The Genetic Characterization of Common Household Flower Species in Forensic Science

Christina Guzman Moumtzis[1], Abhayvir Singh[2], Nathan H. Lents, Ph. D[3]

[1]Frank McCourt High School, [2]Brooklyn Technical High School, [3]John Jay College of Criminal Justice, CUNY

Abstract

The primary application of this work is to explore whether flowering plant residue can be used as a component gathered from a crime scene. We hypothesize that every flower has a unique set of DNA; however, each genus has conserved sequences as well as regions of polymorphisms within that allows for discrimination from one genus to another. To this purpose, plant DNA must be extracted for genomic sequencing. Successful sequencing will allow for us to identify unique DNA sequences which distinguish one species from another. In this project, 6 total species were successfully extracted. The 18S Ribosomal RNA primer (*18S rRNA*) was used for successful amplification with Polymerase Chain Reaction (PCR) and results were then sent for sequencing. Successful amplification of conserved regions allowed for regions of polymorphism to be identified making apparent the uniqueness of each flowering plant's DNA. Species specific primers were carefully chosen from these regions of polymorphism and the Primer3 program was used to design the primers. The designed species specific primers resulted in successful amplification of the species for which they were designed for.

Introduction

•Flowering plants are known for their aesthetic and are popular choices for home décor. They are usually more prevalent during holidays and special occasions such as birthdays, anniversaries, etc. Therefore, people are more likely to come in contact with flowering plants during these occasions.

•Successful extraction of plant DNA is a critical first step in order to sequence plant DNA from the flower species.

•Every plant has certain DNA sequences that are conserved as well as those that are highly variable among species.

•The 18S Ribosomal RNA gene, which encodes the small ribosomal subunit of the ribosome, is common in eukaryotes and exists in all plants.

•The goal is to sequence the DNA of many flowering house plants using the 18S Ribosomal RNA gene and create species specific primers which will allow us to establish the DNA sequences unique to each species.

Methods

•6 flowering plant species (alstroemeria, flamingo flower, helianthus, hyacinth, moth orchid, salvia) were obtained from local florist shops and Home Depot.

•Plant DNA was extracted using the Cetyltrimethylammonium Bromide (CTAB) method. Tiny pieces of leaves (about 1-2grams) were physically grounded in buffer solution (Tris-HCl, EDTA, NaCl, mercapto ethanol, distilled water) with a mortar/pestle. Phase separation using chloroform: isoamyl alcohol (24:1), precipitation in 70% ethanol and dried on heat block. Finally, the purified DNA was suspended in 10mM tris buffer.

•NanoDrop spectrophotometer was used to quantify the plant DNA as well as assess the purity of DNA.

•PCR was used to amplify small amount of DNA. The PCR temperature settings were as follows: Initial pre-denaturation at 95°C - 30 seconds (1 cycle). PCR amplification (28 cycles): Denature at 95°C for 30 seconds. Anneal varies (60°C - 63°C) for 30 seconds. Extension 68°C. Final extension at 68°C for 5 minutes.

•Agarose Gel Electrophoresis was used to check for the amplification of the PCR products. The agarose was melted in Tris-Acetate-EDTA (TAE) buffer along with the addition of ethidium bromide. When the agarose had solidified to a semi-solid state, it was run for 40 minutes and the migration of DNA bands was visualized under UV light.

•DNA Sequence alignment was done by careful selection of a continuous DNA sequence marked by a clean peak representing a base (A, T, G, C) from the chromatogram. Each species has a matching 5' and a 3'-reverse compliment end. Each pair of DNA sequences was copied into the DNASubway program, which was used to perform the alignment.

•Primer design was done using the Primer 3 program, an application of the University of Tartu & Estonian Biocentre Department of Bioinformatics.

Results

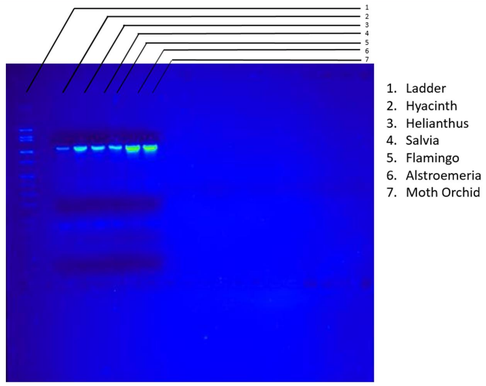

Figure 1. An agarose gel was created from 2.2 grams of agarose dissolved in 150mL TAE buffer and run on electrophoresis for 40 minutes. The gel was visualized under UV light in the presence of ethidium bromide. The set of designed universal primers for amplifying the 18S rRNA gene successfully amplified all 6 species, a second set of sequencing primers were used to produce partial sequences across these same species.

<u>Alstroemeria Composite Sequence</u>

5′-
ACGCCGGCGACGCATCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGGGGC
CTACCATGGTGATGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAG
AAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCTGACACGGG
GAGGTAGTGACAATAAATAACAATACCGGGCTTTTTGAGTCTGGTAATTGGAATGAGTAC
AATCTAAATCCCTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTA
ATTCCAGCTCCAATAGCGTATATTTAAGTTGTTGCAGTTAAAAAGCTCGTAGTCGGACCT
TGGGTTGGGCCGGCCGGTCCGCCTCACGGTGTGCACCTGCCTGCTCGTCCCTTCTGCCGG
CGATGCGCTCCTGGCCTTAACTGGCCGGGTCGTGCCTCCGGCGCCGTTACTTTGAAGAAA
TTAGAGTGCTCAAAGCAAGCCATCGCTCTGCATACATTAGCATGGGATAACATCACAGGA
TTTCGGTCCTATTGTGTTGGCCTTCGGGATCGGAGTAATGATTAACAGGGACAGTCGGGG
GCATTCGTATTTCATAGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGA
-3′
Total: 651bp

Figure 3. The 5' sequence combined with the 3' sequence gives a composite sequence of the plant species for alstroemeria. The sequencing is done with 5' alone and 3' alone so when we overlap we get the full sequence of the species. The overlap consists of 651 base pairs, chosen from the chromatogram sequencing results shown on figure 1.

Specie	Species-Specific Primers
Alstroemeria	Alstroemeria-18S-5′:TGCACCTGCCTGCTC Alstroemeria-18S-3′:AATGTATGCAGAGCGATG
Flamingo Flower	Flamingo Flower-18S-5′: CGGACCTTGGGATGG Flamingo Flower-18S-3′: TACTGCCGGCGATGCGTTC
Helianthus	Helianthus-18S-5′:GGGCTCATACGAGTCTGG Helianthus-18S-3′:GCCCAGTTAAGGGTAGG
Hyacinth	Hyacinths-18S- 5′: CGGGCTCAAAGAGTCTGGTA Hyacinths-18S- 3′: CACGGTGAGCACCGGCC-- G
Moth Orchid	Moth Orchid-18S-5′:GAGACGGCTGCCACA Moth Orchid-18S-3′:CCAATTAAGGCCAGGAA
Salvia	Salvia-18S-5′:ACTTTGGGTTGGAACG Salvia-18S-3′:AGGACCGAAATCCTATAATG

Figure 5. This data table shows the final species specific sequences for each of the species successfully barcoded. Species-Specific sequences were manually selected after looking at the sequence alignment of the species that were successfully sequenced. They were selected from regions of polymorphism that set each species' DNA as unique. These species sequences were then entered into a primer design program known as Primer3 program. This program would design the primers and then they would be tested to check for amplification.

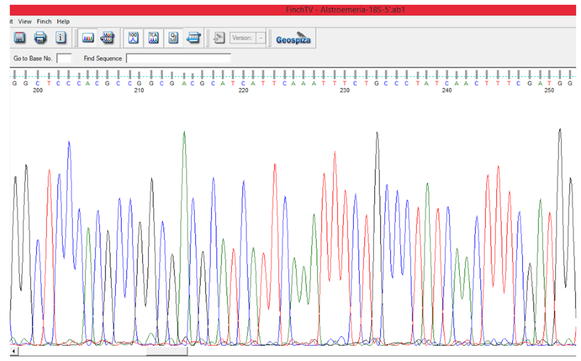

Figure 2. A chromatogram of the 5' end sequence of the plant species alstroemeria produced from Sanger DNA Sequencing by GENEWIZ. Each of the four colored peaks represents a base. Blue = cytosine, green = adenine, black = guanine, and red = thymine.

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Hyacinth        GATAGTGGCCTACCATGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGG
Helianthus      GATAGTGGCCTACTATGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGG
Salvia          GATAGTGGCCTACTATGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGG
Flamingo        GATAGTGGCCTACCATGGTGGTGACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGG
Alstroemeria    GATAGGGGCCTACCATGGTGATGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGG
Moth            GATAGTGGCCTACCATGGTGGTGACGGGTGACGGAGAATTAGGGTTCGATTCCGGAGAGG
                ***** ******* ****** ******** **************************

Hyacinth        GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCC
Helianthus      GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCC
Salvia          GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCC
Flamingo        GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCC
Alstroemeria    GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCC
Moth            GAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCC
                ********************************** ********** ***********

Hyacinth        TGACACGGGGAGGTAGTGACAATAAATAAC-AATACCGGGCTCAAAAGAGTCTGGTAATT
Helianthus      TGACACGGGGAGCTAGTGACAATAAATAACA-ATACCGGGCTCATACGAGTCTGGTAATT
Salvia          TGACACGGGGAGCTAGTGACAATAAATAACA-ATACCGGGCTCT-TGAGTCTGGTAATT
Flamingo        TGACACGGGGAGCTAGTGACAATAAATAACA-ATACCGGGCTCT-TCGAGTCTGGTAATT
Alstroemeria    TGACACGGGGAGCTAGTGACAATAAATAACA-ATACCGGGCTTT-TTGAGTCTGGTAATT
Moth            TGACACGGGGAGCTAGTGACAATAAATAACA-ATACCGGGCTCT-TCGAGTCTGGTAATT
                ********************************** ********** ***********

Hyacinth        GGAATGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAG
Helianthus      GGAATGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAG
Salvia          GGAATGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAG
Flamingo        GGAATGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAG
Alstroemeria    GGAATGAGTACAATCTAAATCCCTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAG
Moth            GGAATGAGTACAATCTAAACCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAG
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Figure 4. Each individual species' sequences are aligned using *ClustalW Multiple Sequence Alignment* tool provided by *ExPASy Bioformatics Resources Portal*. A total of 6 species shown. The * shown at the bottom indicates perfect alignment where all bases are the same across all 6 species. A blank spacing indicates polymorphism of bases between the 9 species in certain regions.

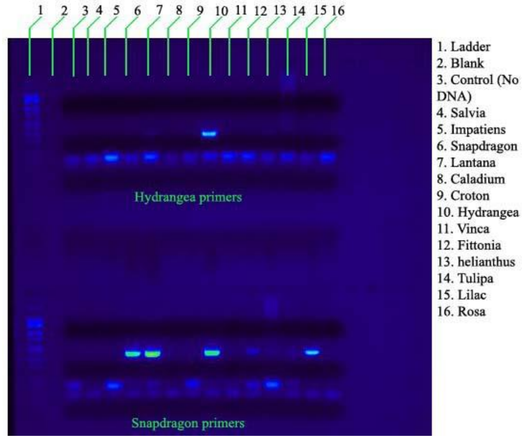

Figure 6. An agarose gel was created from 2.2 grams of agarose dissolved in 150mL TAE buffer and run on electrophoresis for 40 minutes. The gel was visualized under UV light in the presence of ethidium bromide. The plant species hydrangea (top row) and snapdragon (bottom row) was tested against 12 different templates. Hydrangea primer pairs showed amplification on hydrangea template indicated by a bright band (lane 10). Snapdragon primer pairs showed amplification on snapdragon, lantana, hydrangea, and lilac templates.

Conclusions

Our experiment was able to reach up to the point of primer design but not the testing of those designed primers. A member of the Lents Lab, however, was able to test the designed primers and they resulted in successful amplification. This proves regions of polymorphism that set apart one species from another can be used to develop species specific primers. The specie specific primers created can be utilized to identify species left behind at a crime scene. Due to the reserved and unique portions of DNA, if pollen were to be left behind, primers could be used to identify what plant that pollen came from, this being an example of the study being applied to solve real world problems. Doing so can filter a suspect list and can support the forensic aspect of an investigation.

Future Work

•A larger number of flowering plant species will be added to the study in order to add more variety to the experiment which can further increase this research project's applicability in the forensics setting.

•Substrate concentration and annealing temperatures can be optimized for the species specific primers that yield in non-specific results in the experiment.

•Despite the optimization, in case the species specific primers do not show expected results, redesigning of the primers will be attempted with a larger starting sequence.

References

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Acknowledgements

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