NAME _____

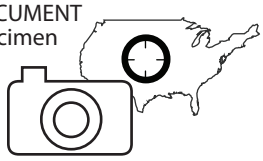
GROUP _____ SPECIMEN TYPE _____ SAMPLE ID # _____

DNA Barcoding

I. COLLECT, DOCUMENT, AND IDENTIFY SPECIMENS

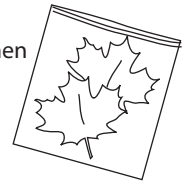
☐ **1** COLLECT specimen

☐ **2** DOCUMENT specimen



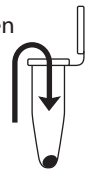
☐ **3** IDENTIFY specimen

☐ **4** STORE specimen

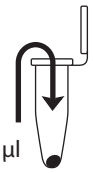


II. ISOLATE DNA FROM PLANT, FUNGAL, OR ANIMAL SAMPLES

☐ **1** ADD specimen tissue sample

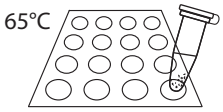


☐ **2** ADD lysis solution

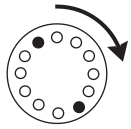


☐ **3** GRIND sample in solution

☐ **4** INCUBATE 10 min TIME IN: _____ TIME OUT: _____



☐ **5** CENTRIFUGE 1 min



Hinges out and balanced

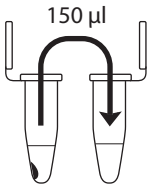
Why is a centrifuge helpful to us? (mark one)

- ☐ Mixes components
- ☐ Separates components
- ☐ Speeds up reactions

After you spin your tube (Step 5) where will the DNA be? (mark one)

- ☐ Supernatant
- ☐ Pellet

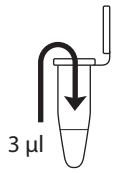
☐ **6** TRANSFER supernatant to fresh tube



Avoid pellet at bottom under hinge

☐ **7** ADD silica resin

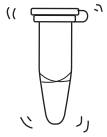
Should look white in pipette



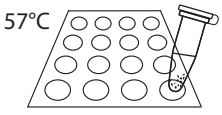
Why did we add silica resin? (mark one)

- ☐ Clean DNA
- ☐ Bind DNA
- ☐ Cut DNA

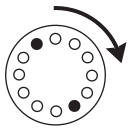
☐ **8** MIX



☐ **9** INCUBATE 5 min TIME IN: _____ TIME OUT: _____



☐ **10** CENTRIFUGE 30 sec



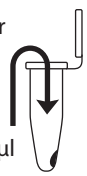
After you spin your tube (Step 10) where will the DNA be? (mark one)

- ☐ Supernatant
- ☐ Pellet

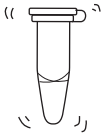
☐ **11** REMOVE supernatant

Either by pouring or pipetting

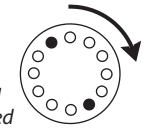
☐ **12** ADD wash buffer



☐ **13** MIX



☐ **14** CENTRIFUGE 30 sec

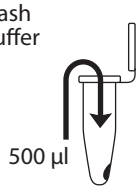


Hinges out and balanced

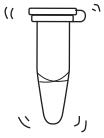
☐ **15** REMOVE supernatant

Either by pouring or pipetting

☐ **16** ADD wash buffer

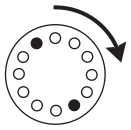


☐ **17** MIX



☐ **18** CENTRIFUGE 30 sec

Hinges out and balanced



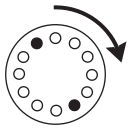
☐ **19** REMOVE remaining supernatant

Either by pouring or pipetting

500 µl

☐ **20** CENTRIFUGE 15 sec

Hinges out and balanced



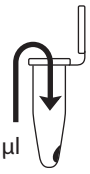
☐ **21** REMOVE remaining supernatant

Amount varies; use small pipette to remove

~50 µl

☐ **22** ADD dH_2O

100 µl



Why did we add distilled water? (mark one)

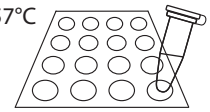
- ☐ Clean DNA
- ☐ Denature DNA
- ☐ Remove DNA from silica

☐ **23** MIX by pipetting in and out

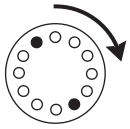
☐ **24** INCUBATE 5 min

TIME IN: _____
TIME OUT: _____

57°C



☐ **25** CENTRIFUGE 30 sec



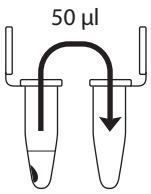
After you spin your tube (Step 25) where will the DNA be? (mark one)

- ☐ Supernatant
- ☐ Pellet

☐ **26** TRANSFER supernatant to fresh tube

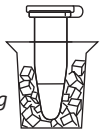
Make sure final DNA solution does not contain silica

50 µl

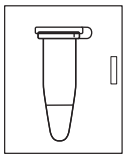


☐ **27** CHILL on ice **OR** STORE at

If proceeding to Part III

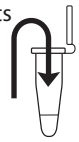


4° C overnight or -20° C longer



III. AMPLIFY DNA BY PCR

☐ **1** ADD PCR reagents

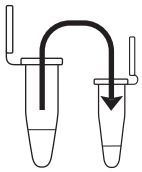


Check one:

- ☐ 23 µl primer mix to PCR beads
  or
- ☐ 12.5 µl *Taq* mix + 10.5 µl primer mix

☐ **2** TRANSFER DNA to PCR tube

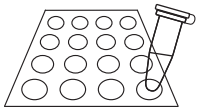
2 µl



Where do you add the DNA? Check one:

- ☐ on the side of the tube
  or
- ☐ directly into PCR mix

☐ **3** AMPLIFY in thermal cycler



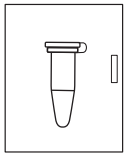
Which gene is being copied?

- ☐ *rbc*L
- ☐ *COI*
- ☐ *ITS*

☐ **4** CHILL on ice **OR** STORE at

If proceeding to Part IV

4° C overnight or -20° C longer

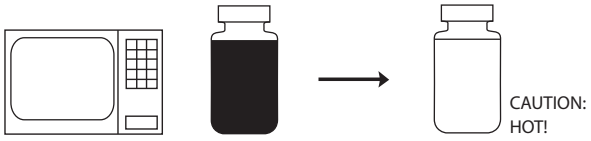


Electrophoresis, Sequencing, and Analysis

IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

1 MELT agarose at 30 sec intervals

Loosen cap

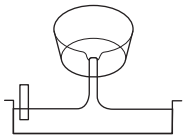


Is the agarose....?
Check all that apply:

- ☐ Clear
- ☐ Boiling
- ☐ Homogeneous

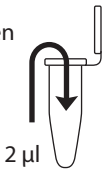
2 COOL 5 min

3 POUR gel

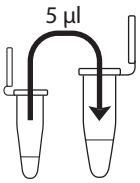


4 SET 20 min

5 ADD SYBR Green to fresh tube



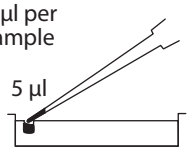
6 TRANSFER DNA from PCR tube to SYBR Green tube

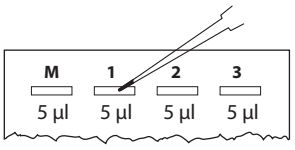


7 LOAD gel

CAUTION!
DO NOT load all 25 µl of sample into the gel or there will be no sample left to sequence!

LOAD 5 µl per sample

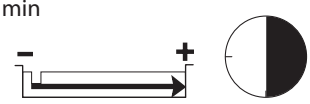




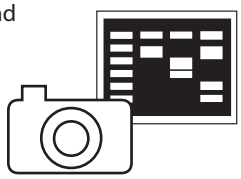
8 STORE PCR tube with remaining 20 µl sample

4° C overnight
or
-20° C longterm

9 ELECTROPHORESE
130 volts
400 mA
30 min

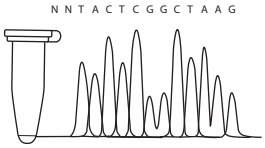


10 PHOTOGRAPH and upload



V. SEQUENCE PCR PRODUCT AND ANALYZE RESULTS

1 SEND sample for sequencing



2 ANALYZE results using bioinformatics