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We use this protocol and it's working

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DNA Barcoding Protocol for Arthropods

Valerie Warhol[1]

[1]Science Club

Valerie Warhol
Science Club

ABSTRACT

This is a basic protocol for doing extraction and amplification of DNA barcodes (COI) for arthropods (typically small insects or spiders).

PROTOCOL MATERIALS

Guanidine Hydrochloride 6M **Carolina Biological Supply Catalog #C33427** Step 5

Prepare sample and equipment

1 Make sure all instruments, such as forceps and pestle, are clean and sterile.

2 Prepare water bath at 65 °C .

3 Dissect sample from specimen (typically 1 leg). Return specimen to freezer.

4 Let sample dry for 5–10 minutes to remove ethanol.

5 Prepare a clean 1.5 mL hinged tube by writing sample ID on it and filling with 250 µL of Guanidine Hydrochloride 6M **Carolina Biological Supply Catalog #C33427**

Lyse cells

11m

6 Put sample in tube. Grind sample with pestle until broken up into tiny pieces.

7 Incubate sample tube in 65 °C water bath for 00:10:00 . 10m

8 Remove tube and lower temperature of water bath to 57 °C .

9 Centrifuge tube for 00:01:00 at maximum speed to pellet debris. 1m

10 Remove Silica Resin **Carolina Biological Supply Catalog #C33426** from refrigerator.

11 Label a clean 1.5 µL tube with sample number. Transfer 150 µL of the supernatant to the clean tube. Discard old tube containing debris.

Bind DNA

5m 30s

12 Add 3 µL of silica resin to tube. Mix well by pipetting up and down several times.

13 Close tube and incubate for 00:05:00 in 57 °C water bath. 5m

14 Centrifuge for 00:00:30 at maximum speed to pellet the resin. 30s

15 Use a pipette with a fresh tip to remove the supernatant, being careful not to disrupt the pellet.

Wash

1m

16 Remove molecular grade water from refrigerator and Wash Buffer **Carolina Biological Supply Catalog #C33428** from freezer.

17 Add 500 µL of ice-cold wash buffer to the pellet. Mix well by pipetting up and down several times to resuspend the silica resin.

18 Close the tube and centrifuge for 00:00:30 at maximum speed to pellet the resin. 30s

19 Use a pipette with a fresh tip to remove the supernatant, being careful not to disrupt the pellet.

20 Again, add 500 µL of ice-cold wash buffer to the pellet. Mix well by pipetting up and down to resuspend the silica resin.

21 Close the tube and centrifuge for 00:00:30 at maximum speed to pellet the resin. 30s

22 Return wash buffer to freezer.

23 Use a pipette with a fresh tip to remove the supernatant, being careful not to disrupt the pellet. Spin the tube briefly to collect any remaining drops of supernatant, and then remove these with a pipette.

Elute DNA

30s

24 Add 100 µL of molecule grade water to the silica resin and mix by pipetting up and down several times.

25 Incubate the mixture at 57 °C for 5 minutes.

26 Centrifuge for 00:00:30 at maximum speed to pellet the resin. 30s

27 Label a clean 1.5 µL tube with sample number. Transfer 90 µL of the supernatant to the clean tube, being careful not to disturb the pellet. Discard old tube containing the resin.

28 Store sample in freezer until ready to PCR. If going directly to PCR, put sample in refrigerator.

Amplify COI (PCR)

10m

29 For each DNA sample, label a PCR microtube with sample ID.

30 Turn on PCR thermal cycler and connect to computer.

31 Remove molecular grade water from refrigerator. Remove template DNA, PCR master mix, and primers fr 10m freezer. Let thaw for 00:10:00 .

	A	B	C	D
	Primer Name	Direction	Sequence	**Concentration**
	LCO1490	Forward	GGTCAACAAATCATAAAGATATTGG	10 µM
	HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	10 µM

32 Add 32 µL molecular grade water to each microtube.

33 Mix forward primer by flicking. Add 1.5 µL forward primer to each microtube.

34 Mix reverse primer by flicking. Add 1.5 µL reverse primer to each microtube.

35 Mix template DNA by flicking. Add 5 µL template DNA to each microtube.

36 Mix EZ PCR Master Mix 5X **miniPCR** by inverting. Add 10 µL PCR master mix to each microtube.

37 Put microtubes in PCR thermal cycler and start touchdown PCR.

1. Denature: 95° C 30 sec; Anneal: 60° C 30 sec; Extend: 72° C 45 sec.
2. Denature: 95° C 30 sec; Anneal: 59° C 30 sec; Extend: 72° C 45 sec.
3. Denature: 95° C 30 sec; Anneal: 58° C 30 sec; Extend: 72° C 45 sec.
4. Denature: 95° C 30 sec; Anneal: 57° C 30 sec; Extend: 72° C 45 sec.

5. Denature: 95° C 30 sec; Anneal: 56° C 30 sec; Extend: 72° C 45 sec.
6. Denature: 95° C 30 sec; Anneal: 54° C 30 sec; Extend: 72° C 45 sec.
7. Denature: 95° C 30 sec; Anneal: 53° C 30 sec; Extend: 72° C 45 sec.
8. Denature: 95° C 30 sec; Anneal: 52° C 30 sec; Extend: 72° C 45 sec. Repeat this step for 28 cycles.
9. Final extension: 72° C 5 min.

(An initial long denature step is not needed for this protocol.)

38 Remove microtubes from PCR thermal cycler. Verify PCR using gel electrophoresis.

39 Store PCR products in freezer (-20 °C).