

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

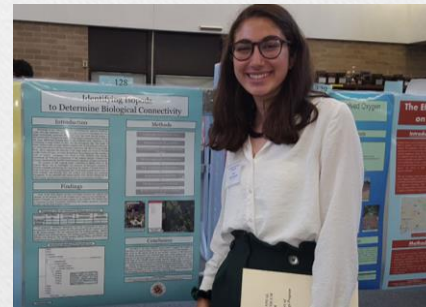
- Avanesyan, A., Sutton, H., and W.O. Lamp. (2020) Choosing an effective molecular approach to diet analysis of insect herbivores: a systematic review.

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 (*trnL*) 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 (*trnL*) 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 (*trnL*) 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 (*trnL*) 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 (*trnL*) 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 (*trnL*) 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 (*trnL*) gene, partial sequence; chloroplast. Direct Submission, *GenBank* Accession no. MK837073

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

DNA Barcoding Protocols

- Shared Google folder

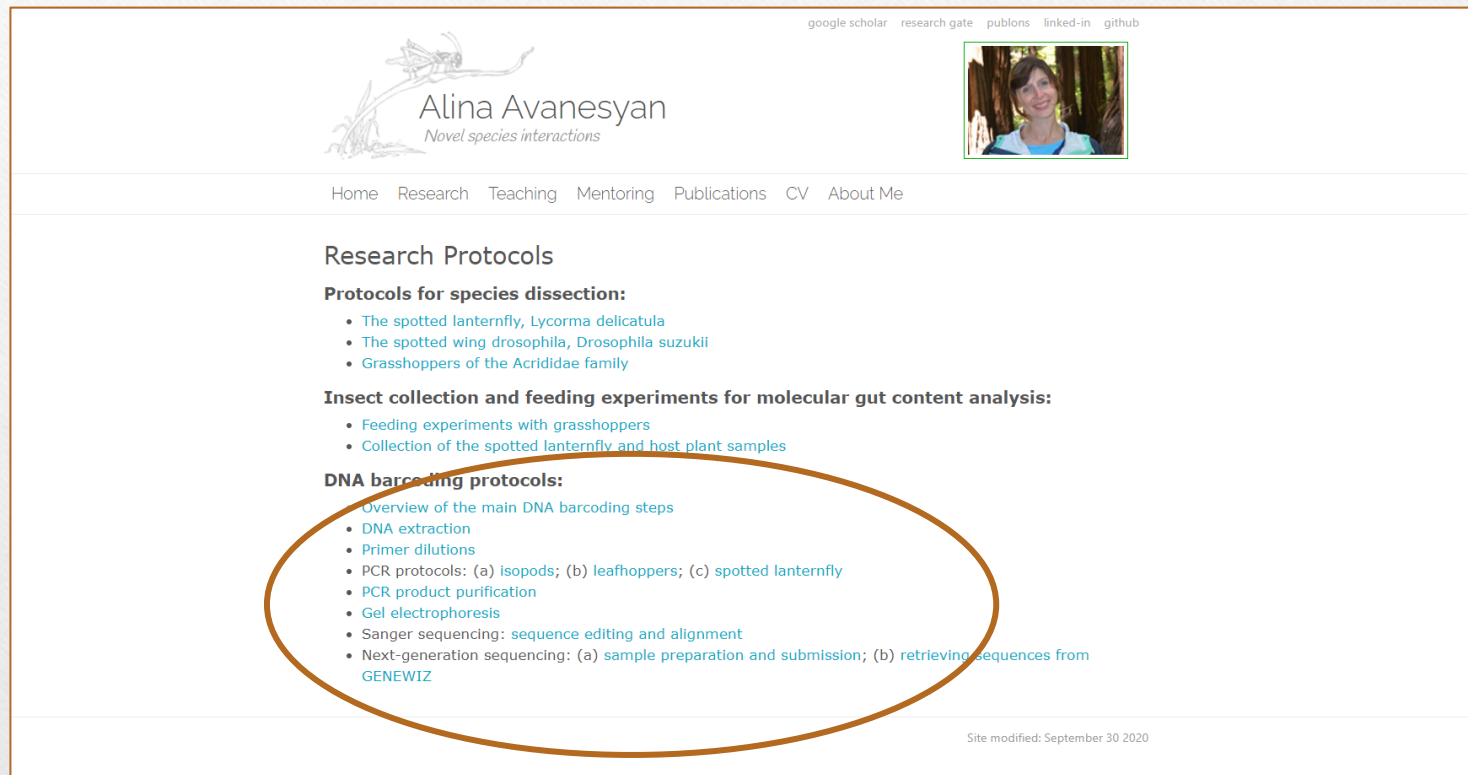
The screenshot shows a Google Drive interface. The left sidebar contains navigation options: 'New', 'Priority', 'My Drive', 'Shared drives', 'Shared with me', 'Recent', 'Starred', 'Trash', and 'Storage' (8.4 GB used). The main area displays the 'DNA barcoding protocols' folder. A table lists the files within the folder.

Name	Owner	Last opened by me	File size
DNAbarcoding_in_pictures.pdf	me	Dec 5, 2019	665 KB
DNAextraction_PCRprotocols_damselflies.pdf	me	Jun 6, 2020	109 KB
DNAextraction_PCRprotocols_feces.pdf	me	Jun 6, 2020	109 KB
DNAextraction_protocol.pdf	me	Dec 5, 2019	182 KB
DNALabWork_GeneralNotes.pdf	me	Oct 8, 2020	95 KB
DNeasybldtissue_handbook.pdf	Margaret Hartman		705 KB
GelElectrophoresis_protocol	Margaret Hartman		—
GelElectrophoresis_protocol.pdf	me	Dec 5, 2019	67 KB
PCR protocol_isopods.pdf	me	Dec 5, 2019	76 KB
PCR protocol_leafhoppers_lanternfly.pdf	me	May 17, 2019	92 KB
PCRpurification_protocol_ExoSAP-IT.pdf	me	Dec 20, 2019	239 KB
RetrievingNGSresults_steps.pdf	me	Jan 29, 2020	1 MB

At the bottom left, there is a blue button that says 'Get Drive for desktop' with a close icon, and below it, 'Download' and 'Learn more' links.

DNA Barcoding Protocols

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



The screenshot displays the website of Alina Avanesyan, titled "Alina Avanesyan Novel species interactions". The navigation bar includes links for Home, Research, Teaching, Mentoring, Publications, CV, and About Me. The "Research Protocols" section is highlighted with a brown oval. It contains three sub-sections: "Protocols for species dissection" with links to Lycorma delicatula, Drosophila suzukii, and Acrididae grasshoppers; "Insect collection and feeding experiments for molecular gut content analysis" with links to grasshopper feeding and lanternfly collection; and "DNA barcoding protocols" which is circled. The DNA barcoding protocols include an overview, extraction, dilutions, PCR protocols for isopods, leafhoppers, and lanternflies, product purification, electrophoresis, Sanger sequencing, and next-generation sequencing.

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](#)

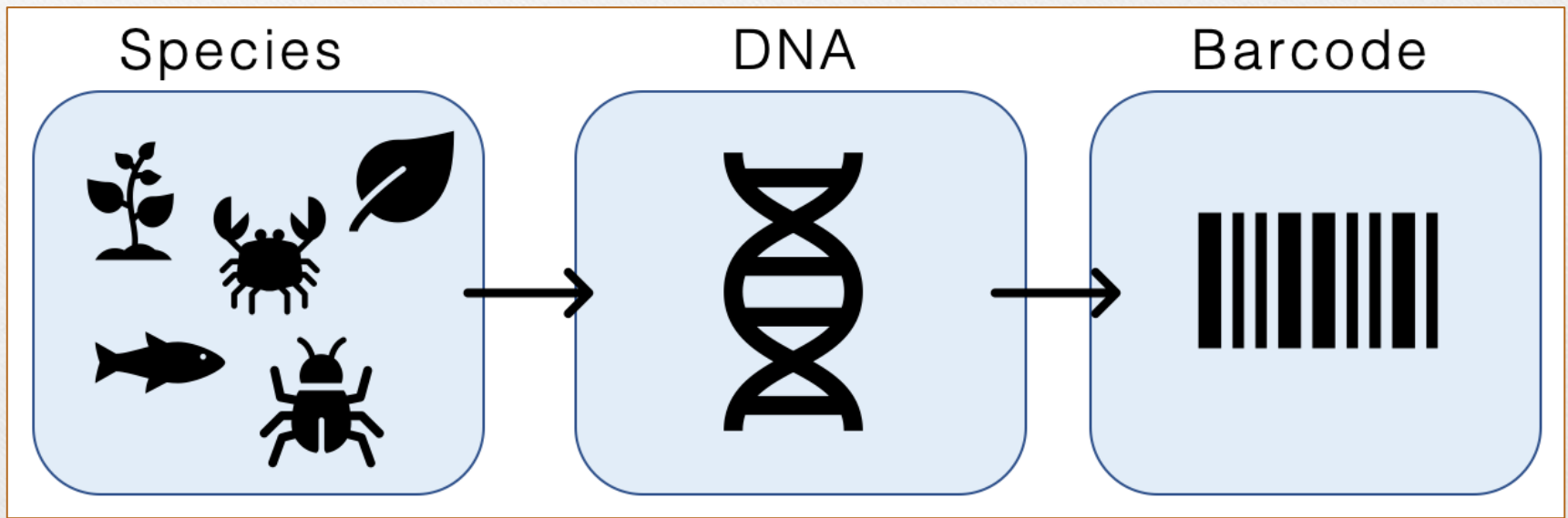
DNA barcoding protocols:

- [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: \(a\) \[sample preparation and submission\]\(#\); \(b\) \[retrieving sequences from GENEWIZ\]\(#\)](#)

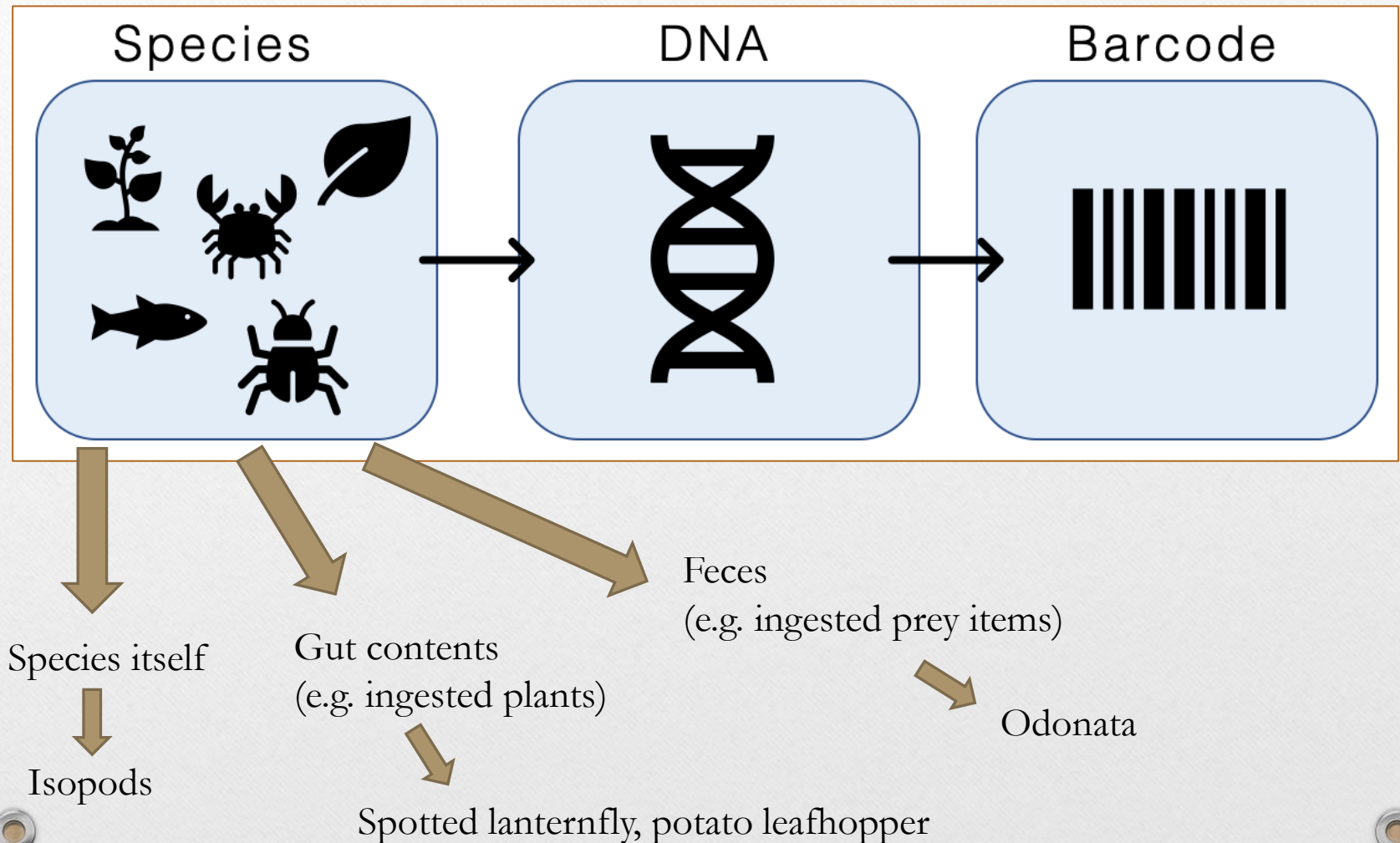
Site modified: September 30 2020

DNA Barcoding

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

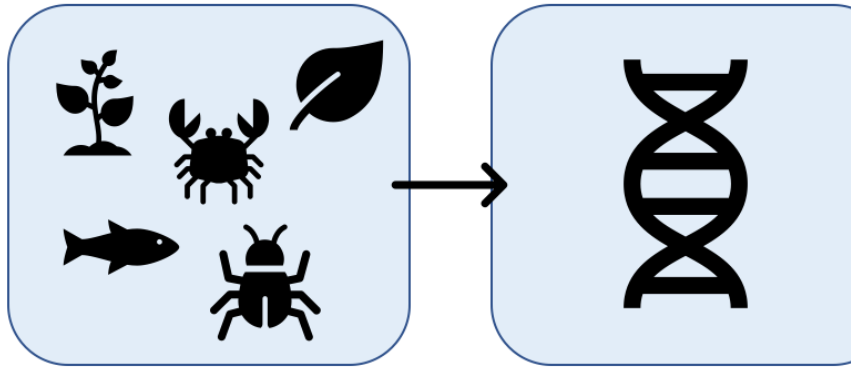


Sources of DNA



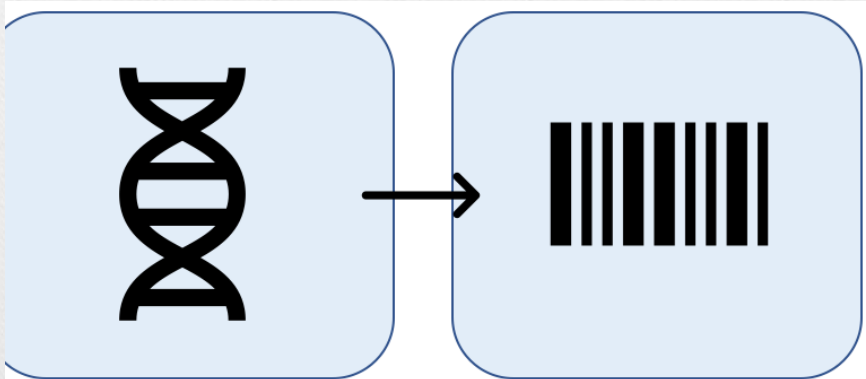
DNA Barcoding: How do we do it?

- Step 1. DNA extraction



Final product: genomic DNA

- Step 2. PCR amplification



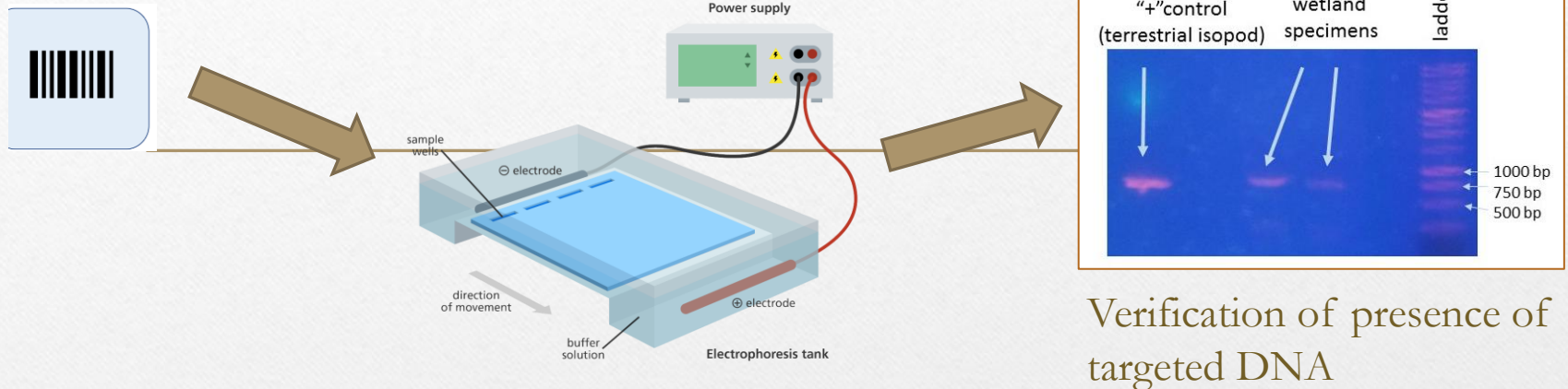
Final product: targeted piece of DNA



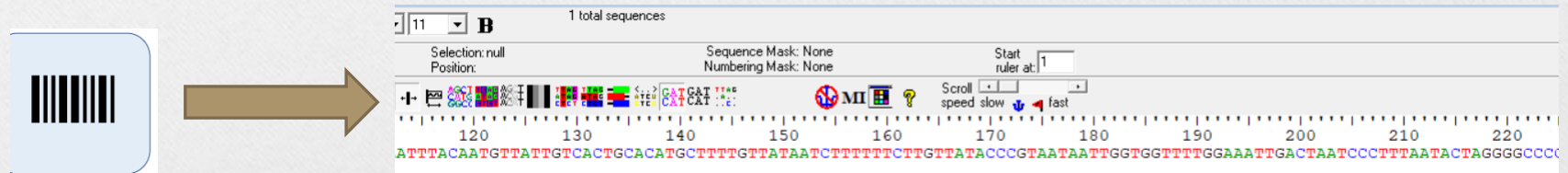
Piece of plant DNA, piece of insect mitochondrial DNA, etc.

DNA Barcoding: How do we do it?

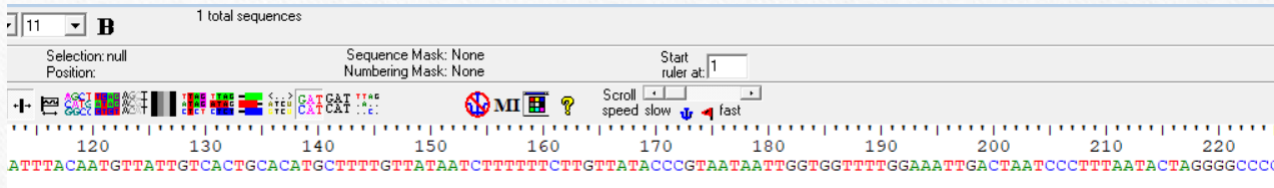
- Step 3. Gel electrophoresis



- Step 4. Sequencing



DNA Barcoding: What's next?



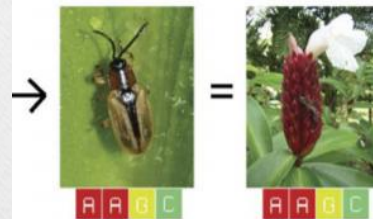
Web BLAST



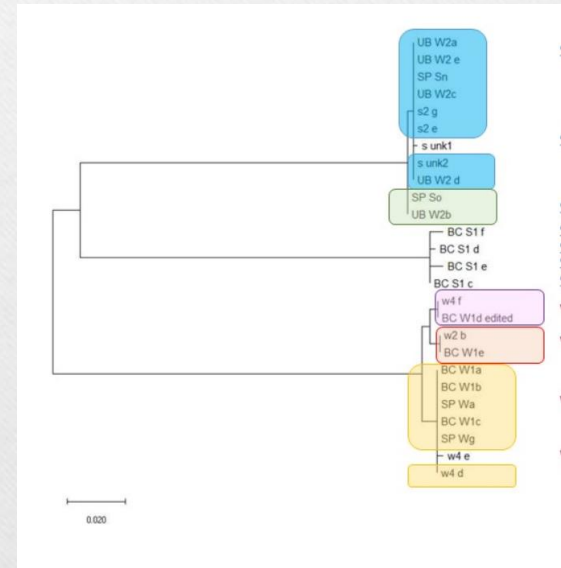
<https://blast.ncbi.nlm.nih.gov/>

Identification of species

Matching DNA sequences and host plant identification



Identification of species
interactions



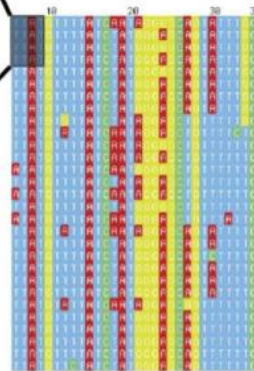
Constructing phylogenetic
relationships

Identification of plant-insect interactions

A. Assembling a host plant DNA barcode library



Host plant DNA barcode library

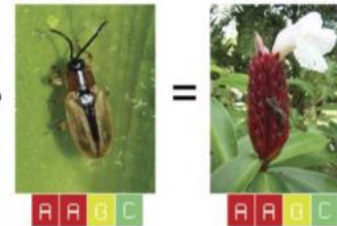


B. Extracting plant DNA from insect herbivores



C. Comparing extracted DNA with sequences in the DNA barcode library

D. Matching DNA sequences and host plant identification



Questions so far?

DNA Barcoding Protocols

- Shared Google folder

My Drive > DNA barcoding > DNA barcoding protocols

Name	Owner	Last opened by me	File size
DNAbarcoding_in_pictures.pdf	me	Dec 5, 2019	665 KB
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DNAextraction_PCRprotocols_feces.pdf	me	Jun 6, 2020	109 KB
DNAextraction_protocol.pdf	me	Dec 5, 2019	182 KB
DNALabWork_GeneralNotes.pdf		Oct 8, 2020	95 KB
DNeasybldtissue_handbook.pdf	Margaret Hartman		705 KB
GelElectrophoresis_protocol			
GelElectrophoresis_protocol.pdf	me	Dec 5, 2019	67 KB
PCR protocol_isopods.pdf			76 KB
PCR protocol_leafhoppers_lanternfly.pdf			92 KB
PCRpurification_protocol_ExoSAP-IT.pdf		Dec 20, 2019	239 KB
RetrievingNGSresults_steps.pdf		Jan 29, 2020	1 MB

Step 1. DNA extraction

General notes

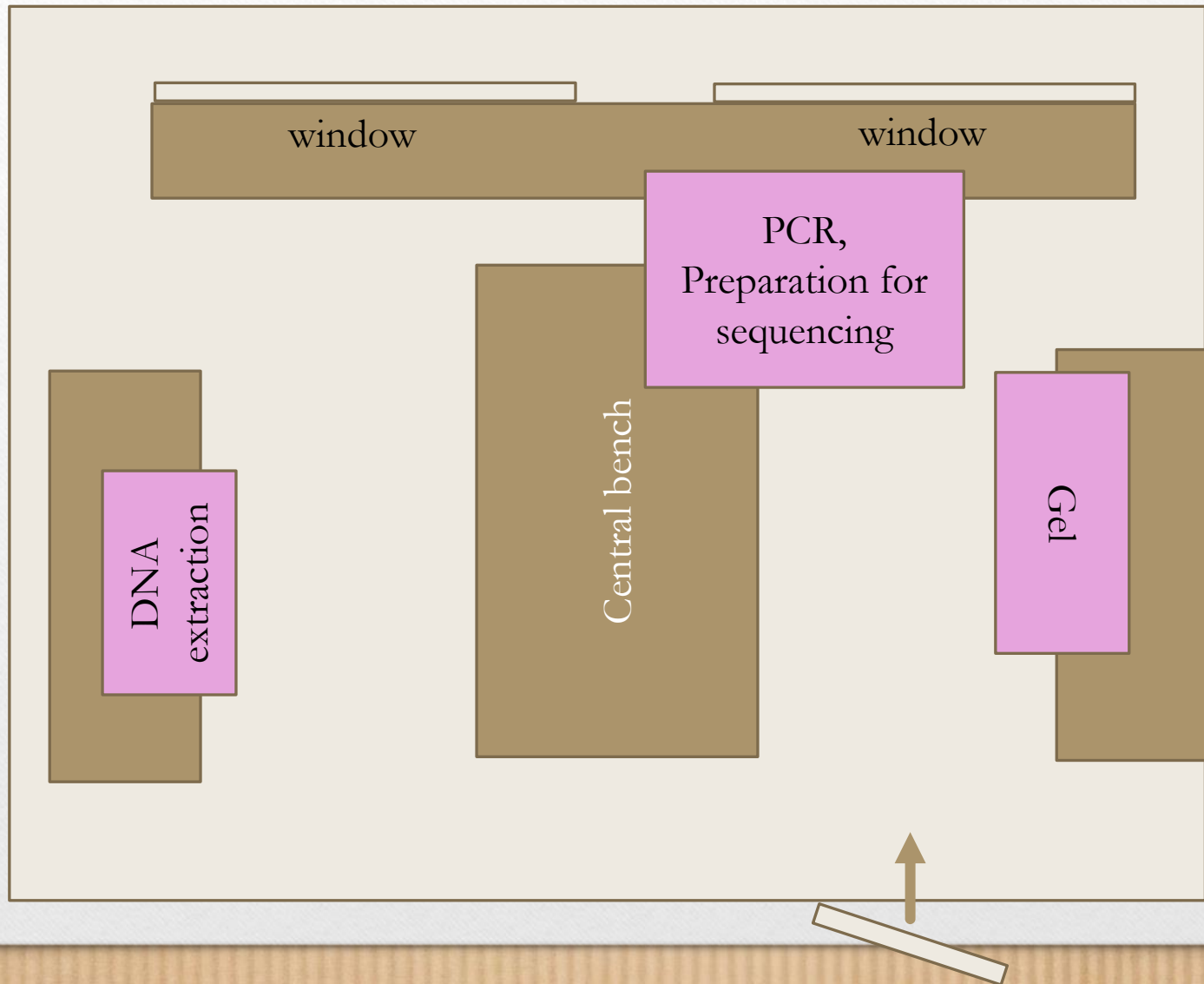
Step 3. Gel electrophoresis

Step 2. PCR amplification

Step 4. Sequencing

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Room 4172

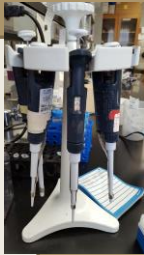


DNA extraction

window



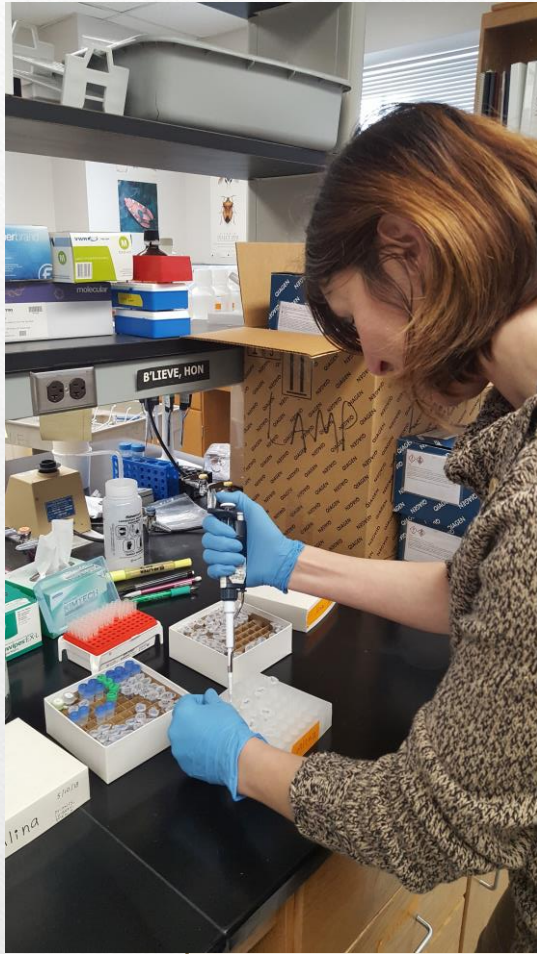
DNA
extraction



Central bench



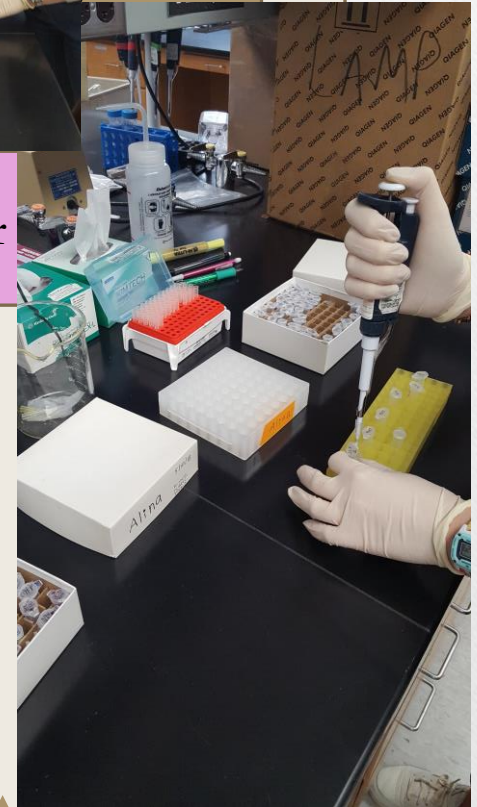
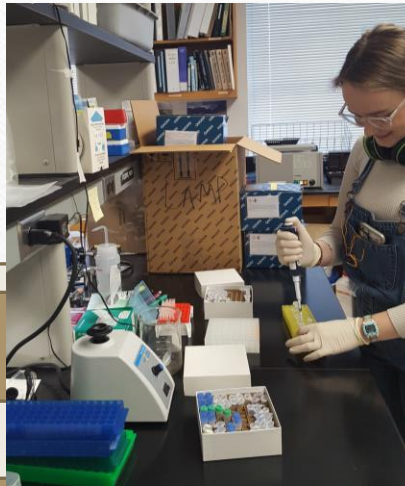
PCR



indow

Central bench

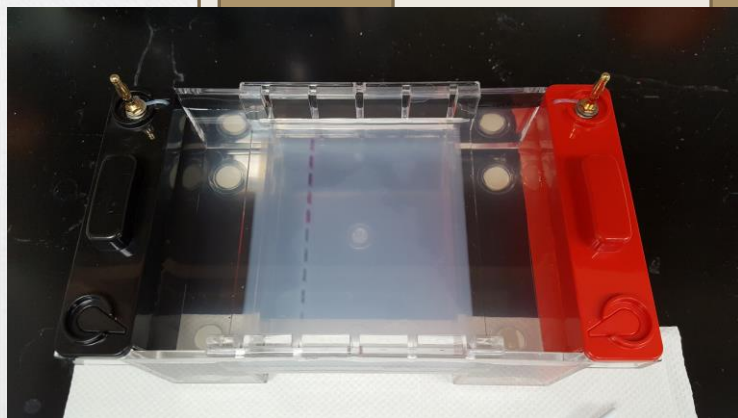
PCR,
Preparation for
sequencing



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!