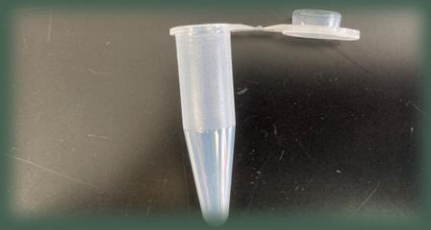


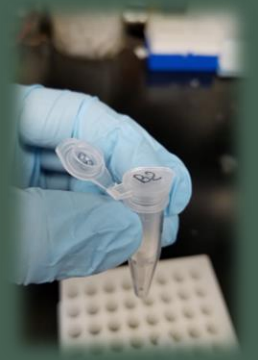


# DNA Extraction:

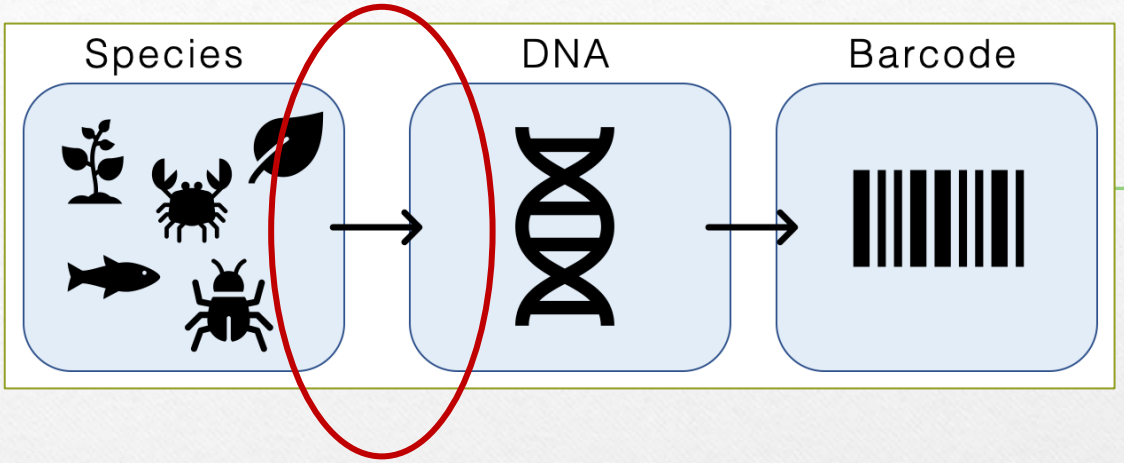
## Step-by-Step







# Isolating Genomic DNA




Final product: genomic DNA

- Insect DNA (mitochondrial DNA, nuclear DNA, etc.)
- Ingested plant DNA (chloroplast DNA, nuclear DNA, etc.)
- Gut microbiota DNA
- DNA of any parasitic organisms (fungi, nematodes, insect parasitoids, etc.)

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

# DNA Extraction Equipment and Materials

Incubator

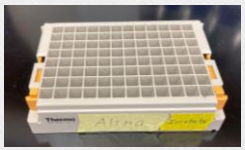

Vortex

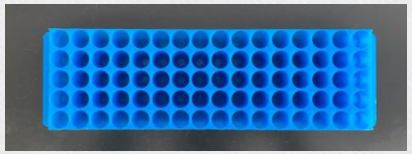

Centrifuge


Qiagen Kit


Pipettes

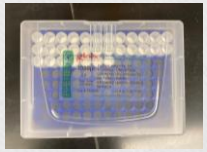
Racks





Microcentrifuge tube 1.5 mL

Pipette tips



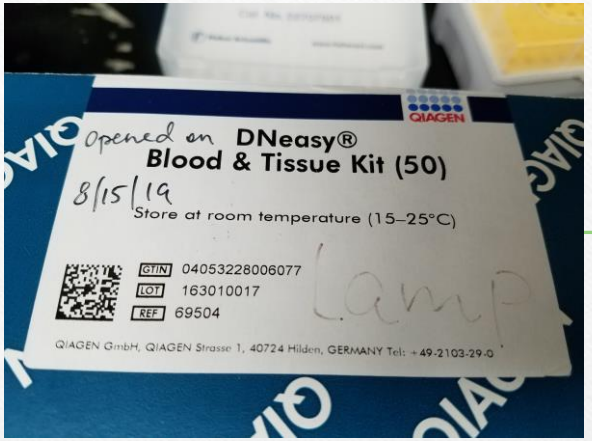
Mortar and pestle (optional)

Marker

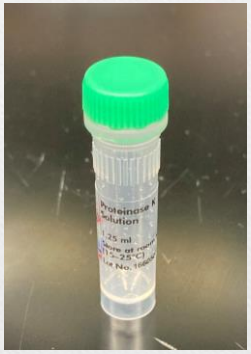

Storage box

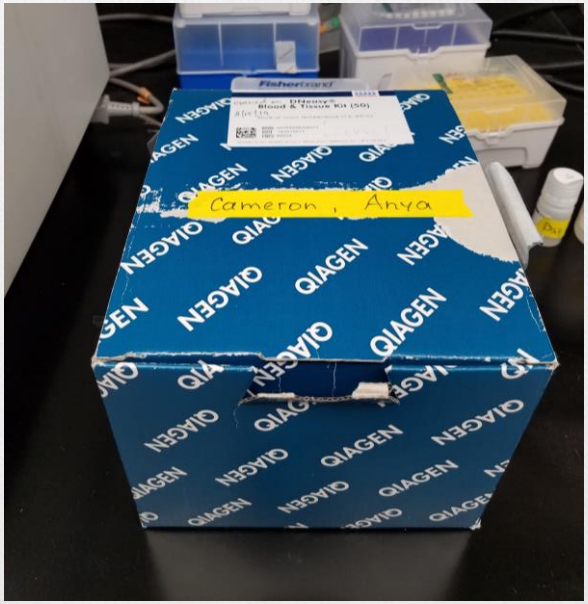
# DNA Extraction Kit

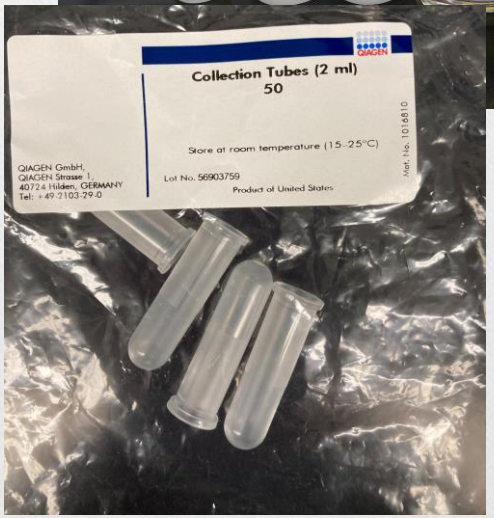
Qiagen DNeasy Blood & Tissue Kit
(www.qiagen.com)










# DNA Extraction Protocol

Day 1
15-30 min

Day 2
30-60 min

**DNA Extraction Protocol**

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

**Day 1**

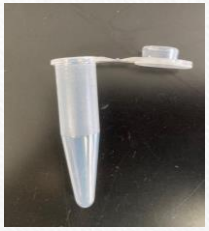
1. Prepare 1.5 µl microcentrifuge tubes (n= # samples), label them.
2. Turn on the incubator, check settings (should be 56$^0$C).
3. Add 180 µl Buffer ATL.
4. Place an insect/tissue in each tube (sterilize forceps between samples, especially if the tissue was cut before).
5. Add 20 µl proteinase K.
6. Vortex the tubes.
7. Place in the incubator at 56$^0$C overnight. Vortex every 2-3 hours if possible.

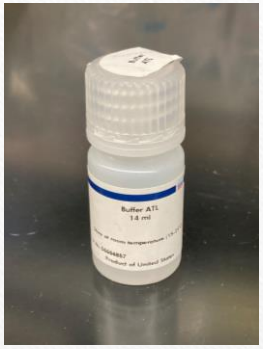
Samples can stay in the incubator for 2-3 hours

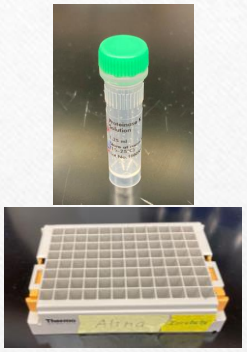
**Day 2**

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 cabinet)
6. Vortex the tubes thoroughly.
7. Prepare DNeasy Mini spin columns placed in a 2 ml collection tubes (come with the kit). Label the spin columns.
8. Set up pipet p1000 to 500 µl. Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube.
9. Centrifuge at 8000 rpm for 1 min
10. Prepare new 2 ml collection tubes (come with the kit).
11. Take the tubes out of the centrifuge. Discard the flow-through and old collection tubes.
12. Place the spin column in a new 2 ml collection tube.
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 the flow-through and old collection tubes.
17. Place the spin column in a new 2 ml collection tube.
18. Add 500 µl Buffer AW2.
19. Centrifuge at 14,000 rpm for 3 min
20. Prepare new 1.5 ml mcirocentrifuge tubes. Label them.
21. Take the tubes out of the centrifuge. Discard the flow-through and collection tubes.
22. Transfer the spin column to a new 1.5 ml mcirocentrifuge tube.
23. Add 200 µl Buffer AE directly to the center of the spin column membrane.
24. Incubate for 1 min at room temperature.
25. Centrifuge at 8000 rpm for 1 min.

# Day 1





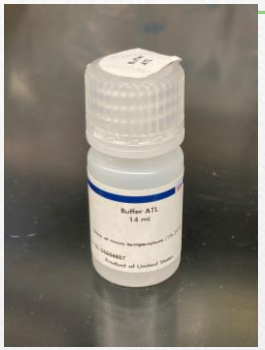


Pipettes:
p1000
p200

# Day 1


Buffer ATL

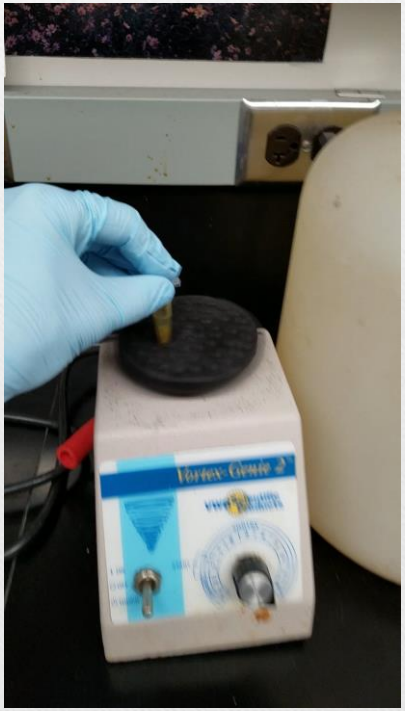
proteinase K

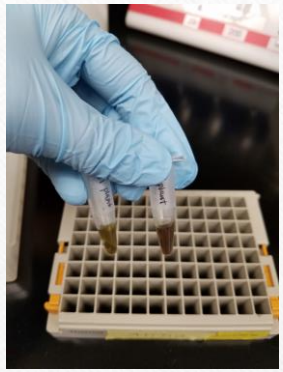




180 µl

20 µl



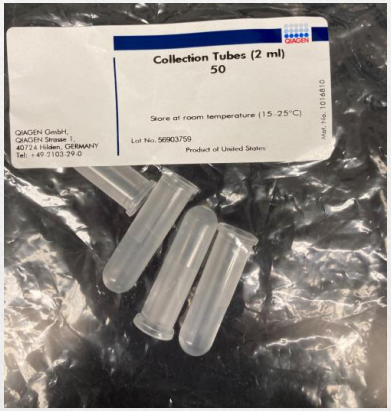

1. Prepare 1.5 µl microcentrifuge tubes (n= # samples), label them.
2. Turn on the incubator, check settings (should be 56$^0$C).
3. Add 180 µl Buffer ATL.
4. Place a plant/tissue in each tube.
5. Add 20 µl proteinase K.
6. Vortex the tubes.
7. Place in the incubator at 56$^0$C overnight (or for 2-3 hours). Vortex every 2-3 hours if possible

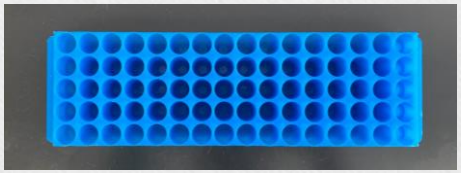
# Day 2

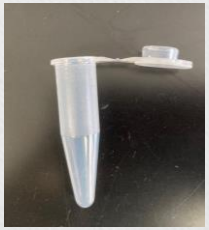

Day 2
Ethyl alcohol
100%


Vortex-Genie 2
VWR Scientific Products


eppendorf
Centrifuge 5417C


Collection Tubes (2 ml)
50
Store at room temperature (15-25°C)
QIAGEN GmbH
QIAGEN Strasse 1
40724 Hilden, GERMANY

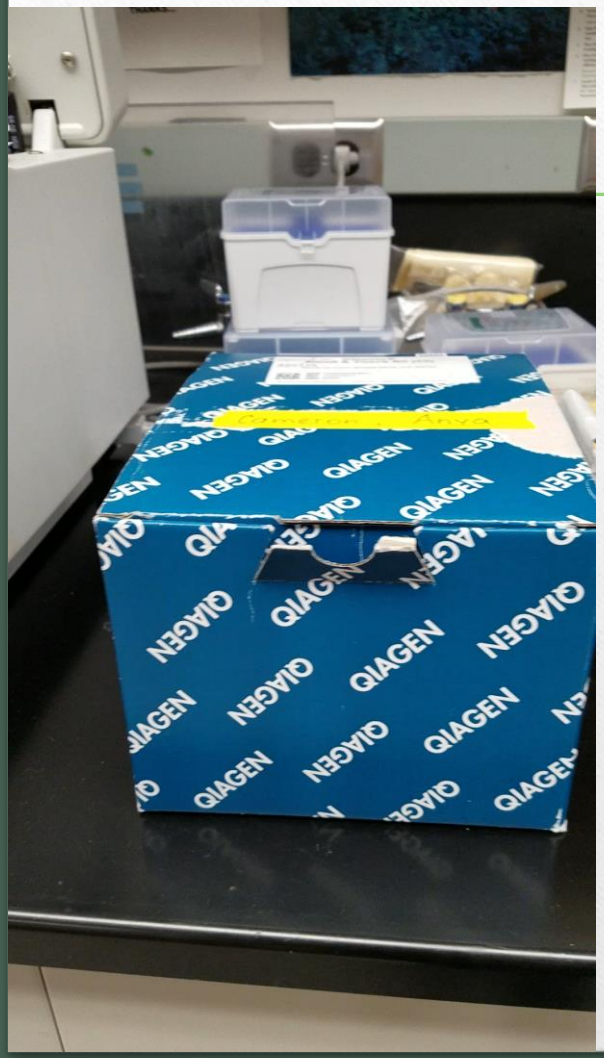




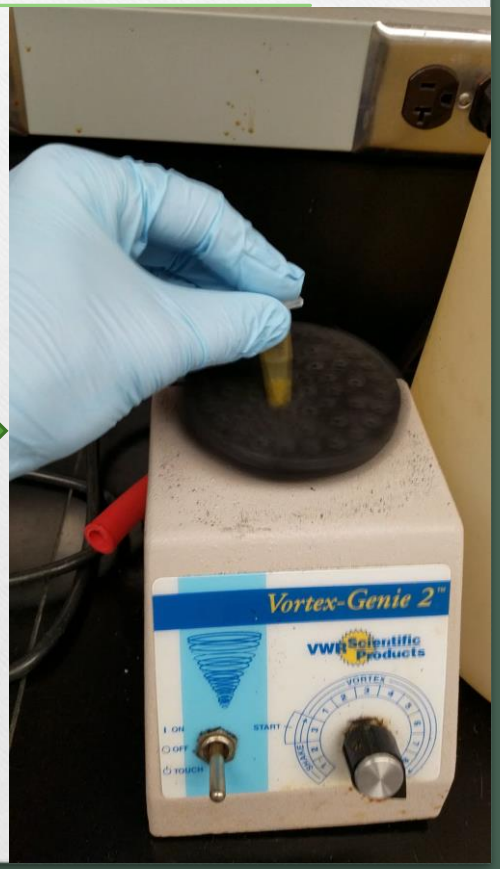
# Day 2



Buffers AL, AW1, AW2, AE
+100%Ethanol



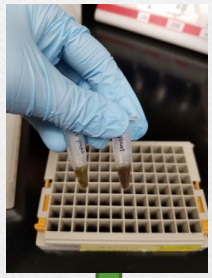

1. Take the tubes out of the incubator. Turn off the incubator.
2. Vortex the tubes 15 sec.

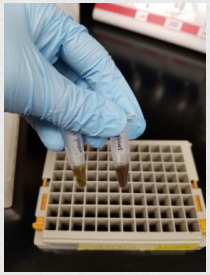
# Day 2


3. Add 200 µl Buffer AL.
4. Vortex the tubes thoroughly.
5. Add 200 µl ethanol (100%, in the metal cabinet)
6. Vortex the tubes thoroughly.

200 µl

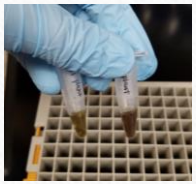



200 µl

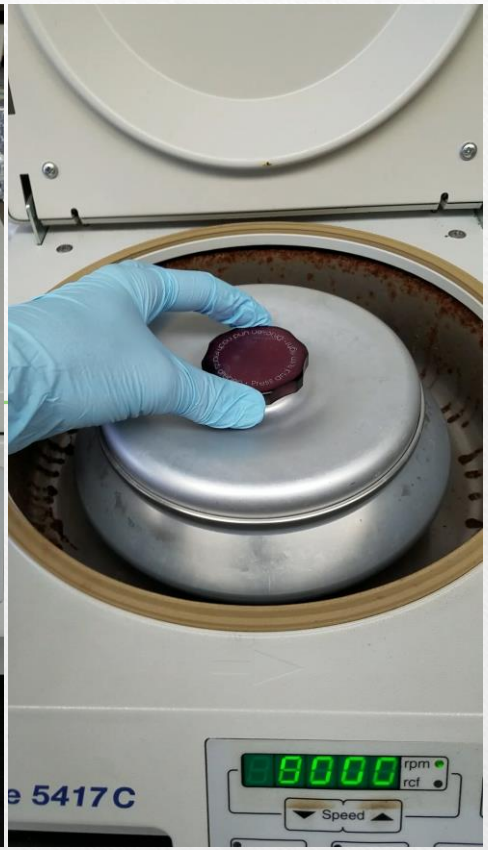



# Day 2

DNeasy Mini spin columns



~500 µl

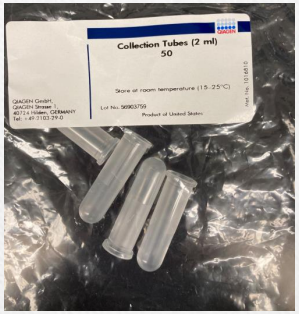

7. Prepare DNeasy Mini spin columns placed in a 2 ml collection tubes (come with the kit).
Label the spin columns.

8. Set up pipet p1000 to 500 µl. Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube.

9. Centrifuge at 8000 rpm for 1 min

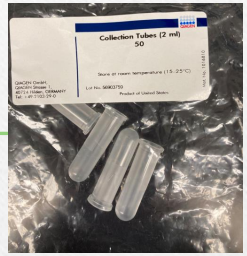
# Day 2


Collection tubes





Spin column → Place in a new collection tube




Collection tube → Discard!

10. Prepare new 2 ml collection tubes (come with the kit).
11. Take the tubes out of the centrifuge. Discard the flow-through and old collection tubes.
12. Place the spin column in a new 2 ml collection tube.

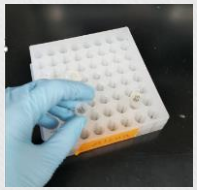
# Day 2 (cont.)

Buffer AW1 Buffer AW2

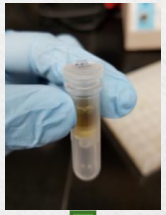


13. Add 500 µl Buffer AW1.
14. Centrifuge at 8000 rpm for 1 min
15. Prepare new 2 ml collection tubes.
16. Take the tubes out of the centrifuge. Discard the flow-through and old collection tubes.
17. Place the spin column in a new 2 ml collection tube.


500 µl

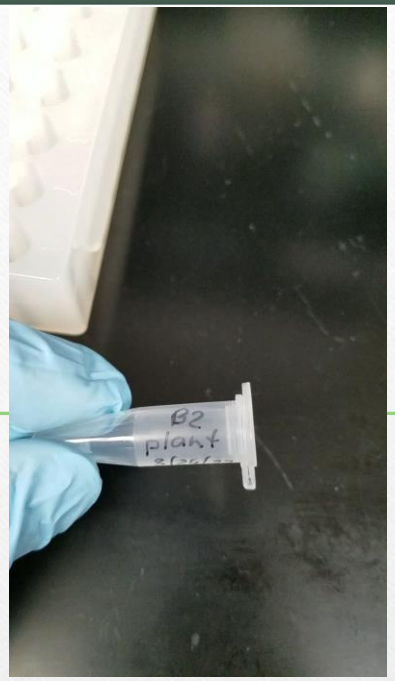

500 µl



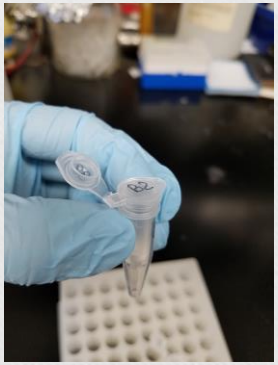

18. Add 500 µl Buffer AW2.
19. Centrifuge at 14,000 rpm for 3 min

# Day 2 (cont.)

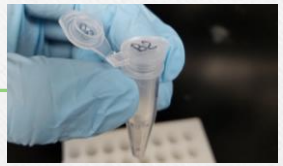
Buffer AE

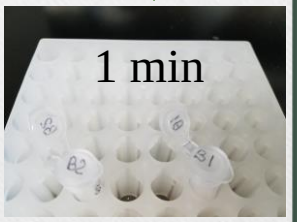







200 µl

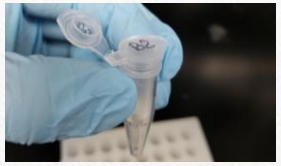




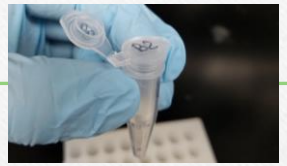


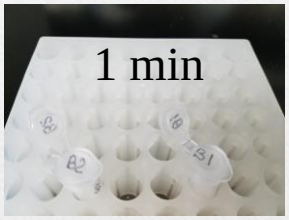
20. Prepare new 1.5 ml microcentrifuge tubes. Label them.
21. Take the tubes out of the centrifuge. Discard the flow-through and collection tubes.
22. Transfer the spin column to a new 1.5 ml microcentrifuge tube.
23. Add 200 µl Buffer AE directly to the center of the spin column membrane.
24. Incubate for 1 min at room temperature.
25. Centrifuge at 8000 rpm for 1 min.

# Day 2 (cont.)

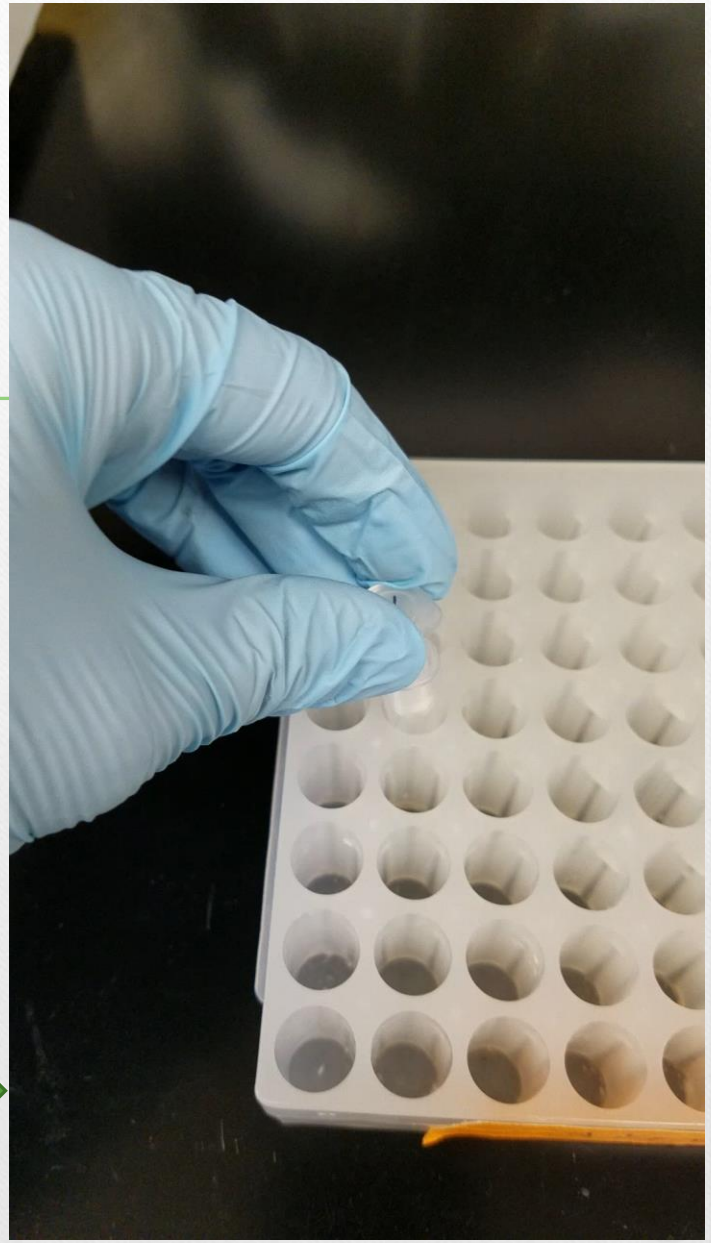



200 µl









26. Keep the spin column and microcentrifuge tube.
27. Repeat steps 23-25 (it will increase DNA yield).
28. Discard the spin column. Close the microcentrifuge tube and store it at +4$^0$C short-term (1-2 months) or at - 20$^0$C long-term.

# DNA extraction is done!

After step 28, you can either store your samples until you need them for PCR, or you can proceed immediately to PCR if needed.

# Image and video credits

- Videos: recording and editing by Alina Avanesyan
- Photos: preparing and editing by 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!
- We also thank Anya Wilkinson for taking the images for the DNA extraction part of the course