Molecular Resources and Protocols: From PLS4178 to PLS4172

Alina Avanesyan

Lab Meeting 10/12/2020

DNA Barcoding Work: Location

- Room 4172, 4th floor
- Dr. David Hawthorne's lab

David Hawthorne, Associate Professor & Director of Education at SESYNC

Office: 4132 Plant Sciences Building
Phone: 301-405-2401
E-mail: djh@umd.edu

Research: The Hawthorne Lab uses population genetics to understand how insects become pests, how they evolve to counter control efforts, and how to use evolutionary thinking to manage them. Additionally, research in the Hawthorne Lab dissects the genetic basis of host-plant associated divides among pest populations and uses phylogeographic analyses to investigate issues in conservation genetics.

DNA Barcoding Work: Research Directions

- Host plant DNA detection from potato leafhopper, *Empoasca fabae*
- Host plant DNA detection from the spotted lanternfly, *Lycorma delicatula*
- Wetland-stream connectivity: DNA barcoding of isopods
- Prey-predator interactions: DNA barcoding of prey items in Odonata gut contents and feces

DNA Barcoding Team
(at different times over the past 2.5 years)

1. Brock Couch**
2. Kevin Clements*
3. Nina McGranahan ♦
4. Bryan Stancliff♦
5. Omar Abdelwahab*
6. Jessica Ho*
7. Darsy Smith**
8. Margaret Hartman**
9. Nurani Illahi*
10. Hannah Sutton*

(*undergraduate students, **graduate students, ♦ high school students)

DNA Barcoding Team: Research results

Journal articles

- Avanesyan, A., Illahi, N. and W.O. Lamp. (2020) Detecting ingested host plant DNA in potato leafhopper, *Empoasca fabae*: potential use of molecular markers for gut content analysis. Journal of Economic Entomology (In Press).
- Avanesyan, A., and W.O. Lamp. (2020) Use of molecular gut content analysis to decipher the range of food plants of the invasive spotted lanternfly, *Lycorma delicatula*. Insects: Special Issue " Molecular Gut Content Analysis: Deciphering Trophic Interactions of Insects", 11(4), 215, doi.org/10.3390/insects11040215.

Manuscript in revision

- Avanesyan, A., Sutton, H., and W.O. Lamp. (2020) Choosing an effective molecular approach to diet analysis of insect herbivores: a systematic review.

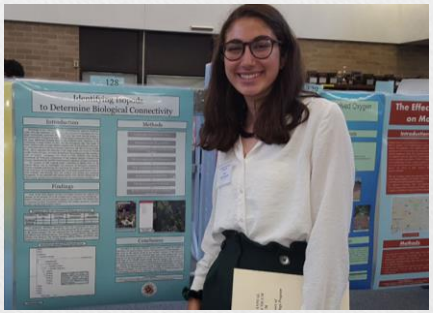
Manuscript in preparation

- Avanesyan, A., Sutton, H., Lamp, W.O., and D. Hawthorne. Identification of host plant use by the invasive spotted lanternfly (*Lycorma delicatula*) using next-gen DNA sequencing technology.

Conference and Research Symposium Presentations

- Avanesyan, A., and W. Lamp (2018) Use of molecular markers for plant DNA to determine host plant usage for potato leafhopper, Empoasca fabae. Annual Meeting of the Entomological Society of America: 2018 ESA, ESC, and ESBC Joint Annual Meeting, Vancouver, BC, Canada. Oral presentation

- McGranahan, N. (2019) Identifying isopods to determine biological connectivity. The ERHS Research Symposium. Poster Presentation.



- Stancliff, B. (2019). The John Carroll School Research Symposium. Oral presentation

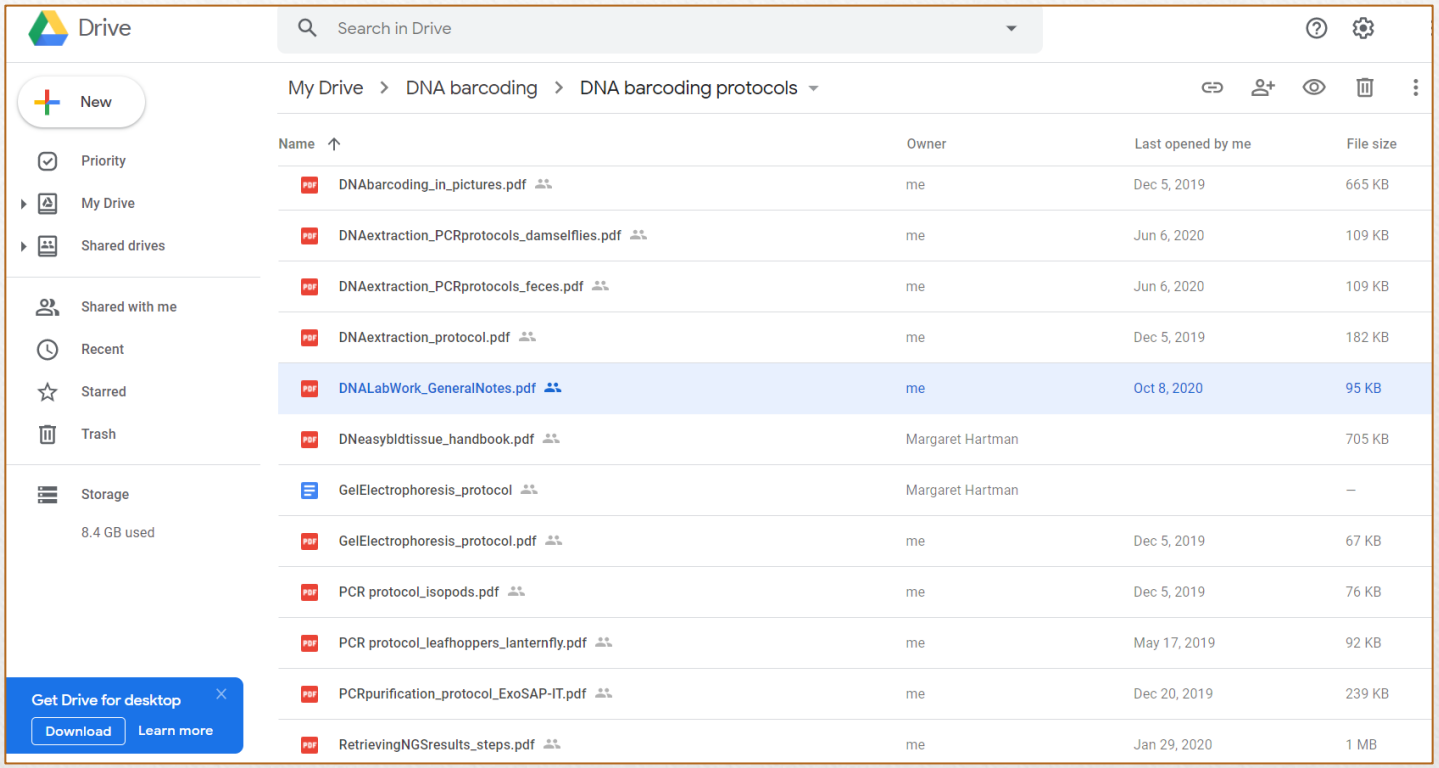
GenBank Submissions

1. Avanesyan, A. and W. O. Lamp. (2020) *Betula pendula* isolate 1E4a ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. Direct Submission, GenBank Accession no. MT119453
2. Avanesyan, A. and W. O. Lamp. (2020) *Acer pseudoplatanus* isolate 1F4b ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. Direct Submission, GenBank Accession no. MT108179
3. Avanesyan, A. and W. O. Lamp. (2020) *Vitis vinifera* isolate 1B3 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. Direct Submission, GenBank Accession no. MN862495
4. Avanesyan, A. and W. O. Lamp. (2020) *Ailanthus altissima* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. Direct Submission, GenBank Accession no. MN853649
5. Avanesyan, A. and W. O. Lamp. (2020) *Celastrus orbiculatus* isolate TT4a ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. Direct Submission, GenBank Accession no. MN862496
6. Illahi, N.*, Avanesyan, A. and W. O. Lamp. (2020) *Lonicera maackii* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. Direct Submission, GenBank Accession no. MN631052
7. Smith, D.K.**, Avanesyan, A. and W. O. Lamp. (2020) *Eupatorium serotinum* tRNA-Leu (*trn*L) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MN395725
8. Smith, D.K.**, Avanesyan, A. and W. O. Lamp. (2020) *Lonicera maackii* tRNA-Leu (*trn*L) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MN365276
9. Smith, D.K.**, Avanesyan, A. and W. O. Lamp. (2020) *Pisum sativum* isolate slf-2 tRNA-Leu (*trn*L) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MN335637
10. Smith, D.K. **, Avanesyan, A. and W. O. Lamp. (2020) *Acer platanoides* tRNA-Leu (trnL) gene, partial sequence; chloroplast. Direct Submission, GenBank Accession no. MN450067
11. Smith, D.K. **, Avanesyan, A. and W. O. Lamp. (2020) *Acer rubrum* tRNA-Leu (trnL) gene, intron; chloroplast. Direct Submission, GenBank Accession no. MN450068
12. Illahi, N.*, Avanesyan, A. and W. O. Lamp. (2019) *Ailanthus altissima* isolate BC4b ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. Direct Submission, GenBank Accession no. MN856629
13. Illahi, N.*, Avanesyan, A. and W. O. Lamp. (2019) *Ailanthus altissima* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast. Direct Submission, GenBank Accession no. MN853649
14. Stancliff, B.♦, Avanesyan, A. and W. Lamp. (2019) *Vicia faba* tRNA-Leu (trnL) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MK934667
15. Stancliff, B.♦, Smith, D.**, Avanesyan, A. and W. Lamp. (2019) *Pisum sativum* tRNA-Leu (*trn*L) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MK919208
16. Stancliff, B.♦, Abdelwahab, O.*, Avanesyan, A. and W. Lamp. (2019) *Vigna unguiculata* tRNA-Leu (*trn*L) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MK883492
17. Stancliff, B.♦, Ho, J.*, Avanesyan, A. and W. Lamp. (2019) *Helianthus annuus* tRNA-Leu (*trn*L) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MK875279
18. Avanesyan, A., and W. Lamp. (2019) *Vicia faba var. major* isolate PLH_fb tRNA-Leu (*trn*L) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MK837073

(*undergraduate students, **graduate students, ♦ high school students)

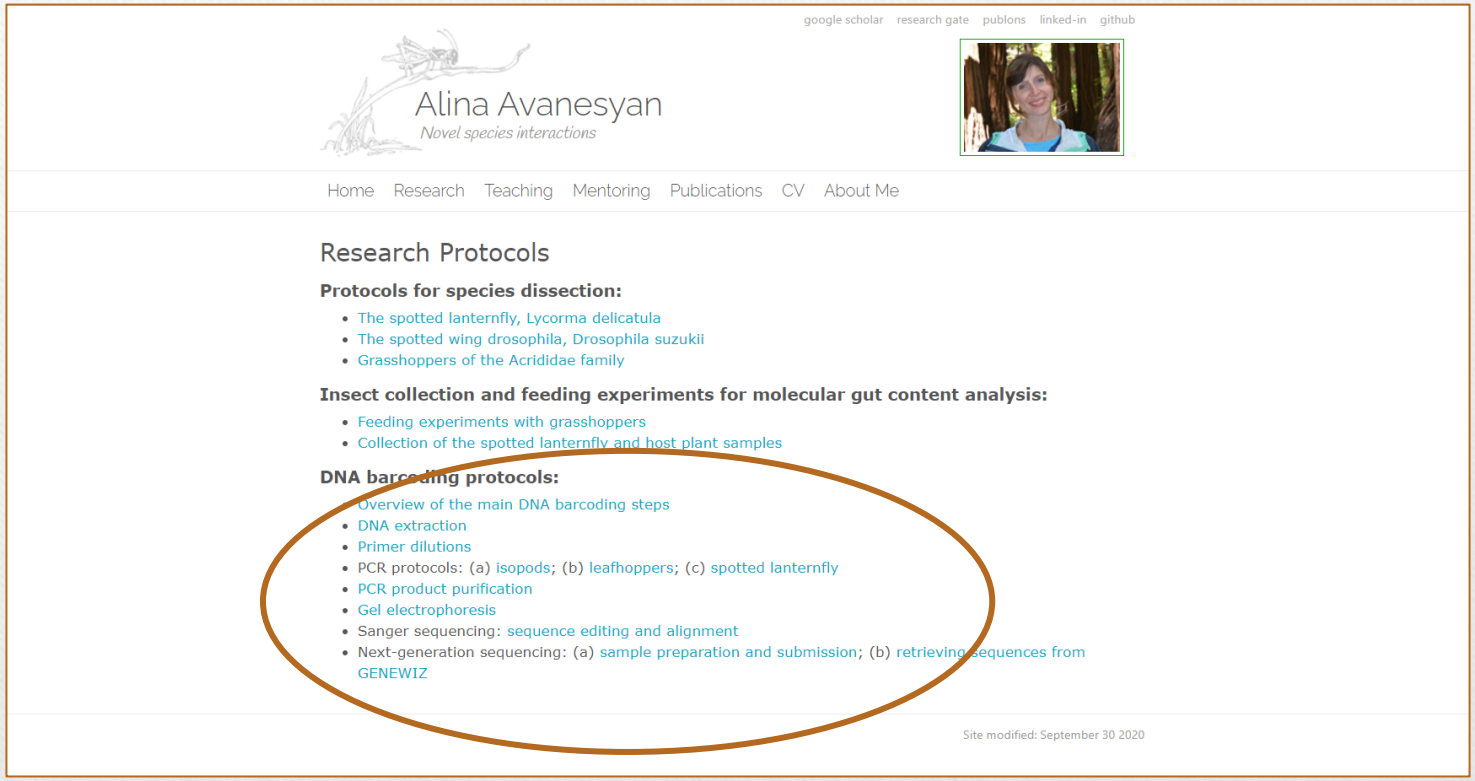
DNA Barcoding Protocols

- Shared Google folder



DNA Barcoding Protocols

- http://alinaavanesyan.com/projects/research-protocols/



google scholar research gate publons linked-in github

Alina Avanesyan

Novel species interactions

Home Research Teaching Mentoring Publications CV About Me

Research Protocols

Protocols for species dissection:

- The spotted lanternfly, Lycorma delicatula
- The spotted wing drosophila, Drosophila suzukii
- Grasshoppers of the Acrididae family

Insect collection and feeding experiments for molecular gut content analysis:

- Feeding experiments with grasshoppers
- Collection of the spotted lanternfly and host plant samples

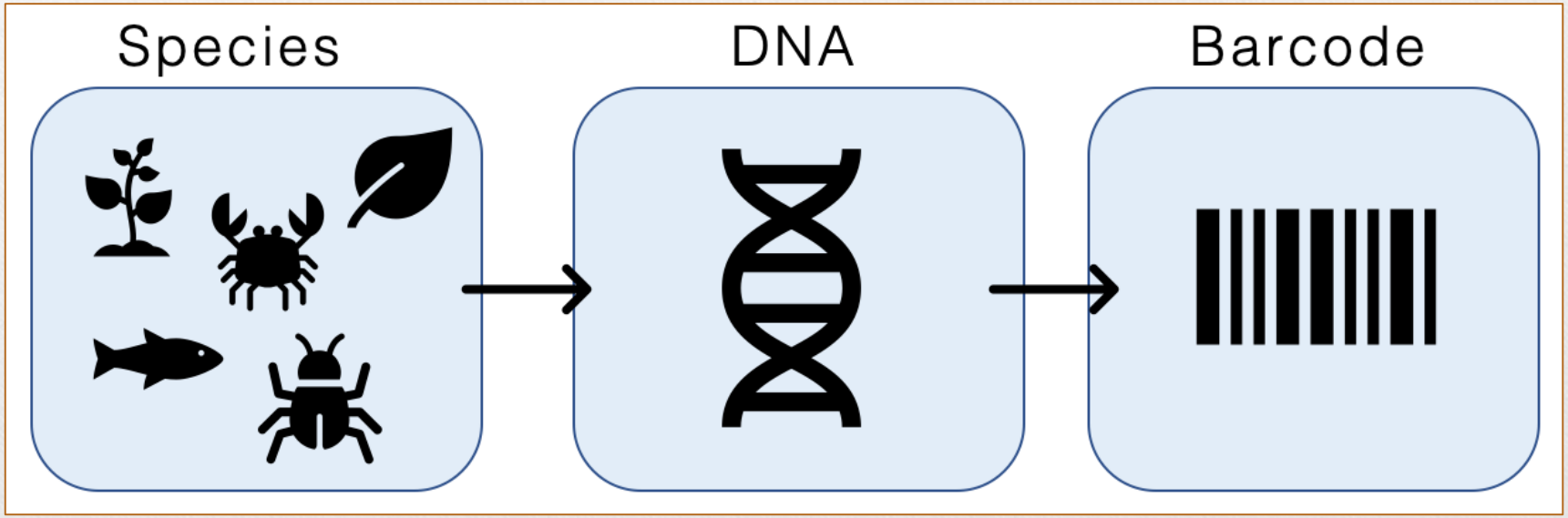
DNA barcoding protocols:

- Overview of the main DNA barcoding steps
- DNA extraction
- Primer dilutions
- PCR protocols: (a) isopods; (b) leafhoppers; (c) spotted lanternfly
- PCR product purification
- Gel electrophoresis
- Sanger sequencing: sequence editing and alignment
- Next-generation sequencing: (a) sample preparation and submission; (b) retrieving sequences from GENEWIZ

Site modified: September 30 2020

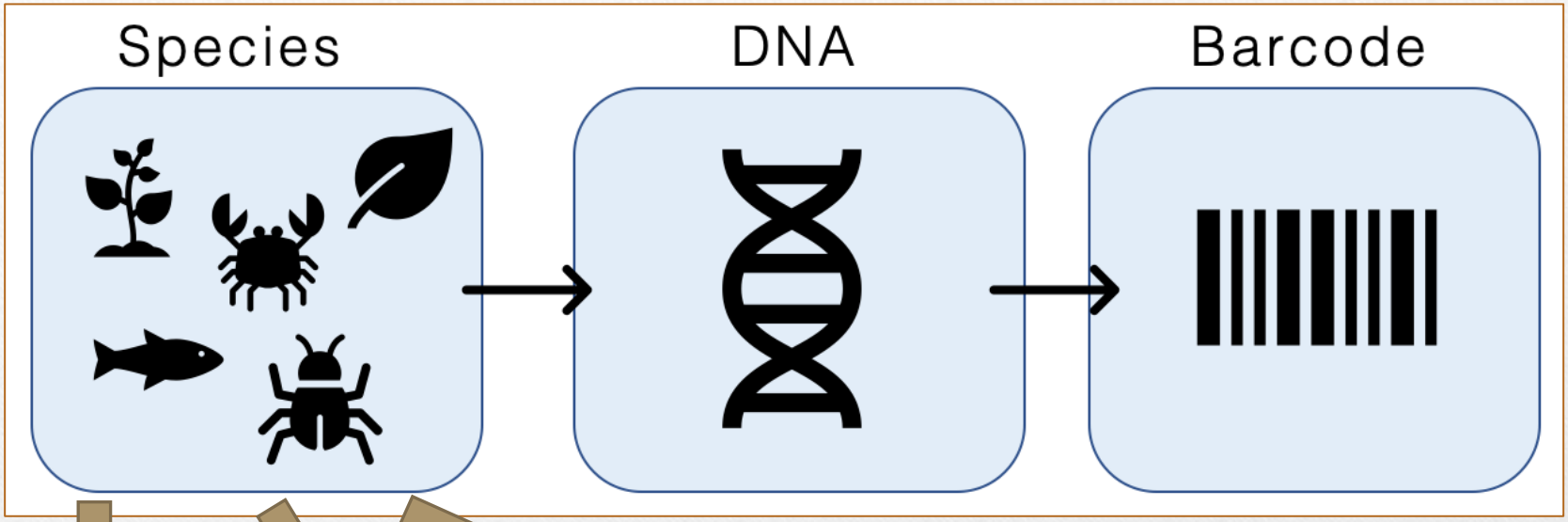
DNA Barcoding

- method of species identification using a short section of DNA from a specific gene or genes



https://en.wikipedia.org/wiki/DNA_barcoding

Sources of DNA



Species itself

Isopods

Gut contents
(e.g. ingested plants)

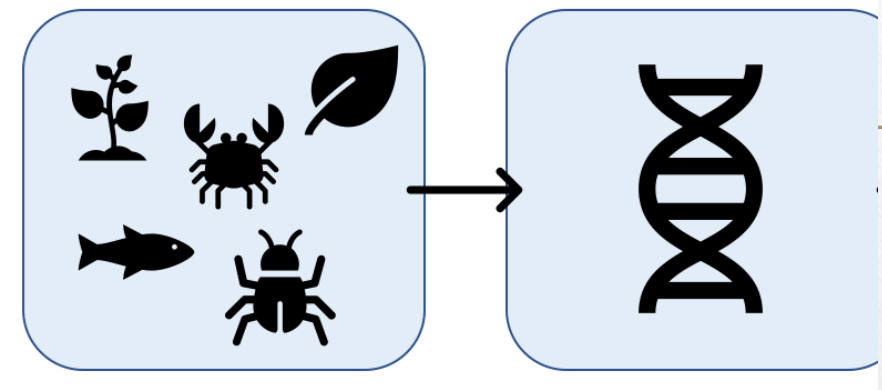
Spotted lanternfly, potato leafhopper

Feces
(e.g. ingested prey items)

Odonata

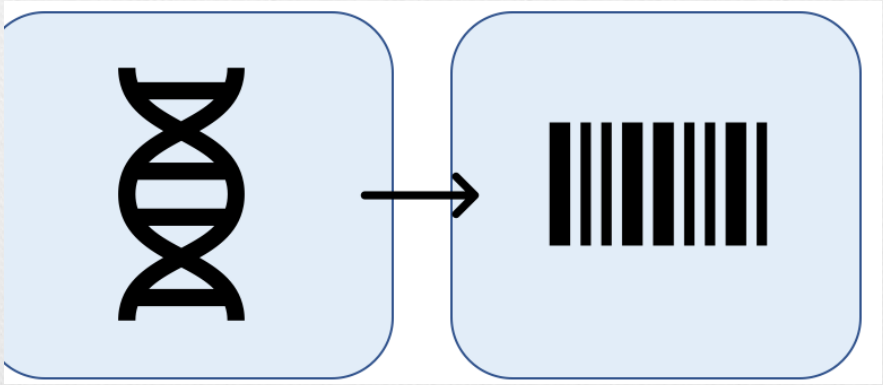
DNA Barcoding: How do we do it?

- Step 1. DNA extraction



Final product: genomic DNA

- Step 2. PCR amplification

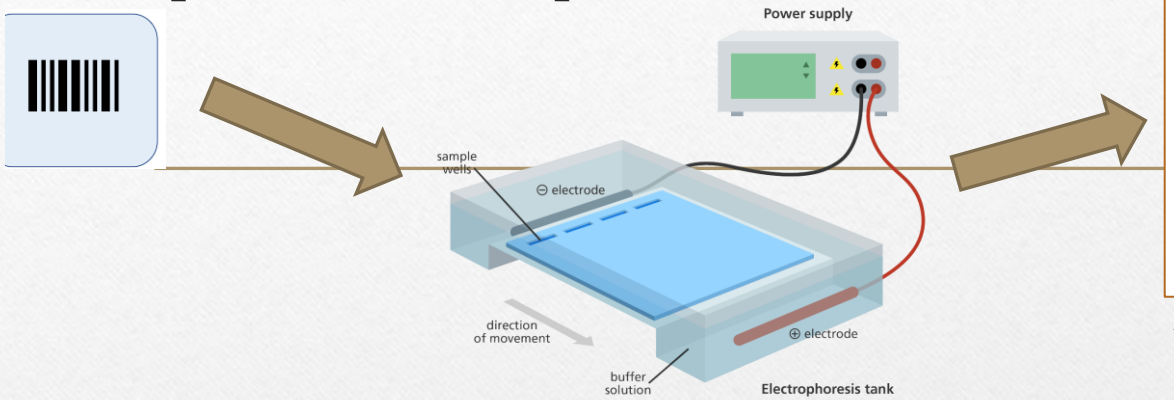


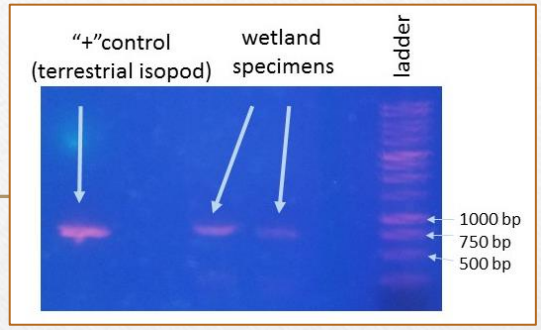
Final product: targeted piece of DNA

Piece of plant DNA, piece of insect mitochondrial DNA, etc.

DNA Barcoding: How do we do it?

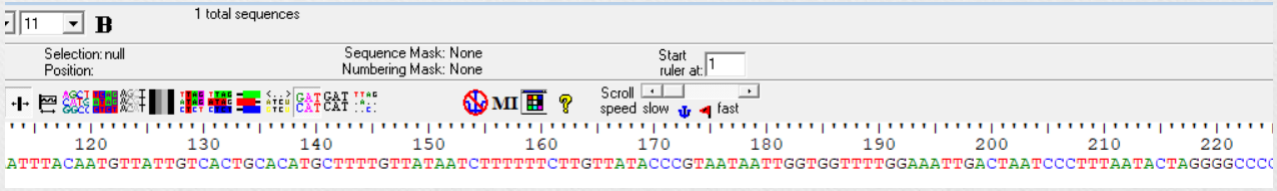
- Step 3. Gel electrophoresis





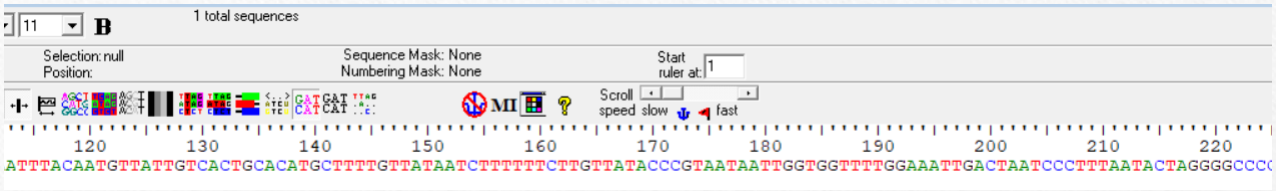
Verification of presence of targeted DNA

- Step 4. Sequencing



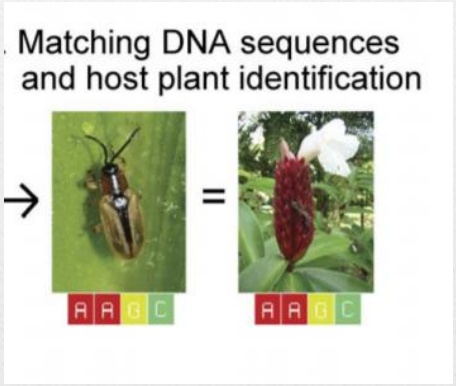
https://www.genewiz.com/

DNA Barcoding: What's next?

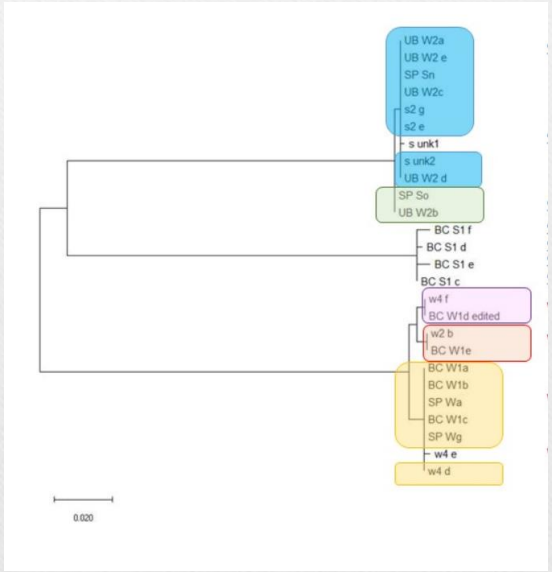


https://blast.ncbi.nlm.nih.gov/

Identification of species

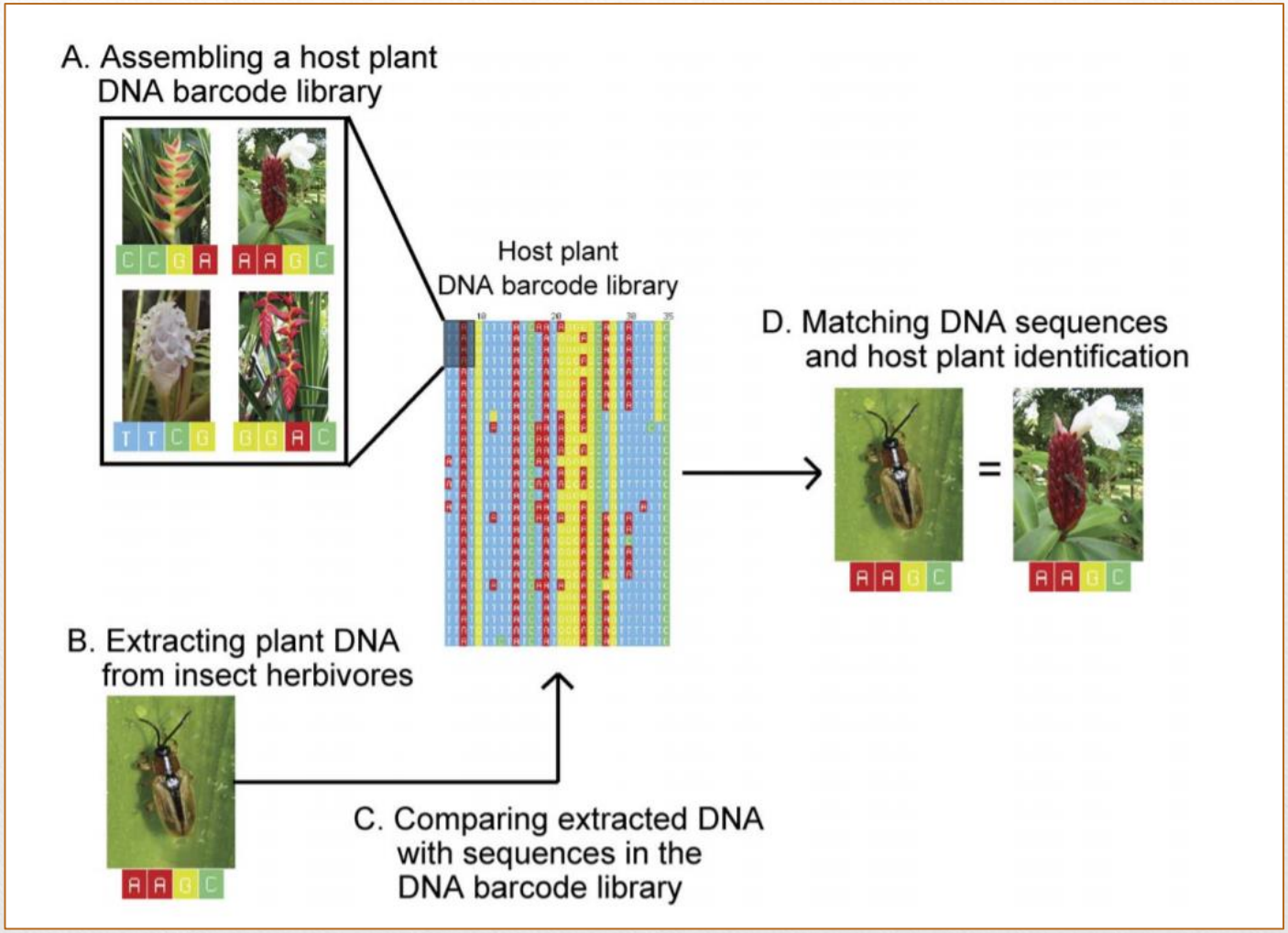


Identification of species interactions



Constructing phylogenetic relationships

Identification of plant-insect interactions



Garcia-Robledo et al. (2013)

Questions so far?

DNA Barcoding Protocols

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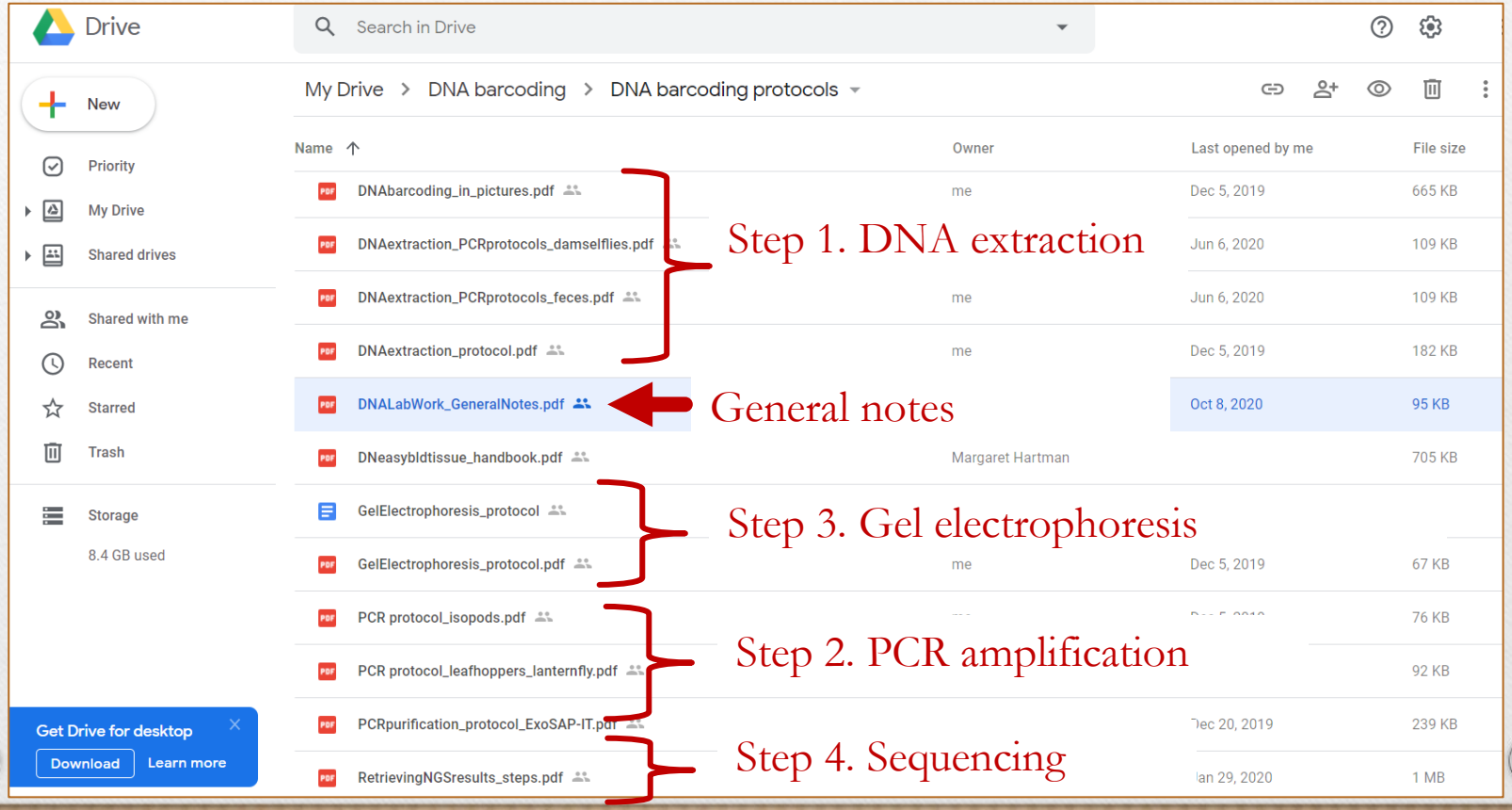
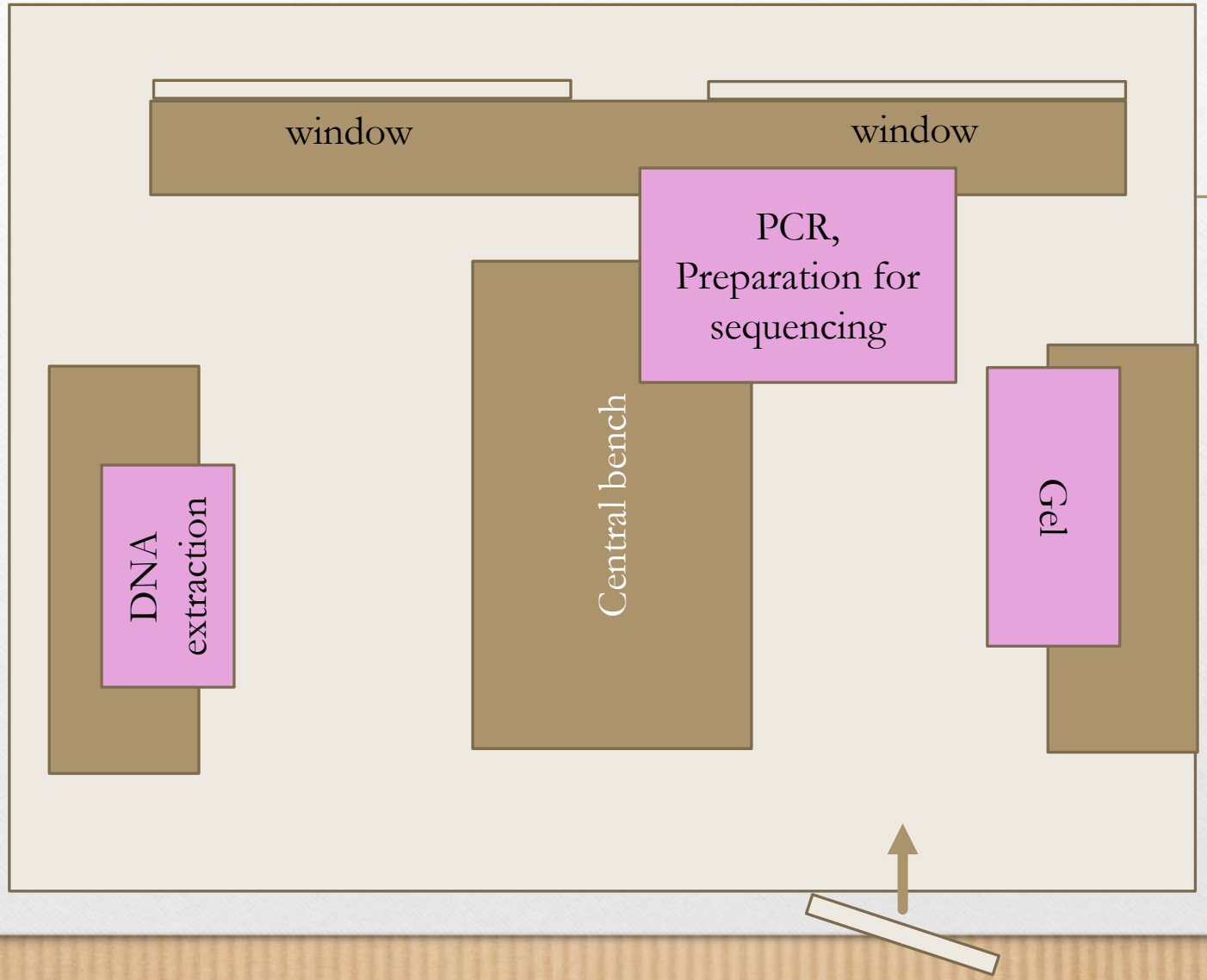


Room 4172


window
window
PCR,
Preparation for
sequencing
Central bench
DNA
extraction
Gel

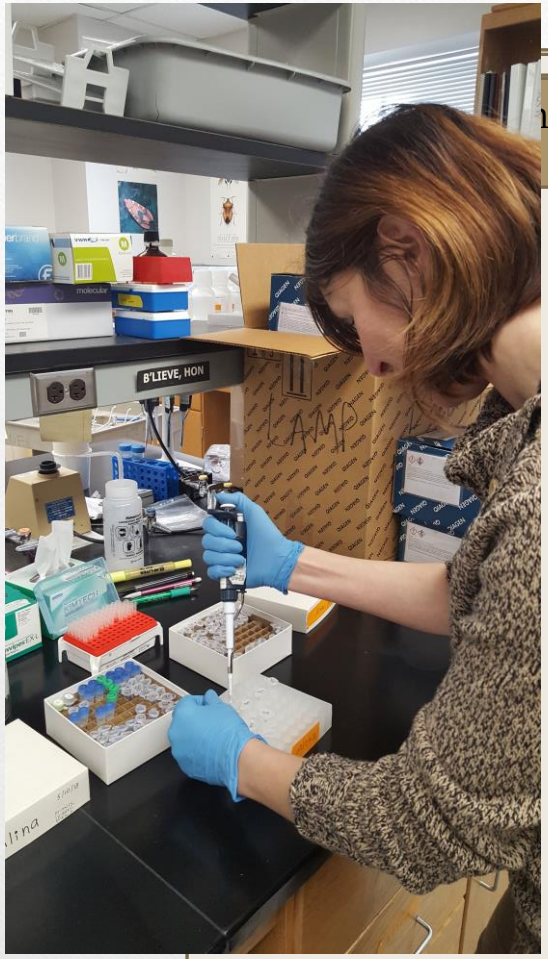
DNA extraction

window

DNA extraction

Central bench

PCR

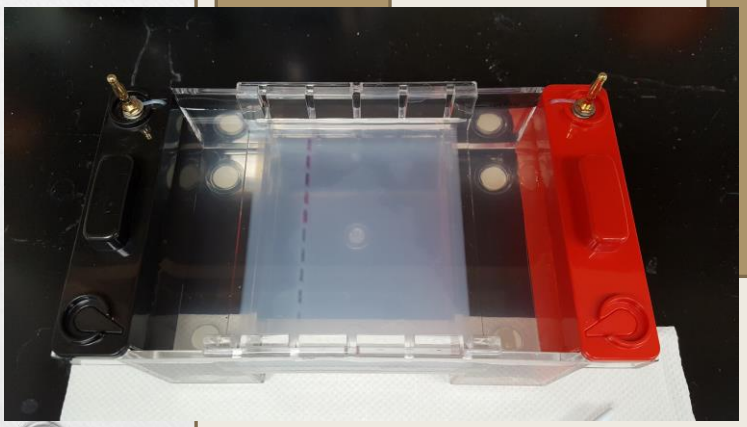


ndow

PCR, Preparation for sequencing

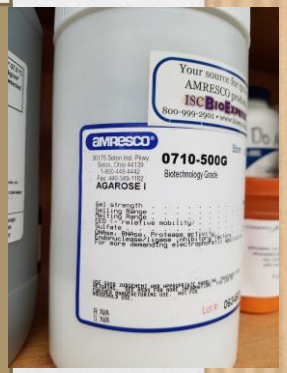
Central bench

Gel



Central bench

Gel



Sequencing

- Open an account at genewiz.com
- Follow the guidelines for sample preparations
- Samples drop-off: dropbox on the 2nd floor

Photo: Dr. Leslie Pick

- Results are ready on the next day (Sanger sequencing) or in ~3 weeks (NGS)
- Contact Genewiz customer service if you have any questions
- Contact me if you have more questions ☺

Our DNA barcoding experts

- Darsy, dsmith28@umd.edu
- Maggie, mehartma@umd.edu
- Nina, nina.e.mcg@gmail.com
- Hannah, hsutton1@terpmail.umd.edu

Helpful Resources

Textbook:

- Genetics: Analysis and Principles, Brooker et al, 6th edition

Coursera:

- Introduction to Genetics and Evolution: https://www.coursera.org/learn/genetics-evolution
- DNA decoded: https://www.coursera.org/learn/dna-decoded

Review papers on DNA barcoding:

- Taylor, H. R., & Harris, W. E. (2012). An emergent science on the brink of irrelevance: a review of the past 8 years of DNA barcoding. Molecular Ecology Resources, 12(3), 377-388.
- Li, X., Yang, Y., Henry, R. J., Rossetto, M., Wang, Y., & Chen, S. (2015). Plant DNA barcoding: from gene to genome. Biological Reviews, 90(1), 157-166.

Thank you!

Happy DNA barcoding!