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
5. CENTRIFUGE 1 min
6. TRANSFER supernatant to fresh tube
7. ADD silica resin
8. MIX
9. INCUBATE 5 min
10. CENTRIFUGE 30 sec
11. REMOVE supernatant
12. ADD wash buffer
13. MIX
14. CENTRIFUGE 30 sec
15. REMOVE supernatant
16. ADD wash buffer
17. MIX

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

INCUBATE 10 min
TIME IN: ___ TIME OUT: ___

Why did we add silica resin? (mark one)
- Clean DNA
- Bind DNA
- Cut DNA

TIME IN: ___ TIME OUT: ___

Why did we add silica resin? (mark one)
- Clean DNA
- Bind DNA
- Cut DNA

Either by pouring or pipetting

Avoid pellet at bottom under hinge

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

INCUBATE 10 min
TIME IN: ___ TIME OUT: ___

INCUBATE 10 min
TIME IN: ___ TIME OUT: ___

INCUBATE 10 min
TIME IN: ___ TIME OUT: ___
III. AMPLIFY DNA BY PCR

1. ADD PCR reagents
   - Check one:
     - 23 µl primer mix to PCR beads
     - 12.5 µl Taq mix + 10.5 µl primer mix

2. TRANSFER DNA to PCR tube
   - 2 µl

3. AMPLIFY in thermal cycler

4. CHILL on ice OR STORE at
   - If proceeding to Part IV
   - 4°C overnight or -20°C longer

If proceeding to Part IV

- 4°C overnight or -20°C longer

Centrifuge 30 sec

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

Why did we add distilled water? (mark one)
- Clean DNA
- Denature DNA
- Remove DNA from silica

INCUBATE 5 min

TIME IN: _____

TIME OUT: _____

57°C

CHILL on ice OR STORE at

If proceeding to Part III

4°C overnight or -20°C longer
IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

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!
8. Store PCR tube with remaining 20 µl sample.
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.

STORE PCR tube with remaining 20 µl sample. 

4°C overnight or -20°C longterm.