The Utilization of Polymerase Chain Reaction, DNA Barcoding and Bioinformatics in Identifying Plant Species

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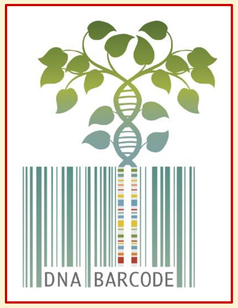
Abstract

Bioinformatics and DNA barcoding is a process used to identify plants, animals, and fungi. DNA barcoding in plants utilizes a key variable region in the genome, the RuBisCo large subunit (RbcL) on Chloroplast DNA. Once the DNA is extracted, Polymerase Chain Reaction (PCR) amplifies that region and that sample is sent off for sequencing. Bioinformatics and DNA barcoding helps taxonomists determine the sequence of the RbcL gene as well as obtain a unique barcode that can be used to identify plants.



Background Information

There are about 400,000 known plant species in the world. Nearly all coniferous plants have been identified, however one-fifth of all vascular plants and an unknown amount of algae remains unnamed.

DNA barcoding and bioinformatics lets scientist identify the species. DNA barcodes are cataloged on the Barcode of Life Database (BOLD), as well as Basic Local Alignment Search Tool (BLAST), which helps analyze, store, and publish records. Using the DNA sequence extracted and these databases we could verify the names of the sample species

Goals:

- **Master the processes of Polymerase Chain Reaction, DNA barcoding and bioinformatics**
- **Determine whether the *Carolina* genomic protocol or the *Wizard* genomic protocol is more efficient**
- **Use the processes mentioned above to identify campus plant biodiversity.**

Methods

Results

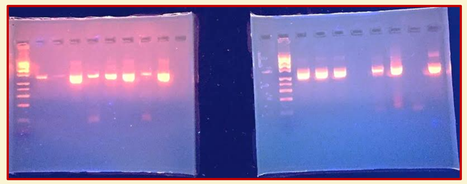


Figure 1- Electrophoresis of samples LT01, LT02, RF01, and RF02

TGCTGGATTCAAAGCGGGCGTTAAAGATTACAGATTGACTTATTATACTCCTGACTATGAAACCAAAGAT
ACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCTGGAGTTCCGCCTGAAGAAGCAGGGGCCGC
GGTAGCTGCTGAATCTTCAACTGGTACATGGACAACTGTGTGGACCGACGGACTTACCAGCCTTGATCG
TTACAAAGGGCGATGCTACCACATCGAGCCCGTTCCTGGAGAAGAAACTCAATTTATTGCTTATGTAGCT
TACCCATTAGACCTTTTTGAAGAAGGTTCTGTTACTAACATGTTTACTTCTATTGTGGGTAATGTATTTGG
GTTCAAAGCCCTGCGCGCTCTACGTCTGGAAGATCTGCGAATCCCTGTCGCTTATGTTAAAACCTTCCAA
GGCCCGCCTCATGGTATCCAAGTTGAGAGAGATAAATTGAACAAGTACGGGCGCCCCCTGTTGGGATG
TACTATTAAACCTAAATTGGGGTTATCTGCTAAAAACTACGGTAGGGCGGTTTATG

Figure 2- Nucleotide Sequence of *Symphoricarpos occidentalis*

Sequence Number	Barcode	*Latin Name* Common Name	QR Code
RF01	0 576	*Ulmus Minor* Field Elm	
RF02	0 579	*Amelanchier Alnifolia* Western Juneberry	
RF03	0 580	*Fraxinus Pennsylvanica* Green Ash	
RF04	0 569	*Acer Pseudoplatanus* Sycamore Maple	
RF05	0 592	*Malus Pumila* Paradise Apple	
RF06	0 574	*Acer Platanoides* Norway Maple	
LT01	0 523	*Symphoricarpos occidentalis* Western Snowberry	
LT02	0 513	*Berberis thunbergii* Japanese Barberry	
LT03	0 581	*Populus tremuloides* Quaking Aspen Tree	
LT04	0 543	*Arctostaphylos uva-ursi* Pinemat Manzanita Bearberry	
LT06	0 540	*Koelreuteria elegans* Goldenrain Tree	

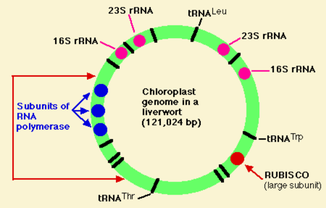
Figure 3-Data Table including DNA barcodes and names for the RbcL gene of the plants

Discussion

- **The *Carolina* genomic protocol was more efficient than the *Wizard* genomic protocol.**
- **If a plant's DNA wasn't extracted using one protocol, the other was sufficient to extract the DNA.**
- **The RbcL gene varied enough across species to identify them.**
- **A variety of plants were identified across the Northwest Nazarene University campus.**
- **LT05 was not identified as the DNA was not extracted successfully from it. There was a waxy layer on the leaf, making extraction difficult.**

Conclusion

- **The DNA barcoding with the use of RuBisCo large subunit from the Chloroplast gene was highly successful.**
- **The DNA barcodes received could be matched with previous submissions on the BOLD and BLAST databases allowing us to confirm plant species.**
- **Further research can help Northwest Nazarene University identify more plants on campus.**



References

Arthur D Chapman. 2009. Numbers of Living Species in Australia and the World.2nd edition. Australian Government, Department of the Environment, Water, Heritage and the Arts. Canberra, Australia.

Bio-Rad Barcode Generator

Gibson. How DNA barcoding can boost quality control for medical plant products. 2015.

Acknowledgments

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