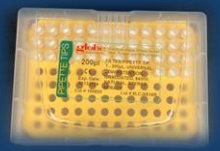
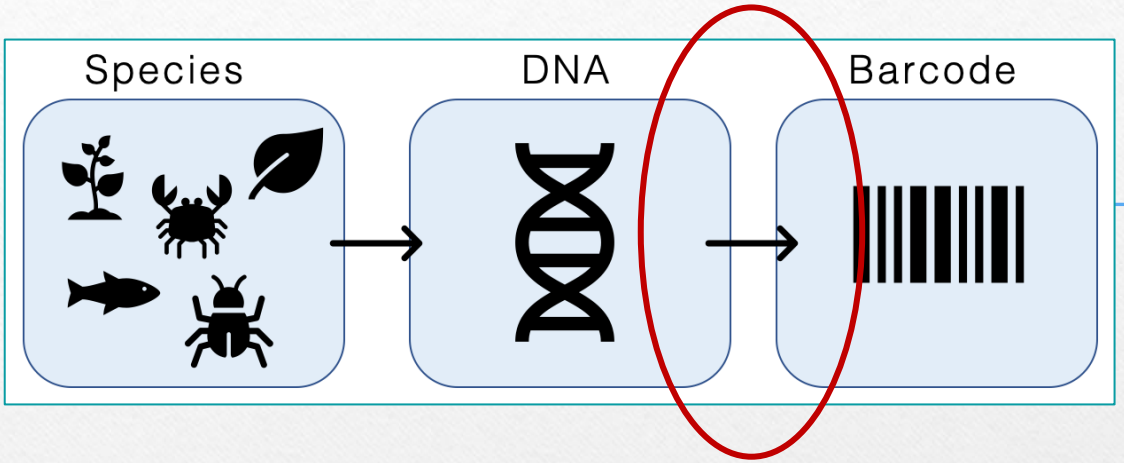
Polymerase Chain Reaction (PCR)



PCR: Amplification of a DNA fragment



Final product:
a targeted piece of DNA
(=DNA barcode)

- Portion of chloroplast DNA
- Portion of mitochondrial DNA
- Portion of bacterial DNA, etc.

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

PCR Equipment and Materials

Vortex

Pipettes

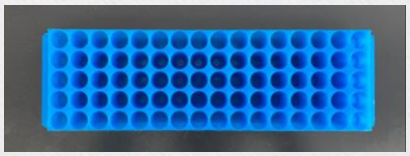
Pipette tips

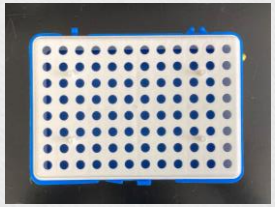
p200

p20

PCR machine

Racks





PCR tube strips

Marker

PCR reagents

PCR reagents and PCR protocol

PCR reagents:
PCR PreMix
Primers 1 and 2
water

Follow the protocol

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_2O (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_2O	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:

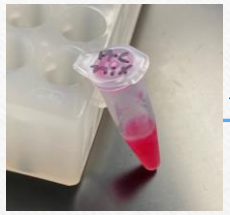
- 94^0C for 4 min
- ×35 cycles:
  - 94^0C for 30 sec
  - 57^0C for 30 sec
  - 72^0C for 30 sec
- 72^0C for 2 min
- Hold at 4^0C for 9 hours

← Typically, PCR runs about 2-2.5 hours

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

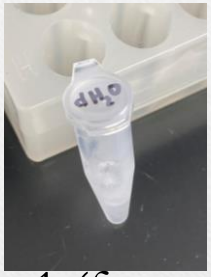
Preparing PCR "cocktail"

PCR PreMix



10 µl

DNase/RNase-Free Distilled Water



5.2 µl

2 µl

Primer 1 (forward)

Primer 2 (reverse)

2 µl

PCR protocol

(plant DNA detection from leafhoppers)

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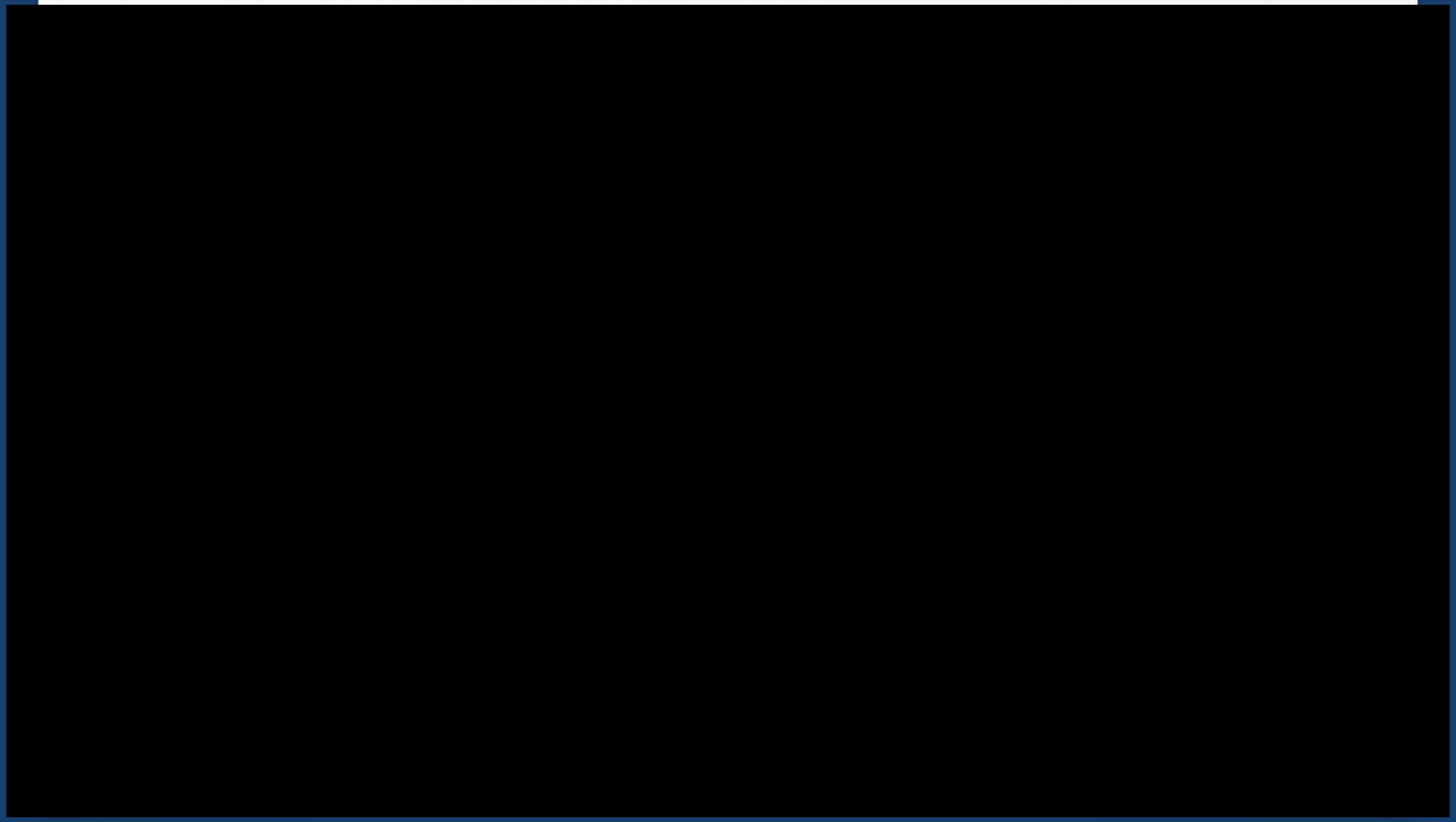
ddH_2O	5.2 µl	26 µl	52 µl

Demo 1: Adding PCR PreMix and Water



Anya Wilkinson, high school student, Lamp Lab (2021-2022)

Demo 2: Adding Primers

Anya Wilkinson, high school student, Lamp Lab (2021-2022)

Before the next step…

After completing step 4, PCR reagents should be placed back to the freezer

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_2O (in the right drawer), one 1.5 ml microcentrifuge tube.
4. Prepare PCR cocktail (#reactions = #samples+1; to cover pipetting error):

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PCR settings:

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- ×35 cycles:
  94^0C for 30 sec
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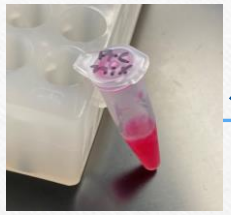
- 72^0C for 2 min
- Hold at 4^0C for 9 hours

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

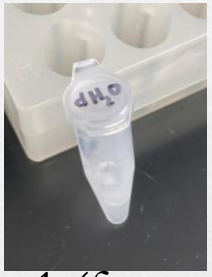
Adding DNA Template

PCR PreMix



10 µl

DNase/RNase-Free Distilled Water



5.2 µl

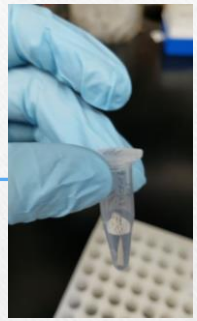
2 µl

Primer 1 (forward)

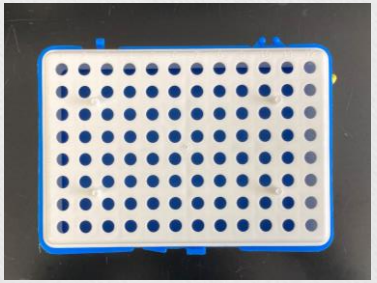
Primer 2 (reverse)

2 µl

19.2 µl (in each tube)



0.8 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.

Demo 3: Transferring PCR "cocktail" to PCR tubes

Anya Wilkinson, high school student, Lamp Lab (2021-2022)

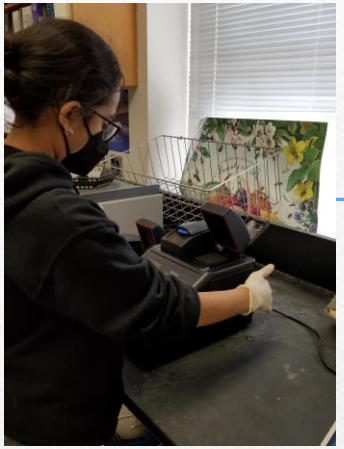
Demo 4: Adding DNA Template

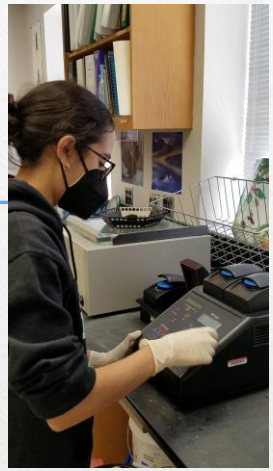
Anya Wilkinson, high school student, Lamp Lab (2021-2022)

Demo 5: Labelling PCR tubes

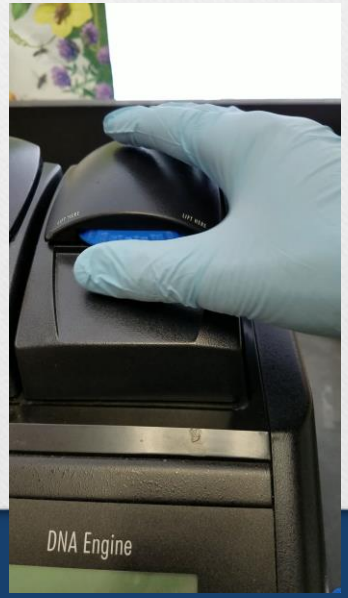
Anya Wilkinson, high school student, Lamp Lab (2021-2022)

Running PCR





~2 hours later…



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:

    - 94^0C for 4 min
    - ×35 cycles:
      94^0C for 30 sec
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      72^0C for 30 sec
    - 72^0C for 2 min
    - Hold at 4^0C 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.

PCR is done!

After PCR is done, you can either store your samples at -20^{0}C until you need to run a gel or to do DNA purification, or you can proceed immediately to DNA purification (~40 min total).

Image and video credits

- Videos: PCR steps were demonstrated by Anya Wilkinson; recording and editing were done by Alina Avanesyan
- Photos: preparing and editing by Anya Wilkinson and Alina Avanesyan
- PCR protocol: prepared by Alina Avanesyan
- 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 demonstrating the main PCR steps for this course