



ESF) Barcoding Micro-Moths using NGS Technology

Introduction



- JIm Steffencollected 600+moths fromMcdonald Woods
- Woods overgrown with weeds after human settlement
- Many insects will pair with specific species of plants, often found together in an environment
- 160,000 moths, perfect choice for studying overall species diversity

Problem

- Many moths are too small to physically identify
- Genetics could help identify these moths though methods are early and underdeveloped
- These micro moths
 Also can not be
 damaged as they are
 expected to end
 up on display



Objectives

- We hope to have enough quality DNA in order to determine the species and taxonomies of these micromoths.
- Want to determine a successful method to extract enough DNA for our needs

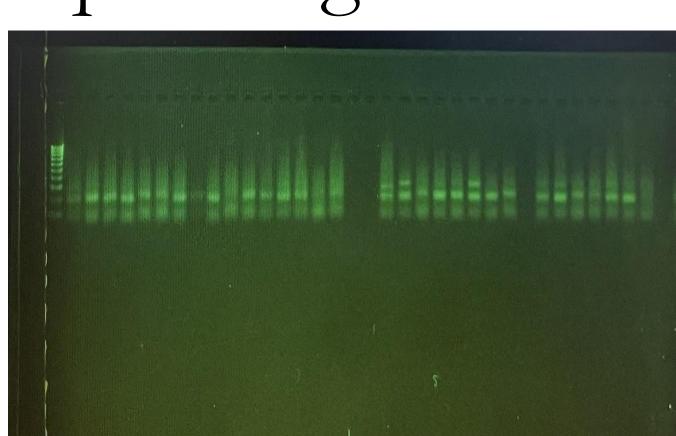
1. Extracting DNA

- Legs removed from moth
- Standard protocol for Insect DNA extraction

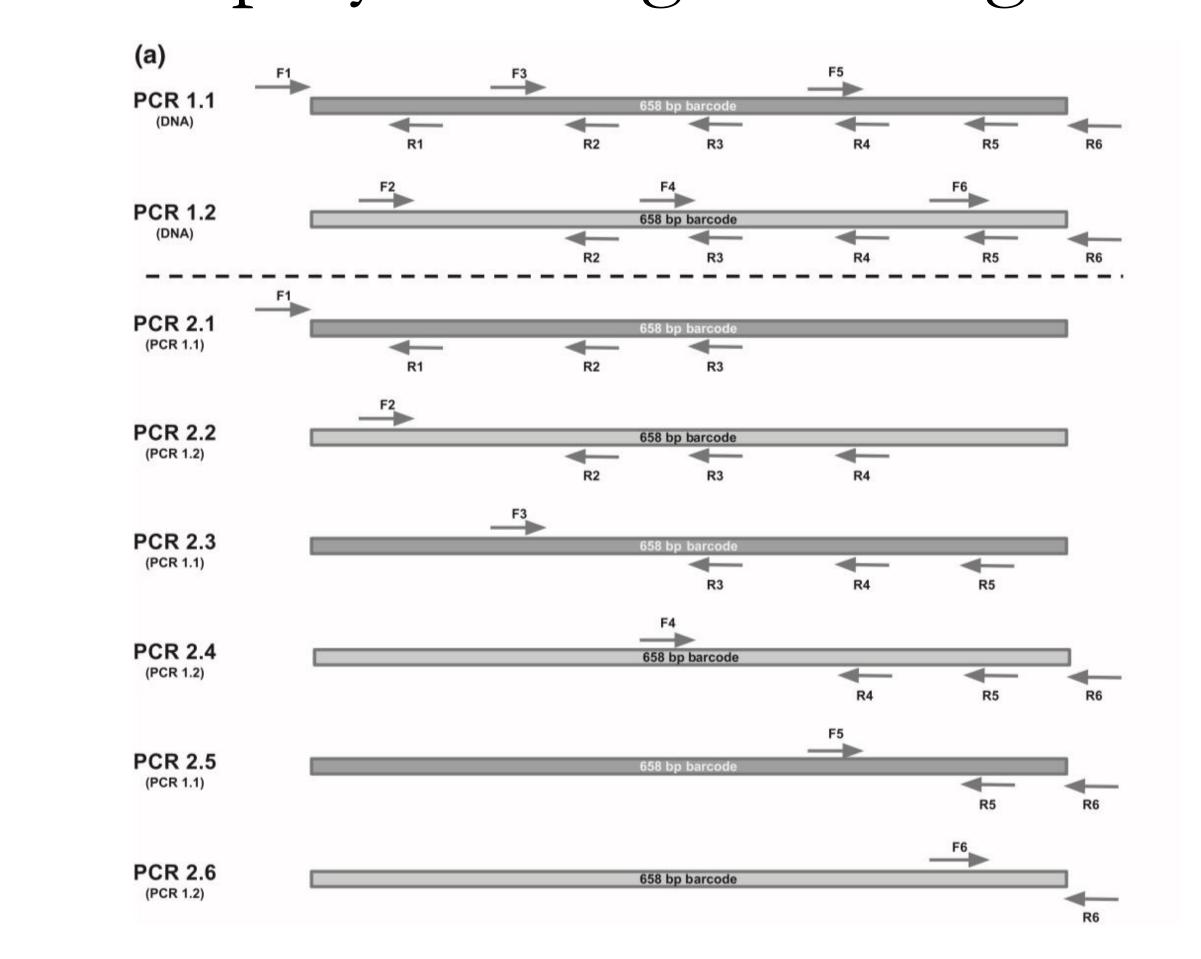


3. Preparing for Sequencing

- Cleaningmagnetic beads
- Removing fragment lengths
- Aiming for 200-300 bp



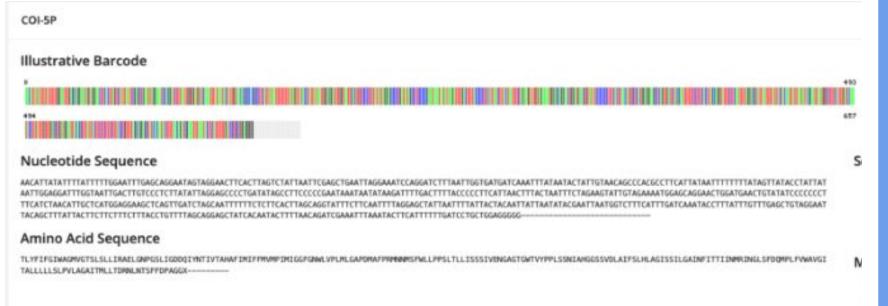
2. Amplify and Tag CO1 Region



- DNA replicated with PCR
- Tagged with unique primers

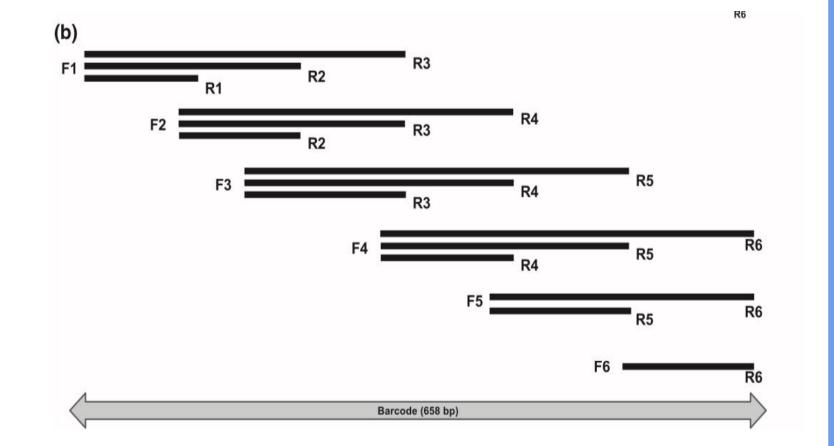
4. Sequencing CO1 Barcode

Pooled samples sent off for sequencing



5. Completing the Genome

Fragments
 "stitched"
 Together to
 make full 658
 bp sequence



Discussion

- Human error could have contributed to a lack of DNA concentration, but still unsure
- Many of the moths were 10+ years old, dna could've degraded
- The project is still experimental and there is a lot to test methods wise. Excited to receive the results and see if this process is successful.

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Works cited RG;, C. J. T. C. G. (n.d.). Chelex without boiling, a rapid and easy technique to obtain stable amplifiable DNA from small amounts of ethanol-stored spiders. Molecular ecology resources. Retrieved August 16, 2022, from https://pubmed.ncbi.nlm.nih.gov/21943065/ Prosser, S., deWaard, J. R., Miller, S., & Hebert, P. (1970, January 1). DNA barcodes from century-old type specimens using next-generation sequencing: Semantic scholar. undefined. Retrieved At 17, 2022, from https://www.semanticscholar.org/paper/DNA-barcodes-from-century%E2%80%90old-type-specimens-using-Prosser-deWaard/c354f11750ea2384a4bc530e3b21104f33389ac3 'to people, they're a nuisance,' but to Jim 'mothman' Steffen, the insects are the key to saving a 100-acre forest in Glencoe. Chicago Tribune.