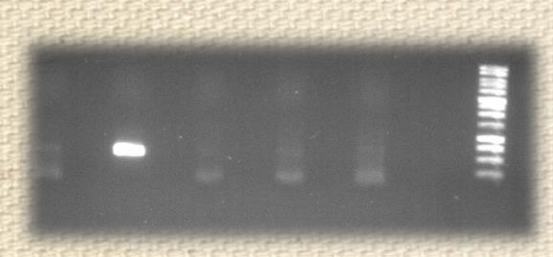
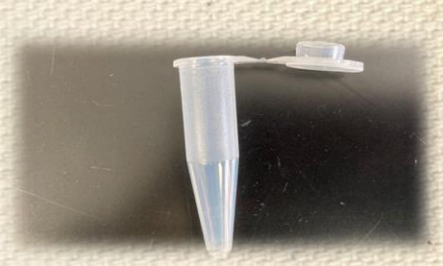
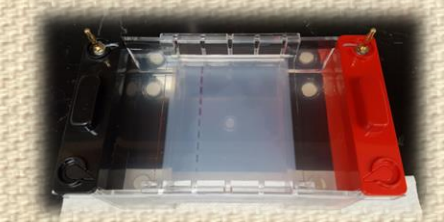


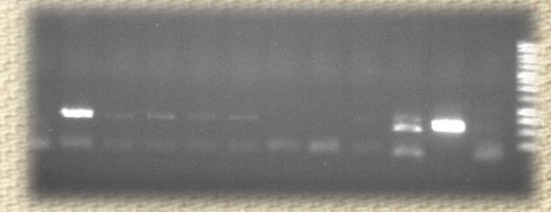





DNA Barcoding For Everyone:

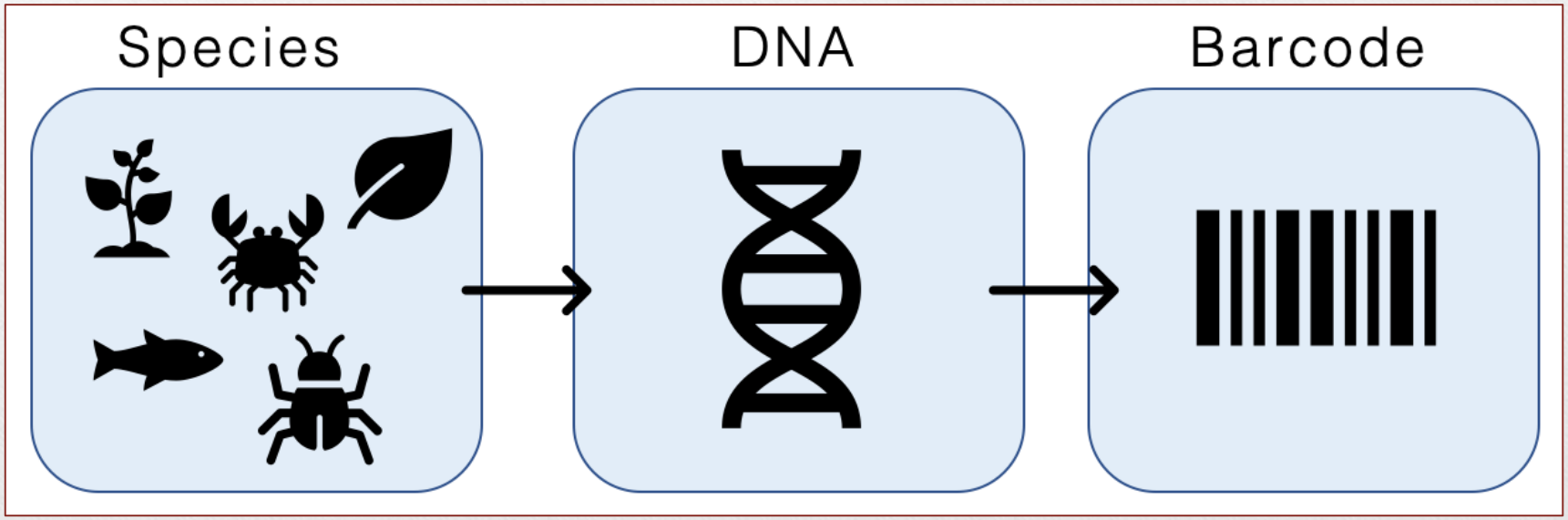
Course Overview





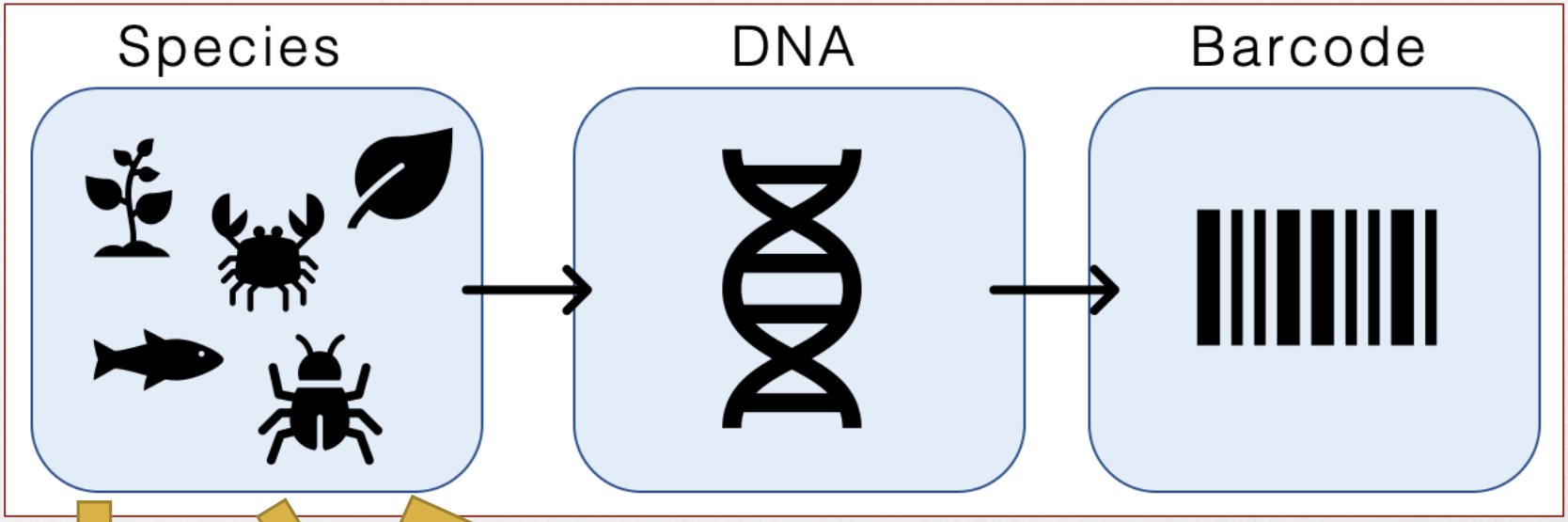
DNA Barcoding

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



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

Sources of DNA



Species itself

Isopods

Gut contents
(e.g. ingested plants)

Spotted lanternfly, potato leafhopper

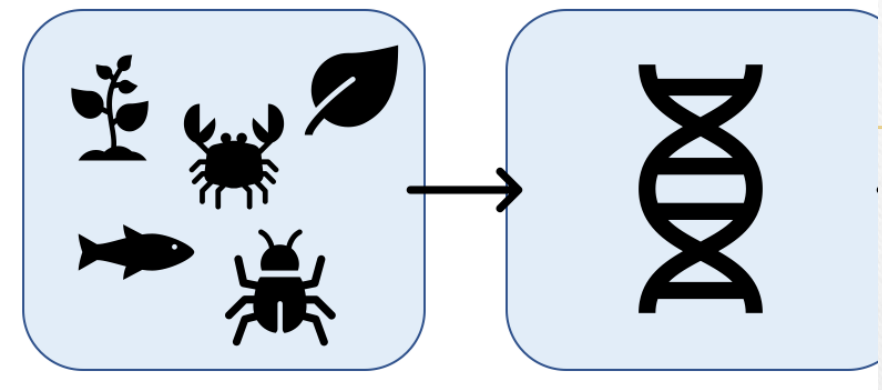
Feces
(e.g. ingested prey items)

Odonata

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

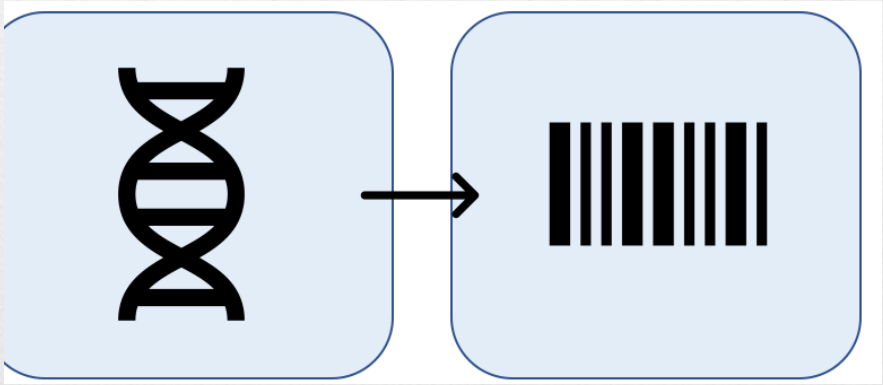
DNA Barcoding: How do we do it?

- Step 1. DNA extraction



Final product: genomic DNA

- Step 2. PCR amplification



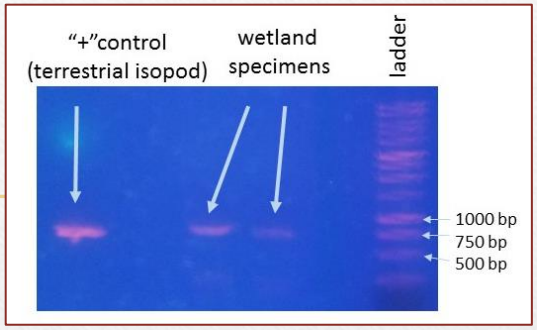
Final product: targeted piece of DNA

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

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

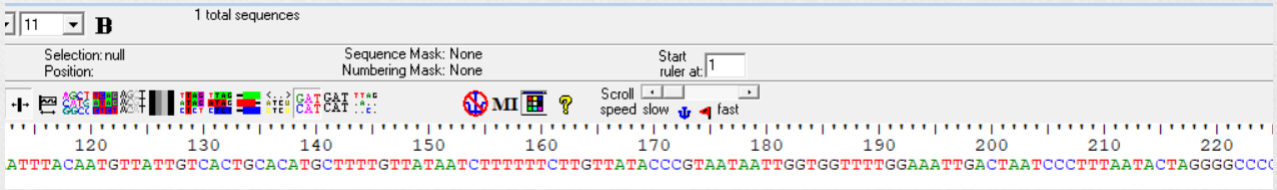
DNA Barcoding: How do we do it?

- Step 3. Gel electrophoresis



Verification of presence of targeted DNA

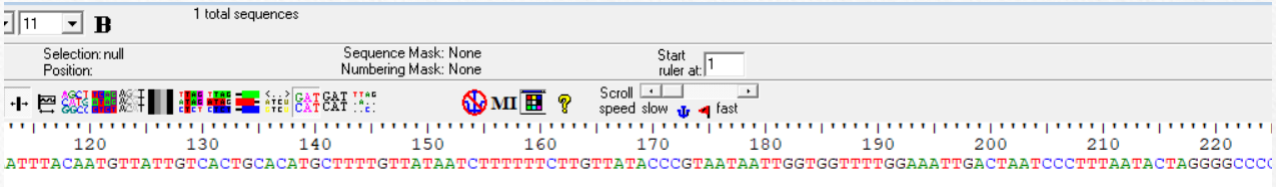
- Step 4. Sequencing



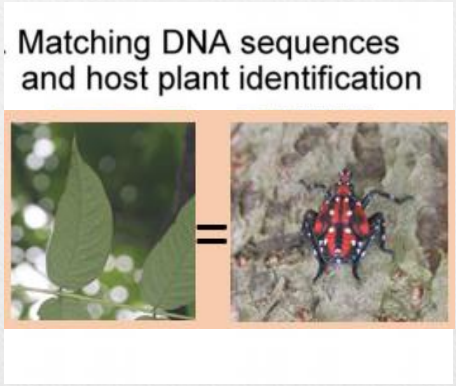
AZENTA LIFE SCIENCES

Images: https://en.wikipedia.org/wiki/DNA_barcoding
https://www.genewiz.com/

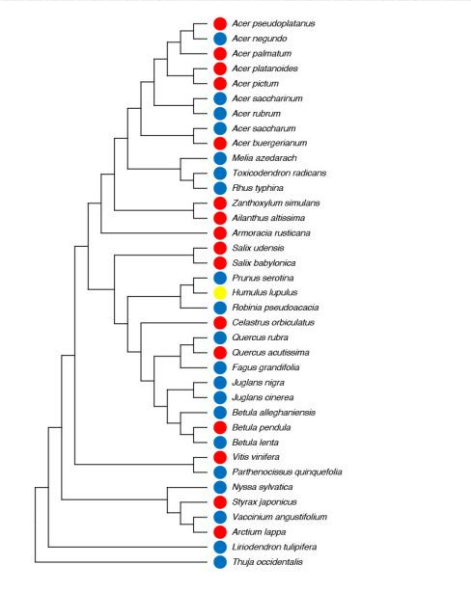
DNA Barcoding: What's next?



Identification of species



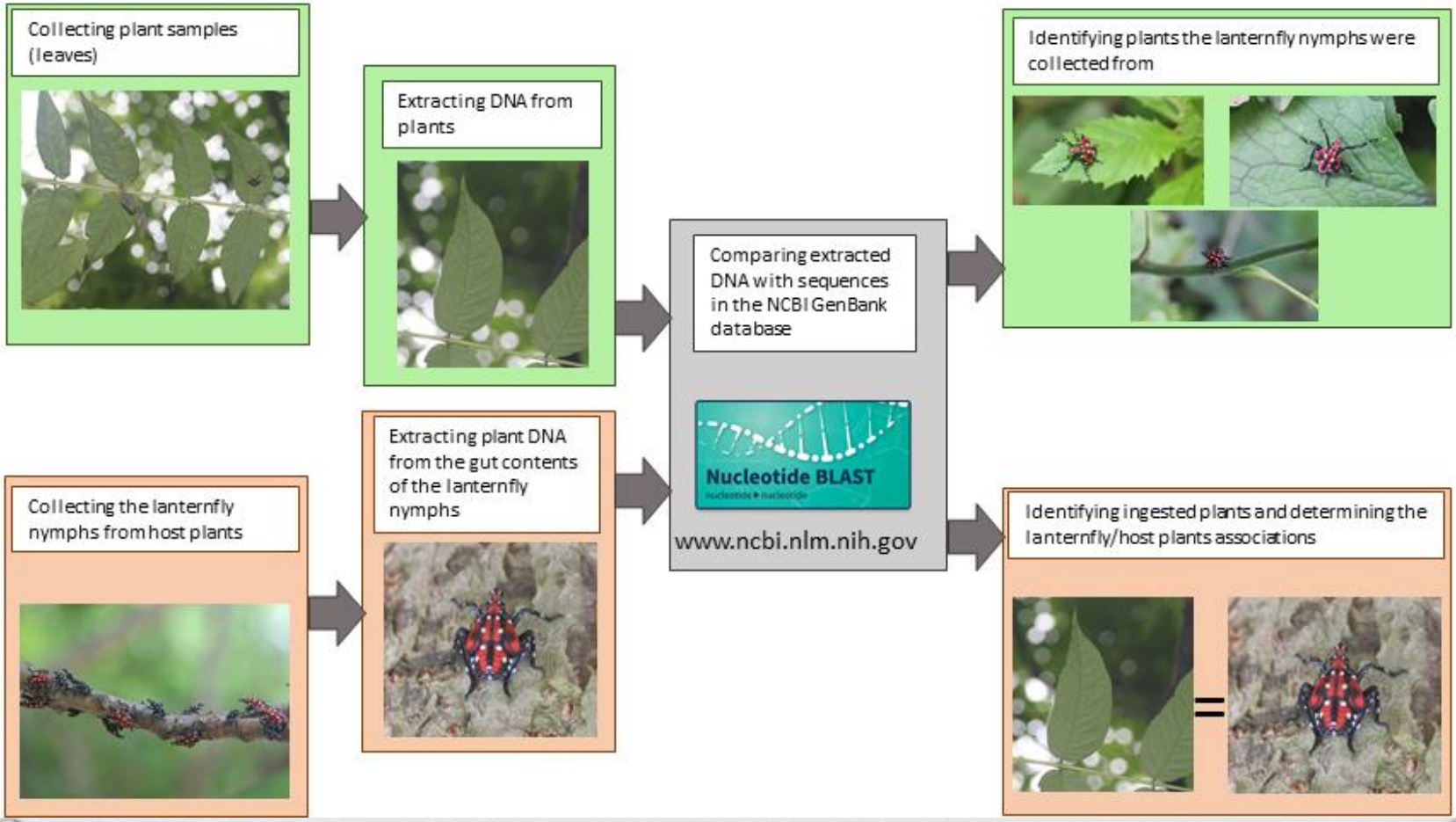
Identification of species interactions



Constructing phylogenetic relationships

Images: https://blast.ncbi.nlm.nih.gov/
Avanesyan et al. 2020
Avanesyan et al. (in prep)

Identification of plant-insect interactions

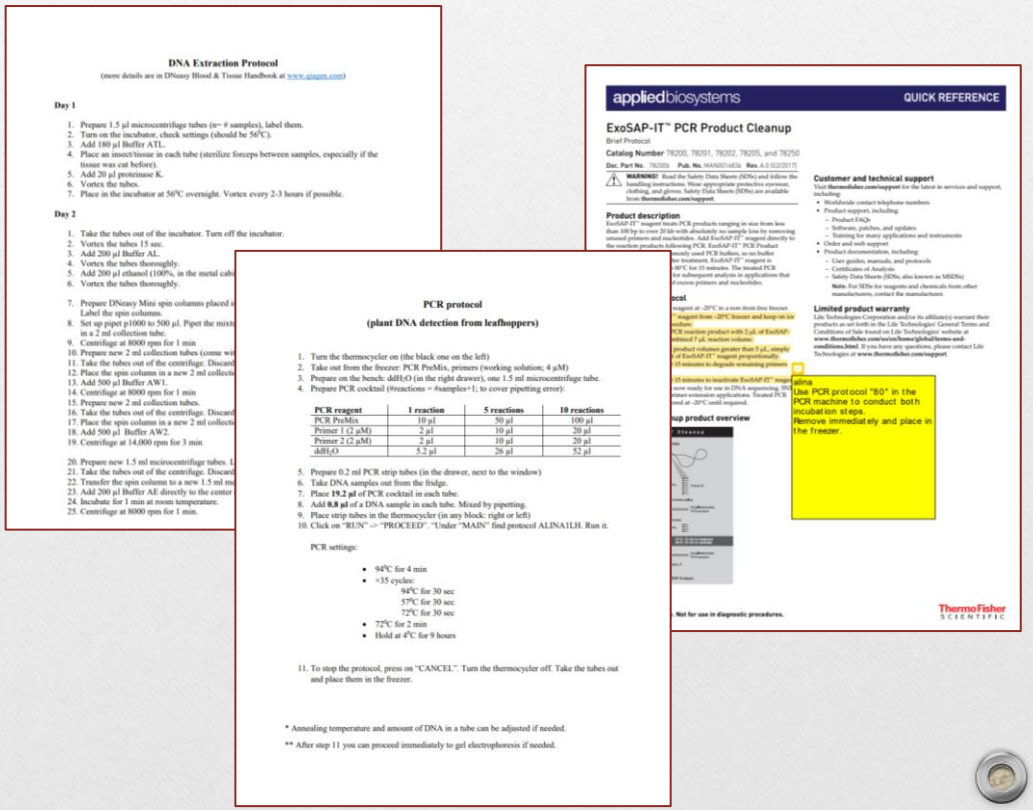


Avanesyan et al. 2020

DNA Barcoding Protocols

- There are protocols for each DNA barcoding step

  - Tissue preparation
  - DNA extraction
  - PCR
  - DNA purification
  - Gel electrophoresis
  - Sequencing analysis



DNA Extraction Protocol

(more details are in DNeasy Blood & Tissue Handbook at www.qiagen.com)

Day 1

1. Prepare 1.5 µl microcentrifuge tubes (n= # samples), label them.
2. Turn on the incubator, check settings (should be 56°C).
3. Add 180 µl Buffer ATL.
4. Place an insect/tissue in each tube (sterilize forceps between samples, especially if the tissue was cut before).
5. Add 20 µl proteinase K.
6. Vortex the tubes.
7. Place in the incubator at 56°C overnight. Vortex every 2-3 hours if possible.

Day 2

1. Take the tubes out of the incubator. Turn off the incubator.
2. Vortex the tubes 15 sec.
3. Add 200 µl Buffer AL.
4. Vortex the tubes thoroughly.
5. Add 200 µl ethanol (100%, in the metal cab
6. Vortex the tubes thoroughly.

7. Prepare DNeasy Mini spin columns placed i
   Label the spin columns.
8. Set up pipet p1000 to 500 µl. Pipet the mixt
   in a 2 ml collection tube.
9. Centrifuge at 8000 rpm for 1 min
10. Prepare new 2 ml collection tubes (come wit
11. Take the tubes out of the centrifuge. Discard
12. Place the spin column in a new 2 ml collecti
13. Add 500 µl Buffer AW1.
14. Centrifuge at 8000 rpm for 1 min
15. Prepare new 2 ml collection tubes.
16. Take the tubes out of the centrifuge. Discard
17. Place the spin column in a new 2 ml collecti
18. Add 500 µl Buffer AW2.
19. Centrifuge at 14,000 rpm for 3 min

20. Prepare new 1.5 ml microcentrifuge tubes. L
21. Take the tubes out of the centrifuge. Discard
22. Transfer the spin column to a new 1.5 ml mi
23. Add 200 µl Buffer AE directly to the center
24. Incubate for 1 min at room temperature.
25. Centrifuge at 8000 rpm for 1 min.

PCR protocol

(plant DNA detection from leafhoppers)

1. Turn the thermocycler on (the black one on the left)
2. Take out from the freezer: PCR PreMix, primers (working solution; 4 µM)
3. Prepare on the bench: ddH$_2$O (in the right drawer), one 1.5 ml microcentrifuge tube.
4. Prepare PCR cocktail (#reactions = #samples+1; to cover pipetting error):

PCR reagent	1 reaction	5 reactions	10 reactions
PCR PreMix	10 µl	50 µl	100 µl
Primer 1 (2 µM)	2 µl	10 µl	20 µl
Primer 2 (2 µM)	2 µl	10 µl	20 µl
ddH$_2$O	5.2 µl	26 µl	52 µl

5. Prepare 0.2 ml PCR strip tubes (in the drawer, next to the window)
6. Take DNA samples out from the fridge.
7. Place **19.2 µl** of PCR cocktail in each tube.
8. Add **0.8 µl** of a DNA sample in each tube. Mixed by pipetting.
9. Place strip tubes in the thermocycler (in any block: right or left)
10. Click on "RUN" -> "PROCEED". "Under "MAIN" find protocol ALINA1LH. Run it.

PCR settings:

- 94°C for 4 min
- ×35 cycles:
  - 94°C for 30 sec
  - 57°C for 30 sec
  - 72°C for 30 sec
- 72°C for 2 min
- Hold at 4°C for 9 hours

11. To stop the protocol, press on "CANCEL". Turn the thermocycler off. Take the tubes out and place them in the freezer.

* Annealing temperature and amount of DNA in a tube can be adjusted if needed.

** After step 11 you can proceed immediately to gel electrophoresis if needed.

appliedbiosystems QUICK REFERENCE

ExoSAP-IT™ PCR Product Cleanup

Brief Protocol

Catalog Number 78200, 78201, 78202, 78205, and 78250

Doc. Part No. 78203b Pub. No. MAN0014636 Rev. A.0 02/2017

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

ExoSAP-IT™ reagent treats PCR products ranging in size from less than 100 bp to over 20 kb with absolutely no sample loss by removing unused primers and nucleotides. Add ExoSAP-IT™ reagent directly to the reaction products following PCR. ExoSAP-IT™ PCR Product

Customer and technical support

Visit thermofisher.com/support for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

alina
Use PCR protocol "80" in the PCR machine to conduct both incubation steps.
Remove immediately and place in the freezer.

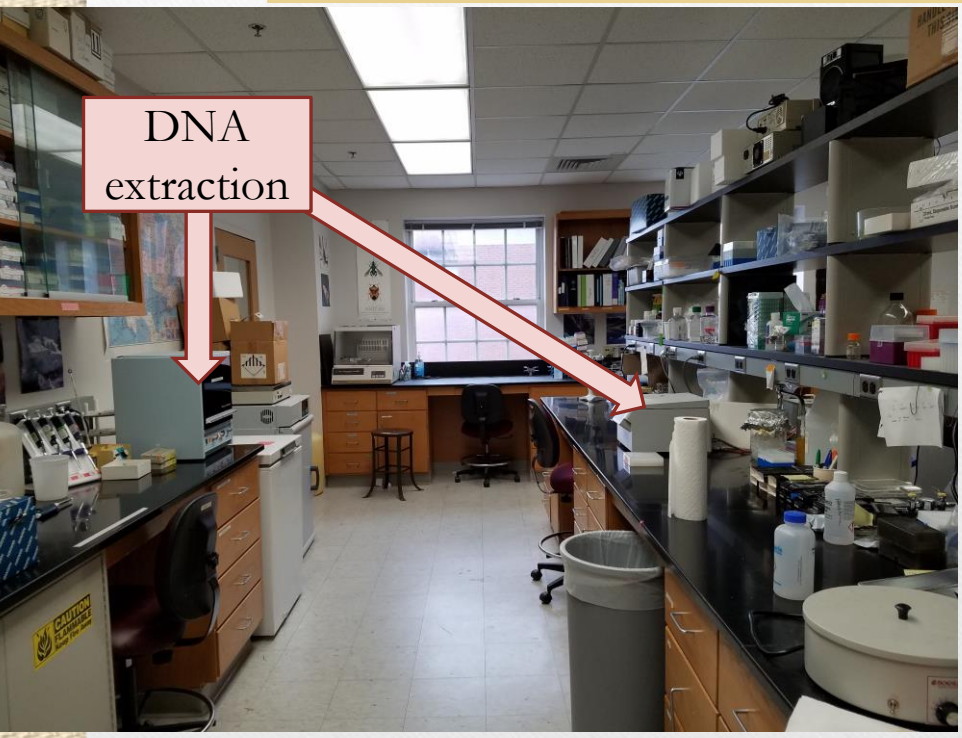
up product overview

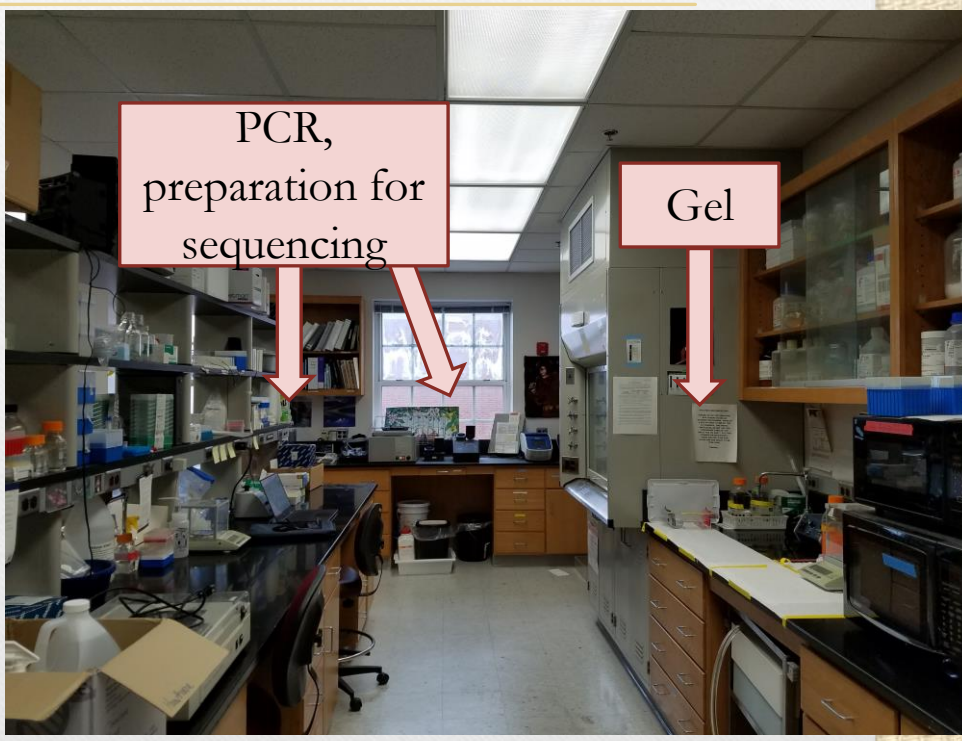
Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

DNA lab

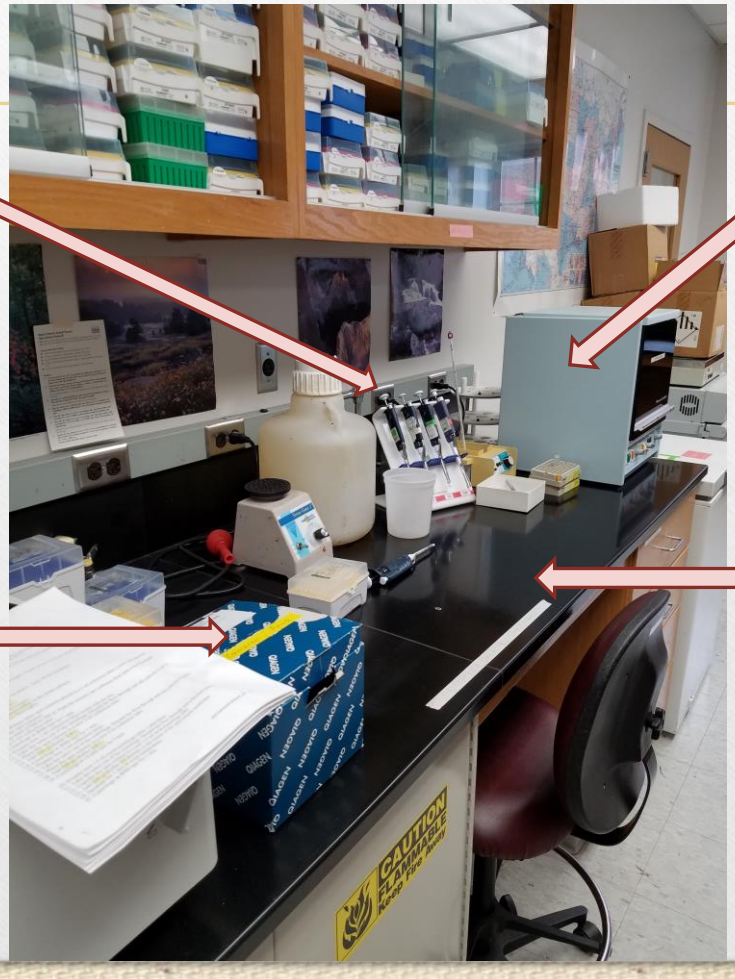
- There are designated places ("stations") for each DNA barcoding step

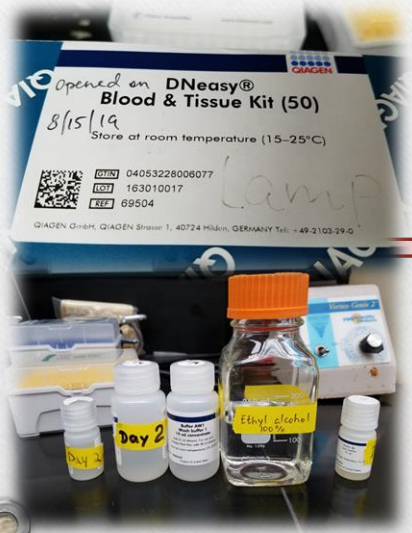


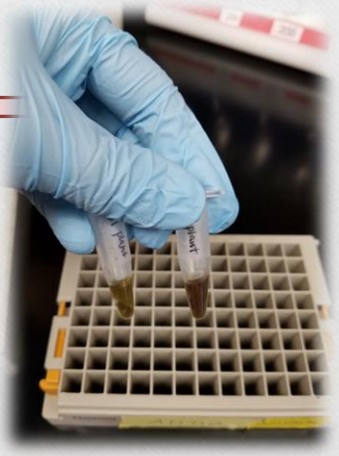


Dr. David Hawthorne's lab: 4172 Plant Science Building, Department of Entomology, University of Maryland

DNA extraction station







Dr. David Hawthorne's lab: 4172 Plant Science Building, Department of Entomology, University of Maryland

PCR station

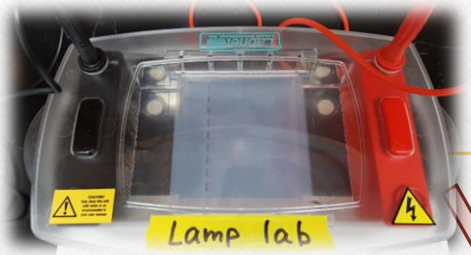
Lab bench for sample preparations

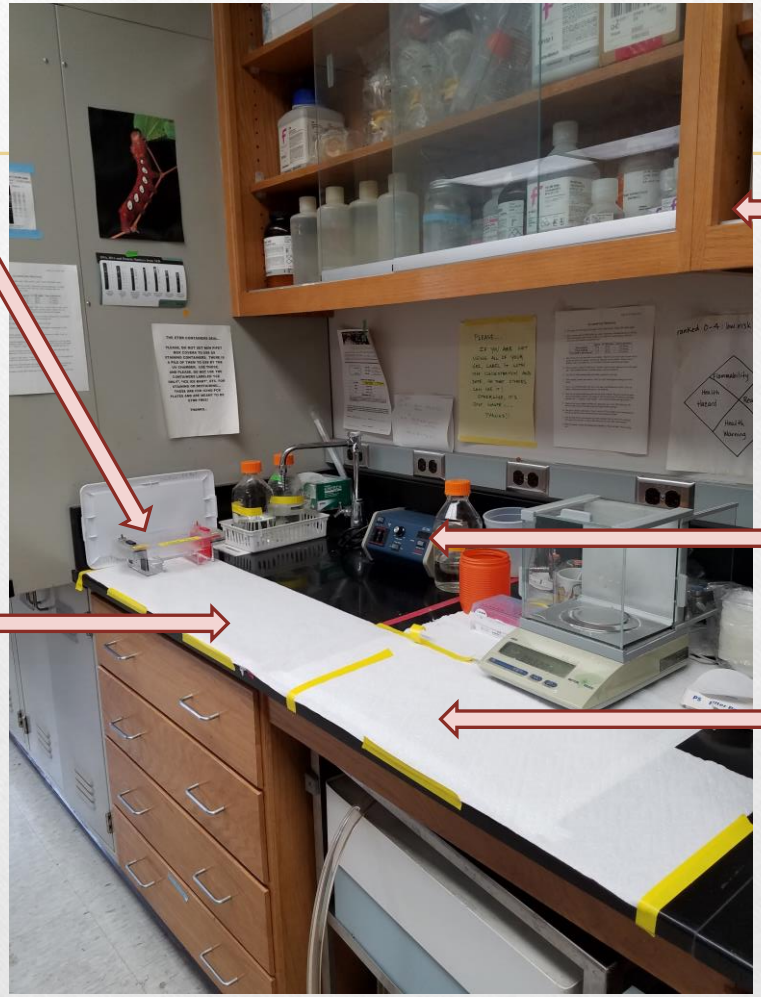
Thermocycler, or PCR machine

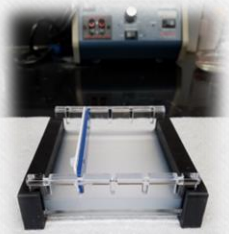
- PCR
- DNA purification
- Preparing samples for sequencing

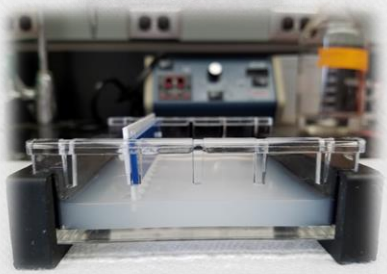
Dr. David Hawthorne's lab: 4172 Plant Science Building, Department of Entomology, University of Maryland

Gel station





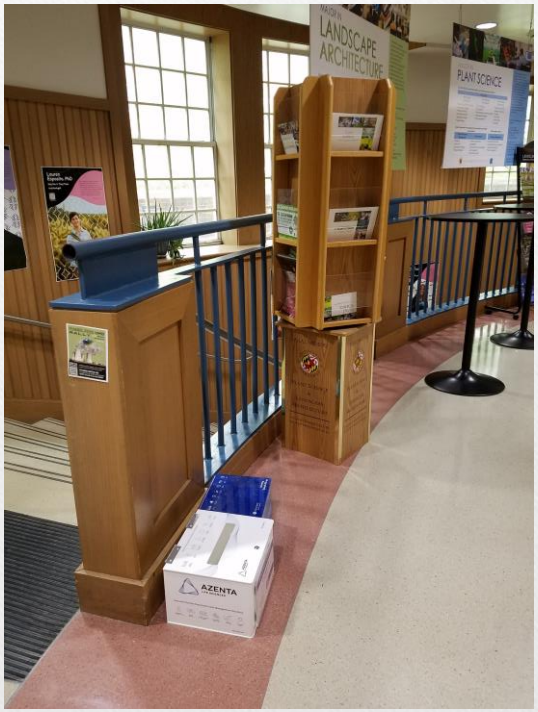


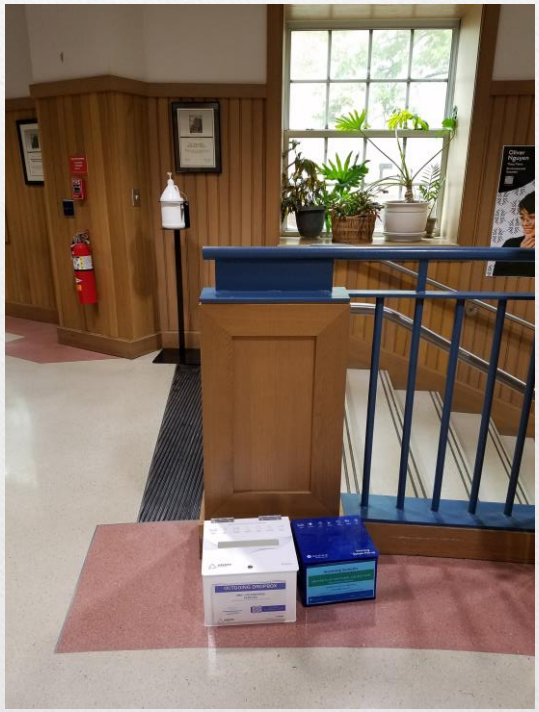


Dr. David Hawthorne's lab: 4172 Plant Science Building, Department of Entomology, University of Maryland

Sequencing

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





- Results are ready on the next day (Sanger sequencing) or in ~2-3 weeks (NGS)
- Contact Azenta customer service if you have any questions

Helpful Resources

Textbook:

- Genetics: Analysis and Principles, Brooker et al, 6th edition, 2017

Coursera:

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

Cold Spring Harbor Laboratory, DNA Learning Center: https://dnabarcoding101.org/lab/index.html

Review papers on DNA barcoding:

- Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. Trends in ecology & evolution, 24(2), 110-117.
- 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.

Our research articles:

- Avanesyan, A., Sutton, H., and W.O. Lamp. (2021) Choosing an effective PCR-based approach for diet analysis of insect herbivores: A systematic review. Journal of Economic Entomology 114(3), 1035–1046.
- Avanesyan, A., Illahi, N. and W.O. Lamp. (2021) Detecting ingested host plant DNA in potato leafhopper, *Empoasca fabae*: potential use of molecular markers for gut content analysis. Journal of Economic Entomology, 114(1), 2021, 472–475
- 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.

Thank you!

Happy DNA barcoding!

Image and video credits

- Videos: recording and editing by Alina Avanesyan
- Photos: preparing and editing by Hannah Sutton, Anya Wilkinson, and Alina Avanesyan
- DNA extraction protocol using Qiagen kit: modified from the manufacturer's protocol; the original protocol can be found at www.qiagen.com
- Videos were recorded and photos were taken in Dr. David Hawthorne's lab: 4172 Plant Science Building, Department of Entomology, University of Maryland, College Park, MD

Acknowledgements

- We thank Dr. David Hawthorne (Department of Entomology, University of Maryland) for providing lab equipment and lab space for our DNA barcoding work; for providing lab space to take the photos and record the videos needed for developing this course; and for continuous support and encouragement!