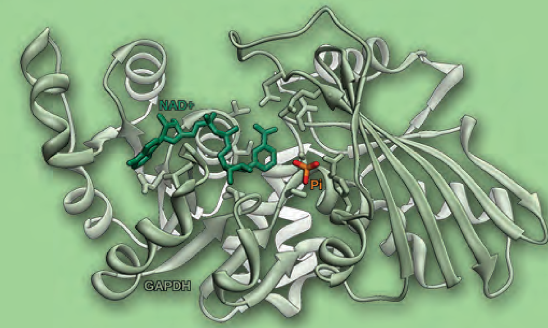
Background



Over the past 20 years there has been a deep desire among scientists to understand the role DNA plays as the "blueprint of life" and how it can be compared between organisms.



As more advanced technology and protocols were developed, scientists have been able to gather enough information about the genes of an organism to pioneer a whole new field of systematics; molecular phylogeny.

The GAPC gene has been a standard used in this field of science because of its relative ease of isolating and sequencing.

Delosperma cooperi, a recently diverged species of succulent plant native to South Africa, has yet to be fully understood. Using modern DNA replication and sequencing techniques we are seeking to compare the GAPC of this species with other plants of the same family.

Goals

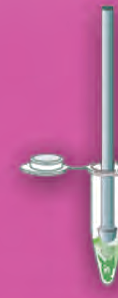
Learn and understand the methods and procedures of DNA cloning and gene sequencing

Accurately sequence the Delosperma cooperi GAPC gene

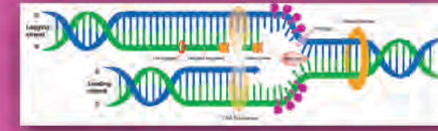
Submit results to GeneBank so other scientists have access to our results

Methods

Isolate DNA from source



Amplify gene with initial and nested Primers in PCR

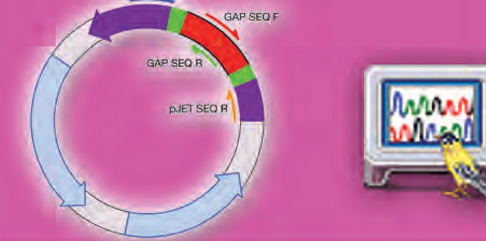


Electrophoresis

Plasmid preperation and ligation

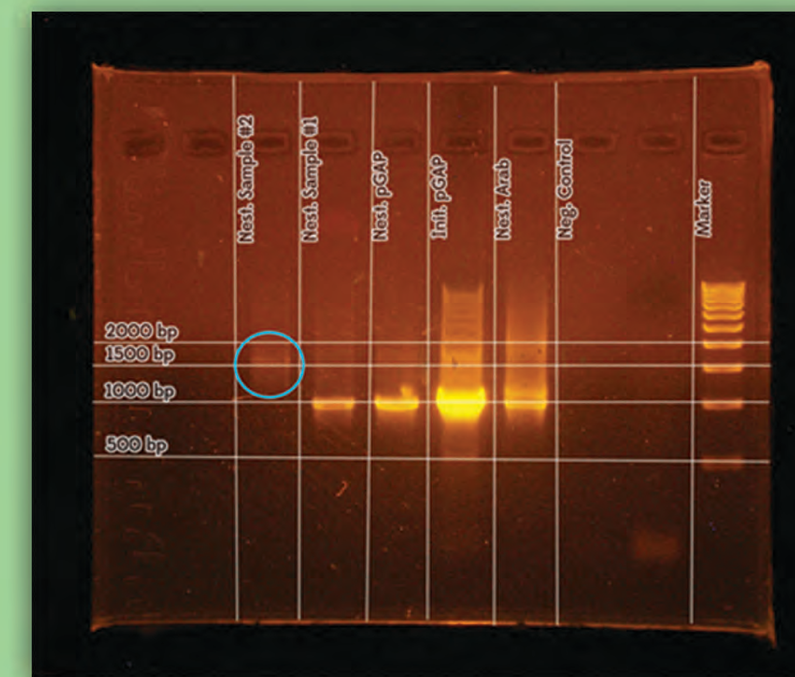
E. Coli DNA cloning and plasmid purification

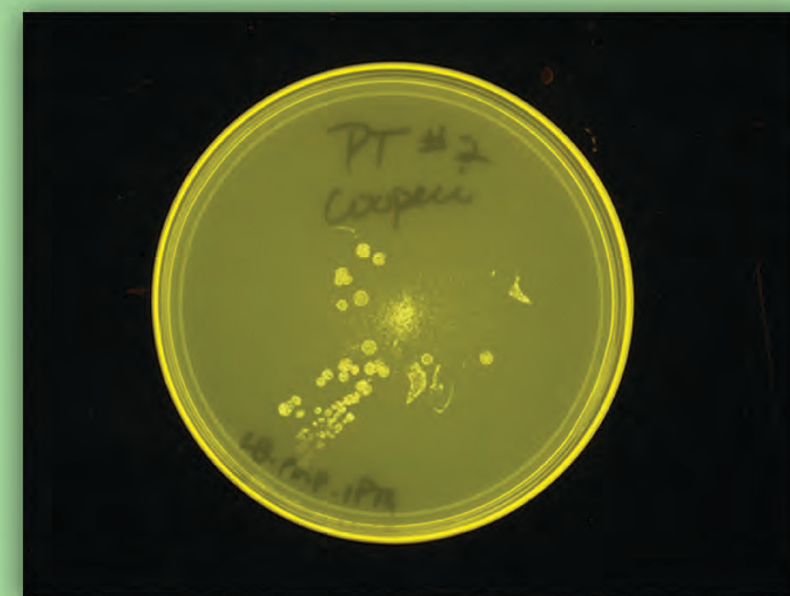
Analyze and interpret sequenced genome



Data and Results

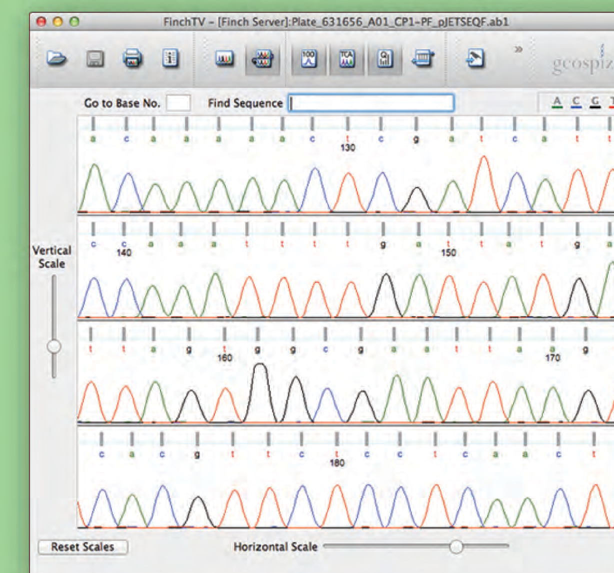
By comparing the size of the DNA fragments against positive and negative controls, gel electrophoresis was used as a test to confirm whether or not the 1500 base pair target gene had been isolated and amplified. One of the major complications with the PCR process was the contamination of our sample by the positive control, Arabidopsis thaliana. Because the kit was designed for a wide range of plants, the primers were partial to the Arabidopsis thaliana genome rather than the target organism.





Cloning was accomplished by inserting the gene into a bacterial plasmid vector. The growth of bacterial colonies on a specialized medium (along with further electrophoresis) showed that the plasmids had been successfully cloned; but this was only achieved after a few unsuccessful growth attempts. This could be attributed to a poor quality "one size fit all" plasmid from the supplier. A single bacterial colony from four different plates was extracted and prepared for electrophoresis and only one of the samples was high enough quality to be sequenced.

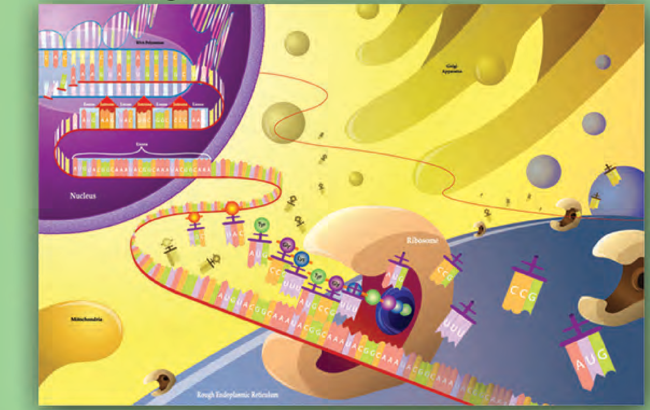
Raw genomic data was returned tfrom the sequencing lab, which was then analyzed. Using a bioinformatics program the genomic code of the target gene along with its amino acid protein code could be deciphered. In addition, the data was analyzed for quality purposes. The raw sequence data then needed to be processed. The extra plasmid DNA was removed, then the data was categorized as introns and exons. From this a mRNA sequence was able to be arranged and the origin of the DNA could be verified.



Amino Acid Translation

Conclusions

Through multiple confirmation tests conducted during the cloning process, along with comparisons of the deciphered genomic sequence against previously published works in genomic libraries, it was concluded that the Delosperma cooperi GAPC gene had been successfully sequenced. Though it was confirmed that the target gene originated from the Delosperma cooperi GAPC gene, the protein sequence differed by four amino acids from a previous attempt to sequence this gene. This is a significant enough difference that further sequencing experiments need to be performed to verify the correct sequence. Through the many successes and failures we encountered during our time on this project, we believe that we have gained knowledge of many of the procedures used in genomic cloning and sequencing along with an appreciation for the genetic theories behind these methods.



STGIFTDKEKAAAHLKGGAKKVVISAPSRDAPMFVGVNEKEYKPELNIVSNASCTTNCLAPL (V, G, K)

AKVINDRFGIVEGLMTTVHSITATQKTVDGPSSKDWRGGRAASFNIIPSSTGAAKAVGKVLPVL

GKLTGMSFRVPTVDVSVVDLTARLEKAATYEDIKQGGIRGEPQRHLGVHRRRRCLHRFHX (V)

Future Research

Developing techniques to minimize/eliminate contamination with positive controls

Constructing specific primers to target specimen more accurately

Clone more genes to confirm results

Accurately place the species Delosperma cooperi in a phylogenetic tree with respect to the GAPC gene

Acknowledgements

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