

DEC 22, 2023

OPEN ACCESS



Protocol Citation: Valerie Warhol 2023. DNA Barcoding Protocol for Arthropods.

protocols.io

https://protocols.io/view/dnabarcoding-protocol-forarthropods-c6i7zchn

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working We use this protocol and it's working

Created: Dec 19, 2023

Last Modified: Dec 22,

2023

PROTOCOL integer ID:

92479

ONA Barcoding Protocol for Arthropods

Valerie Warhol¹

¹Science Club

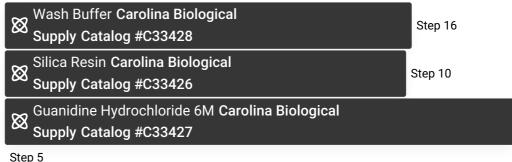


Valerie Warhol

ABSTRACT

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

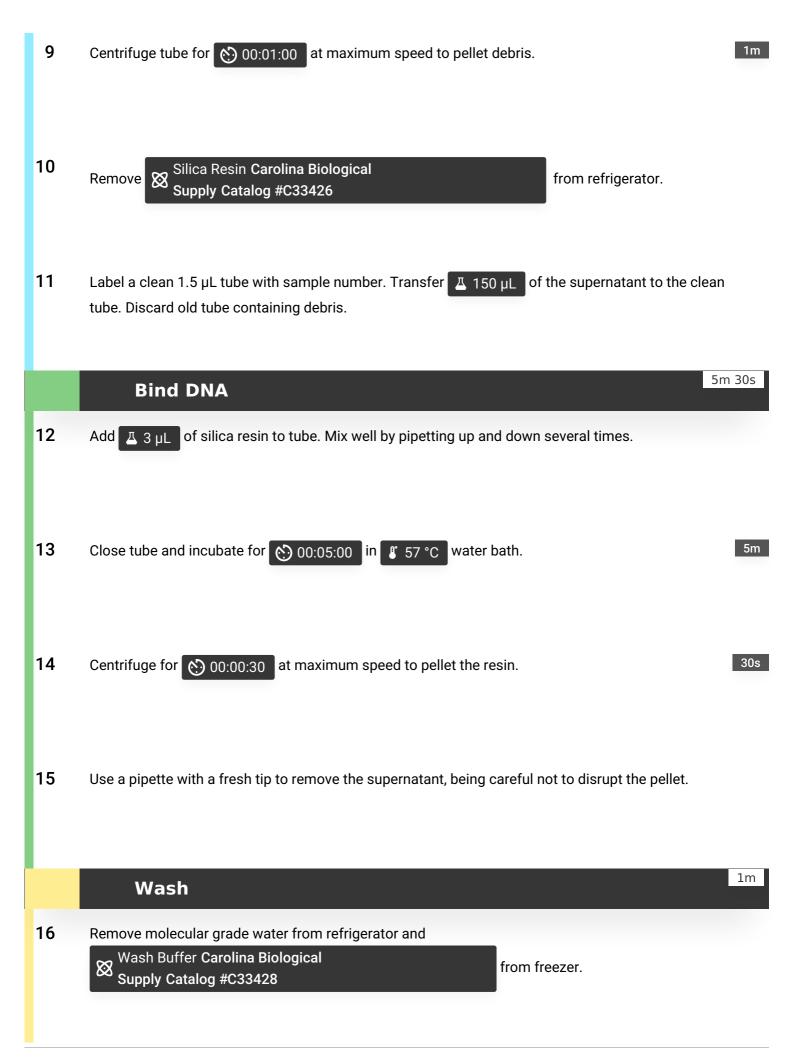
PROTOCOL MATERIALS





Step 36

Prepare sample and equipment 1 Make sure all instruments, such as forceps and pestle, are clean and sterile. 2 Prepare water bath at \$\circ\$ 65 °C 3 Dissect sample from specimen (typically 1 leg). Return specimen to freezer. 4 Let sample dry for 10 minutes to remove any ethanol. 5 Prepare a clean 1.5 mL hinged tube by writing sample ID on it and filling with Z 250 µL Guanidine Hydrochloride 6M Carolina Biological Supply Catalog #C33427 11m Lyse cells 6 Put sample in tube. Grind sample with pestle until broken up into tiny pieces. 7 10m Incubate sample tube in \$\ 65 \circ\$ water bath for \$\ 00:10:00\$



17 Add A 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 30s Close the tube and centrifuge for 00:00:30 at maximum speed to pellet the resin. 19 Use a pipette with a fresh tip to remove the supernatant, being careful not to disrupt the pellet. 20 Again, add A 500 µL of ice-cold wash buffer to the pellet. Mix well by pipetting up and down to resuspend the silica resin. 21 30s Close the tube and centrifuge for 00:00:30 at maximum speed to pellet the resin. 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. 30s **Elute DNA** 24 Add A 100 µL of molecule grade water to the silica resin and mix by pipetting up and down several times. 25 Incubate the mixture at \$\mathbb{I}^\circ 57 \circ \text{for 5 minutes.}

- 26

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

- 30s
- 27 Label a clean 1.5 μ L tube with sample number. Transfer \perp 90 μ L 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 prime 10m from freezer. Let thaw for 00:10:00.

A	В	С	D
Primer Name	Direction	Sequence	Concentration
LC01490	Forward	GGTCAACAAATCATAAAGATATTGG	10 μΜ
HCO2198	Reverse	TAAACTTCAGGGTGACCAAAAAATCA	10 μΜ

32 Add 🗸 31 µL molecular grade water to each microtube.

